To Fear or not to Fear: Unraveling the (Oculo)motor and Autonomic Components of Defensive States in Humans

Vor Furcht Erstarren: Charakterisierung der (Okulo)motorischen und Autonomen Komponenten Menschlicher Defensivreaktionen

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Table of Contents

Abstract

Defensive behaviors in response to threats are key factors in maintaining mental and physical health, but their phenomenology remains poorly understood. Prior work reported an inhibition of oculomotor activity in response to avoidable threat in humans that reminded of freezing behaviors in rodents. This notion of a homology between defensive responding in rodents and humans was seconded by concomitant heart rate decrease and skin conductance increase. However, several aspects of this presumed defense state remained ambiguous. For example, it was unclear whether the observed oculomotor inhibition would 1) robustly occur during preparation for threat-avoidance irrespective of task demands, 2) reflect a threat-specific defensive state, 3) be related to an inhibition of somatomotor activity as both motion metrics have been discussed as indicators for freezing behaviors in humans, and 4) manifest in unconstrained settings.

We thus embarked on a series of experiments to unravel the robustness, threat-specificity, and validity of previously observed (oculo)motor and autonomic dynamics upon avoidable threat in humans. We provided robust evidence for reduced gaze dispersion, significantly predicting the speed of subsequent motor reactions across a wide range of stimulus contexts. Along this gaze pattern, we found reductions in body movement and showed that the temporal profiles between gaze and body activity were positively related within individuals, suggesting that both metrics reflect the same construct. A simultaneous activation of the parasympathetic (i.e., heart rate deceleration) and sympathetic (i.e., increased skin conductance and pupil dilation) nervous system was present in both defensive and appetitive contexts, suggesting that these autonomic dynamics are not only sensitive to threat but reflecting a more general action-preparatory mechanism. We further gathered evidence for two previously proposed defensive states involving a decrease of (oculo)motor activity in a naturalistic, unconstrained virtual reality environment. Specifically, we observed a state consisting of a cessation of ongoing behaviors and orienting upon relatively distal, ambiguous threat (Attentive Immobility) while an entire immobilization and presumed allocation of attention to the threat stimulus became apparent upon approaching potential threat (Immobility under Attack).

Taken together, we provided evidence for specific oculomotor and autonomic dynamics upon increasing levels of threat that may inspire future translational work in rodents and humans on shared mechanisms of threat processing, ultimately supporting the development of novel therapeutic approaches.

Zusammenfassung

Angemessen auf Gefahren zu reagieren, ist überlebensnotwendig, wissenschaftlich jedoch wenig verstanden. Eine frühere Studie wies auf, dass ProbandInnen ihre Augen weniger bewegten, wenn sie mit einer Bedrohung konfrontiert waren, der sie mit einer schnellen Bewegung entkommen konnten. Dieses eingeschränkte Blickbewegungsmuster wurde von einer Herzraten-Dezeleration und einem Anstieg der Hautleitfähigkeit begleitet und wies damit Ähnlichkeiten mit bestimmten Erstarrungsreaktionen auf Bedrohungen (Freezing) bei Nagetieren auf. Es blieb jedoch unklar, ob die eingeschränkten Augenbewegungen 1. robust und unabhängig von spezifischen Aufgabenstellungen als Reaktion auf eine vermeidbare Bedrohung auftreten, 2. eine bedrohungsspezifische Komponente eines Defensivzustands darstellen, 3. von einer körperlichen Bewegungsreduktion begleitet und 4. im freien Raum auftreten würden.

Wir haben daher untersucht, ob dieses eingeschränkte Blickbewegungsmuster sowie seine autonomen Begleiterscheinungen robust, bedrohungsspezifisch und valide sind. In unseren Studien traten verringerte Augenbewegungen robust und bedrohungsspezifisch als Reaktion auf vermeidbare Bedrohungen auf und sagten schnellere Reaktionszeiten vorher. Die eingeschränkten Augenbewegungen wurden von verringerten Körperbewegungen begleitet, deren zeitliche Verläufe miteinander korrelierten. Dies könnte auf ein zugrundeliegendes gemeinsames Konstrukt hinweisen. Wir beobachteten außerdem eine Herzraten-Dezeleration sowie erhöhte Hautleitfähigkeit und Pupillendilation in bedrohlichen und appetitiven Kontexten, was darauf hindeutet, dass diese autonomen Dynamiken nicht nur durch Bedrohungen, sondern auch allgemein handlungsvorbereitend ausgelöst werden können. Zuletzt konnten wir in einer frei explorierbaren, virtuellen Umgebung Hinweise auf zwei Defensivzustände liefern, deren Unterscheidung zuvor postuliert, jedoch noch nicht weitreichend belegt war. Bei relativ weit entfernter und ambivalenter Gefahr hielten die ProbandInnen inne und drehten sich, vermutlich zur Orientierung, zum potentiellen Ort der Bedrohung hin (Attentive Immobility). Wenn sich die Bedrohung jedoch näherte, verringerten sich sowohl Körper- als auch Augenbewegungen, als würden die ProbandInnen ihre Aufmerksamkeit auf die Bedrohung ausrichten (Immobility under Attack).

Zusammenfassend lieferten unsere Erhebungen damit Belege für spezifische (okulo)motorische und autonome Dynamiken bei steigender Bedrohung, die zukünftige translationale Forschungen über Homologien von Defensivzuständen zwischen Nagetieren und Menschen inspirieren und damit womöglich die Entwicklung neuer therapeutischer Verfahren unterstützen können.

1. Theoretical and Empirical Background

Through the process of evolution, nature has developed remarkable behaviors that enhance the likelihood of a species' survival and reproductive success (Blanchard & Blanchard, 2008). These behaviors include intricate and specialized defense mechanisms that protect organisms from harm (Adolphs, 2013; Lang & Bradley, 2010; LeDoux & Daw, 2018). For example, certain types of fish may hide in crevices in coral reefs and remain still while sharks patrol the area. Armadillos curl up into tight, spiky balls of armor, skunks release a pungent odor, and mice swiftly run to safety in the underbrush when sensing a nearby predator. Similarly, when confronted with a car approaching at high speed, humans may experience an abrupt adrenaline rush, allowing them to move out of the way just in time.

However, failing to display appropriate defensive responses can jeopardize an individual's mental and physical health and, in extreme cases, lead to death (e.g., the fish leaving its hiding place before the coast is clear; Blanchard & Blanchard, 2008). In humans, their impact on psychopathology is well-recognized. Disproportionate or persistent defensive behaviors are considered as key features of anxiety-related disorders, which are among the most prevalent mental disorders of our time (Pittig et al., 2018, 2020). Anxious psychopathology thereby not only burdens those affected but also their social and work-related environments (Chisholm et al., 2016; Grupe & Nitschke, 2013; Jacobi et al., 2014; Kessler et al., 2012; Wittchen et al., 2011). However, the underlying mechanisms of fear, anxiety, and related defensive behaviors remain poorly understood, and current treatments, while effective, have high recurrence rates (Bandelow et al., 2013; Bandelow & Michaelis, 2015; Carpenter et al., 2018; Otto et al., 2010; Springer et al., 2018). Rigorous scientific research is therefore crucial for improving our understanding of these behaviors and eventually developing novel therapeutic techniques to maintain or restore mental health.

Although defensive behaviors vary widely across species, research has demonstrated that basal fear- and anxiety-related defense circuits are evolutionarily conserved, allowing for translation from animal models to human studies (Adolphs, 2013; LeDoux & Daw, 2018; Mobbs et al., 2015). These similarities can be attributed to consistencies in the types of threats and corresponding responses across species, irrespective of their individual characteristics (Blanchard & Blanchard, 2008). Drawing from insights from different disciplines (e.g., Biology, Psychology, Neuroscience), such translational research offers promising avenues to comprehensively grasp fear- and anxiety-related defensive states from different angles. Additionally, its approach provides possibilities to compensate for methodological constraints of respective fields, which helps to build more complete theoretical models (Hamm, 2020; Johnson et al., 1995; McTeague, 2016).

However, previous research on defensive states in both rodents and humans is assailed by a lack of methodological standardization, a lack of conceptual consensus, and simplified assessments of complex defensive behaviors based on single measures (Maren, 2008; Signoret-Genest et al., 2022; Volchan et al., 2017). This has yielded inconsistent findings and outstanding questions, which render conclusions elusive.

Given the presumed clinical significance of these states, it is crucial to promote clear communication within and across research disciplines and comprehensively characterize defensive states and their individual components. This can eventually establish a basis for distinguishing healthy from aberrant defensive responses and developing appropriate preventive and intervention strategies. To contribute to this ultimate goal, this thesis will examine the limitations and outstanding questions of previous translational research on defensive states and offer both theoretical and experimental suggestions for addressing them.

1.1. A conceptual framework of fear, anxiety, and defensive behaviors

The definitions of fear and anxiety as well as their experimental investigation are subject to vigorous debates (Mobbs et al., 2019). Still, on a coarse level, many scientists largely agree that fear is an emotional response to a realistic, often tangible threat, while anxiety refers to an overlapping yet distinct concept applied when the source of a threat is uncertain, ambiguous, or unrealistic (Elman & Borsook, 2018). This conceptualization has been adopted and elaborated in multiple theoretical models.

One particularly influential model, the Predatory or Threat Imminence Continuum model (TICM), has been put forward by Fanselow and Lester (1988) who proposed that fear and anxiety and their related defensive behaviors can be linked to threat imminence. Their theory states that the probabilistic, spatiotemporal, or psychological distance of a threat elicits distinct defensive behaviors, ranging from hard-wired automatic reactions to specific goal-directed behaviors (LeDoux & Daw, 2018; Mobbs, 2018; Pittig et al., 2020). Based on increasing levels of threat, the TICM classifies four contexts that are associated with specific defensive states. Later, the theory has been extended by contiguous subcategories of fear and anxiety, but these remain to be scientifically supported (Mobbs et al., 2020).

During the *Safety* stage, there is no foreseeable prospect of danger (e.g., the mouse is in its mousehole, Figure 1). Organisms can thus invest in crucial non-defensive behaviors such as building borrows and mating to ensure their long-term survival. Humans, as Mobbs and colleagues (2020) suggest, may exhibit signs of ‘Intermittent Anxiety’, characterized by worries, strategizing about potential future threats and appraisal of changes in the environment that may require adaptation of current behaviors to remain safe.

In the *Pre-encounter* stage, it is increasingly likely to encounter a threat but there is still no visible sign of danger (e.g., the mouse has left the safety of its mousehole to forage). Now, hypervigilance and transient inhibitions of appetitive behavior may be mandated as measures of precaution. To reduce risks, places that have been previously associated with danger may be avoided and novel contexts only cautiously explored. Based on the still uncertain, ambiguous situation, humans may be in a state of ‘Anticipatory Anxiety’, involving strategizing and planning for when real danger appears (Mobbs et al., 2020).

The *Post-encounter* stage means that a threat has been detected but the prey cannot perceive acute aggressiveness or believes itself to stay undetected by the predator. Depending on the (perceived) proximity of the threat, this phase may elicit different defensive behaviors. If the defensive distance is relatively far and an acute threat seemingly unlikely (e.g., the mouse has sensed the odor of a cat but not seen it yet), a form of ‘Encounter Anxiety’ may manifest, entailing orienting behaviors and risk assessment (Blanchard et al., 2011). When the distance of the threat is more tangible and it is more likely to be attacked (e.g., the mouse has detected a lurking cat), however, ‘Cognitive Fear’ may ignite, comprising preparations for fast subsequent defensive actions in case the danger becomes real. Movement inhibitions (i.e., freezing) are considered typical behaviors in this phase as they have been suggested to help in optimizing sensual uptake and action preparation, while staying undetected by a predator (Roelofs & Dayan, 2022).

The most imminent stage of threat is called C*irca-strike*. The organism’s life is now in utmost danger (e.g., the cat strikes to attack the mouse). To increase chances of survival, the prey must flee from or fight the threat. Mobbs and colleagues (2020) subsume these presumably hard-wired, automated reactions to realistic and acute threat under the term ‘Reactive Fear’. However, if there is not enough time to properly perform these defensive behaviors, panic may be sparked resulting in uncoordinated flight or tonic immobility as the last resort to thwart a deadly attack.

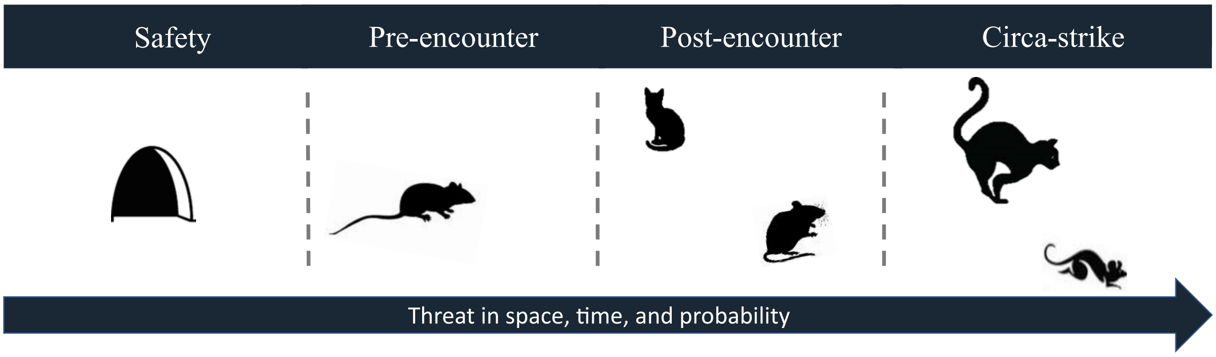

Figure 1. Illustration of the Threat Imminence Continuum Model (Fanselow & Lester, 1988; Mobbs et al., 2020).

A compellingly naturalistic model accounting for complex and dynamic features of the real world, the TICM has gained increasing popularity. Extended by additional factors that may contribute to the expression of defensive states such as the context (e.g., availability of escape routes; Blanchard & Blanchard, 1989; Volchan et al., 2017), specificities of the threat stimulus (e.g., novelty; Trott et al., 2022) as well as characteristics of the affected individual (e.g., age, sex, previous experience; Maren, 2008), it has gathered scientific support in both animal and human work (e.g., Hamm, 2020; Hoffman et al., 2022; Mobbs et al., 2009, 2020; Qi et al., 2018).

However, methodological issues, inconsistent findings and conceptual imprecision have prompted questions about the integrated phenomenology of certain defensive states and their individual components. Of particular concern is the concept of freezing, which has been subject to theoretical imprecision and methodological confusion. For example, the term freezing has been applied to both a single behavioral measure (i.e., movement cessation in animals and reduced postural sway in humans) and to a broader defensive state comprising dynamics of multiple measures (e.g., autonomic and behavioral), which has hampered inner- and interdisciplinary communication. Moreover, there is no standardized method on how to assess freezing behaviors within and across different disciplines. The term freezing as well as parts of its experimental investigation and interpretation have hence been rendered misleading (Volchan et al., 2017). Clear definitional distinctions and a rigorous evaluation of its measurement, interpretation, and integration into broader defense patterns seem crucial to warrant meaningful comparisons between studies and different research disciplines.

1.2. Freezing in response to threats in animals

In animal research, freezing in response to threats has been defined as an absence of all behavior except respiration (Bolles & Collier, 1976; Fanselow, 1980). It is one of the most extensively studied defensive behaviors in rodents, popular for its noninvasive, inexpensive, and robust measurement (Maren, 2008; Trott et al., 2022).

Freezing in rodents is usually assessed using video tracking or force transducers that record displacements of an animal within a chamber. While earlier researchers manually coded the length and number of freezing bouts within a certain period, which was time-consuming and, although relatively reliable, prone to unwanted biases (Anagnostaras et al., 2010; Bolles & Riley, 1973; Fanselow, 1980; Phillips & LeDoux, 1992; Sigmundi et al., 1980), this task is nowadays typically performed by computerized programs based on rules that are predetermined by the experimenter (Anagnostaras et al., 2010; Curzon et al., 2009; Kopec et al., 2007). These rules usually entail a definition of a Motion Index Threshold below which freezing is scored as well as a definition of a Minimum Freeze Duration specifying the time in which the motion index must remain below the Motion Index Threshold. Despite carefully evaluated scoring protocols (e.g., Anagnostaras et al., 2010), the exact definition of these parameters varies between studies (e.g., Minimum Freeze Durations of at least 1 sec, Chang et al., 2009; Trott et al., 2022; or 2 sec, Herry et al., 2008; Tovote et al., 2016).

Freezing in rodents has been mainly investigated in fear conditioning paradigms where an inherently aversive unconditioned stimulus (US) such as a foot-shock is paired with an initially neutral stimulus such as a tone (conditioned stimulus (CS), Anagnostaras et al., 2010; Bouton & Bolles, 1980; Grewe et al., 2017; Kim & Fanselow, 1992; Kwon et al., 2015; Nader et al., 2001). After a training session of repeated US-CS pairings, the CS usually evokes conditioned fear. This conditional response is typically expressed as freezing, which, in turn, has been majorly relied on to quantify fear and associative learning (Tovote et al., 2015; Trott et al., 2022).

However, the assessment of fear based on a single behavioral readout has aroused heavy critique for several reasons. For example, the absence of freezing does not necessarily indicate an absence of fear. Freezing is just one of many defensive behaviors in response to threat and, depending on the context, not the only reaction to conditioned stimuli (Colom-Lapetina et al., 2019; Gruene et al., 2015; Mitchell et al., 2022). Moreover, it has been suggested that different types of freezing exist that have been linked to qualitatively different neuroanatomical profiles (Brandão et al., 2008; Mobbs et al., 2015). This idea is supported by seemingly paradoxical autonomic patterns that have been reported to accompany freezing, including heart rate deceleration (de Toledo & Black, 1966; Gentile et al., 1986; Roelofs & Dayan, 2022; Fanselow, 1984; Vianna & Carrive, 2005; Walker & Carrive, 2003) and acceleration (Carrive, 2000; Iwata & LeDoux, 1988; LeDoux et al., 1984; Sakaguchi et al., 1983; Stiedl & Spiess, 1997).

Relying solely on freezing as a marker for fear-related behavior thus seems problematic. While further research is needed to address these issues and outstanding questions, theoretical classifications of defensive states that involve immobility are necessary to enable meaningful interdisciplinary communication. Volchan and colleagues (2017) proposed such a classification, identifying three threat-related behavioral defense states characterized by immobility: Attentive Immobility, Immobility under Attack, and Tonic Immobility. Thereby, they used contextual features, including the spatiotemporal distance of the threat (Fanselow & Lester, 1988) and the availability of escape routes (Blanchard et al., 2011), as distinction criteria. Given the artificiality of laboratory procedures, such an ethological approach seems particularly useful for understanding and distinguishing defensive behaviors based on the context in which they evolved.

However, fear conditioning paradigms present a challenge for clear classification into these categories, as they usually do not entail a manipulation of escape routes. Moreover, their results appear to defy categorization based on threat imminence. For the latter issue, theoretical solutions have been suggested. Accordingly, freezing as a response to a CS (mere association with a threat) represents a Post-encounter reaction (Fanselow, 1989) while activity bursts in response to a US (predatory contact) display Circa-strike reactions (Fanselow, 1989; Perusini & Fanselow, 2015; but see extensions to this selection rule of different defensive responses by Trott et al., 2022). With this in mind, the following categories are introduced as operational definitions until more suitable terms are developed.

ATTENTIVE IMMOBILITY. Attentive Immobility occurs when a threat is present, but not attacking yet (i.e., Post-encounter stage, Mobbs et al., 2020; Roelofs & Dayan, 2022). Also labeled as an ‘active form of freezing’ (Mobbs et al., 2020), it has been associated with enhanced visual and olfactory processing, risk assessment (Blanchard et al., 2011) and decision-making (Gallup & Rager, 1996; Marx et al., 2008) while decreasing the risk of predator detection (Trott et al., 2022). For example, Blanchard and Blanchard (1989) showed that in rodents, novel or innately aversive stimuli such as the odor of a cat elicit alternations between transient episodes of freezing and slow orienting movements. The latter were indicated by ears pointing forward, slow head movements or even approach of the source of the potential threat (Blanchard & Blanchard, 1990). Another study reported freezing or hiding in mice that were exposed to a dark, rapidly expanding, overhead looming stimulus that resembled an aerial predatory threat (Yilmaz & Meister, 2013).

IMMOBILITY UNDER ATTACK. Immobility under Attack is exhibited when the animal is attacked but flight is not an option. For example, when approached by a threat in an inescapable context, rats have been shown to freeze at defensive distances greater than 2 m. When the threat stimulus approaches even closer, the response switches to a more active mode (i.e., vocalization and display of teeth and increasing levels of jumping, Blanchard et al., 1986, 1991; Blanchard & Blanchard, 1989). Freezing in this scenario may help the prey in predicting the predator’s behaviors and preparing fast subsequent defensive actions (Roelofs, 2017; Roelofs & Dayan, 2022).

TONIC IMMOBILITY. Tonic Immobility is exhibited as a last resort to a life-threatening situation (i.e., after the predator has stricken; Carli & Farabollini, 2021). In experimental scenarios, it is typically elicited by physically restraining or inversing an animal until it stops struggling (Marx et al., 2008), which usually persists after the restraint has been removed (Carli & Farabollini, 2021). This behavior is supposedly adaptive as it may signal danger to conspecifics and make it harder for the predator to hold on to the prey (Carli & Farabollini, 2021; Ewell et al., 1981; Jones, 1986; Sargeant & Eberhardt, 1975; Thompson et al., 1981). It can last from seconds to hours and is marked by an unresponsiveness to external stimuli (Gallup & Rager, 1996). Still, animals seem to be able to process and learn from the situation (Ewell et al., 1981; Gallup et al., 1972, 1980; Klemm, 1971; Sigman & Prestrude, 1981).

Besides the described behaviors, defensive states also entail physiological dynamics. However, the challenges in classifying fear conditioning studies based on the distinction criteria of this conceptualization (i.e., availability of escape routes and proximity of a threat) as well as inconsistent findings in the literature (i.e., bradycardia and tachycardia, see Signoret-Genest and colleagues (2022) for an approach to the problem) make it difficult to clearly match immobile behaviors with autonomic dynamics. Still, a particular defense state has been proposed to consist of freezing, transient bradycardia (Bagur et al., 2021; Hermans et al., 2013; Liu et al., 2017; Roelofs & Dayan, 2022; Vianna & Carrive, 2005; Walker & Carrive, 2003) and a concomitant activation of the sympathetic nervous system, indicated by increased muscle tone and skin conductance (Leaton & Borszcz, 1985; Liu et al., 2017; Yoshimoto et al., 2010). This cardio-behavioral pattern has been suggested to support sensual uptake, attentional processing, and action preparation and may thus be associated with either Attentive Immobility or Immobility under Attack (Roelofs, 2017; Roelofs & Dayan, 2022). Tonic Immobility, in contrast, has been mainly linked to tachycardia (Nagaraja & Jeganathan, 1999; Carli & Farabollini, 2021) but, depending on the experimental design, context, the animal species, previous experience and the intensity of the threat stimulus, bradycardia may also be observed (Carli & Farabollini, 2021; Paylor et al., 1994).

Overall, pinpointing the autonomic dynamics accompanying these different defensive behaviors remains an important question for future research that is crucial to validating their proposed classification. Given the behavioral evidence, however, this conceptualization offers an approach to disentangling distinct defensive behaviors that have been interchangeably referred to as freezing, helping in unraveling inconsistent findings in freezing research. We will therefore adopt, apply, and discuss these concepts in human research to yield maximal comparability between animal and human work but treat them as subjects to debate when clear scientific evidence calls for conceptual modifications.

1.3. Freezing in response to threats in humans

To assess freezing in humans, researchers commonly use a stabilometric platform to track the standard deviations of weight shifts from the center of pressure (Roelofs, 2017; Roelofs et al., 2010; Roelofs & Dayan, 2022; for an approach to assess freezing in infants, see Buss et al., 2004). However, there is no standard way to analyze this data. Some researchers calculate the standard deviation of weight shifts in small time windows (e.g., Gladwin et al., 2016), while others use longer time windows and different filters (Niermann et al., 2015, 2017). Additionally, some researchers look at changes in body sway on either the anterior-posterior (e.g., Niermann et al., 2017; Roelofs et al., 2010), mediolateral axis (e.g., Azevedo et al., 2005; Facchinetti et al., 2006), or both (Hagenaars, Roelofs, et al., 2014), while others do not assess any measure of body movement but infer freezing from changes in heart rate (e.g., Lojowska et al., 2015; Wendt et al., 2017).

The absence of standardization and conceptual clarity hinders the interpretability of freezing behaviors in humans, and raises concerns about comparability with animal research, where freezing is typically indicated by complete body immobility. It is therefore not surprising that previous findings are mixed. For example, freezing has been observed in response to both inevitable (e.g., while passively viewing aversive pictures, Hagenaars et al., 2012) and avoidable threats (e.g., while preparing for active threat avoidance; Gladwin et al., 2016; Hashemi et al., 2021; Niermann, 2018). Some studies thereby reported associations between reductions in body sway and heart rate, respectively, and clinical constructs (e.g., Hagenaars et al., 2012; Roelofs et al., 2010), suggesting freezing as a vulnerability marker for psychopathologies, while others did not corroborate such associations (e.g., Gladwin et al., 2016; Rösler & Gamer, 2019), stressing its adaptive function in action preparation, attentional processing, and decision-making (Roelofs, 2017). To avoid ambiguity resulting from the interchangeable use of the term "freezing" for different defensive behaviors, we thus need clearer theoretical classifications. Drawing on evidence from animal research, we will therefore apply the previously introduced categories to humans, using features of experimental designs (i.e., the availability of escape routes and threat proximity) as criteria for categorization (Volchan et al., 2017).

ATTENTIVE IMMOBILITY. Based on Volchan and colleague's (2017) classification, Attentive Immobility is a Post-encounter response. Experimental designs indicating such state should hence involve a manipulation of present but relatively distal, potential threat. An example of such scenario was introduced by Gladwin and colleagues (2016). Specifically, participants stood on a stabilometric platform and watched as one of two men appeared on screen. After a short period of anticipation (6.5 sec), the trial outcome was revealed. The man would either draw a gun to shoot at the participant, resulting in delivery of an electric shock (Threat opponent) or visual feedback only (Safe opponent), or hold up a mobile phone. Participants were thereby armed or unarmed themselves. If they had a gun, they could thwart an attack by shooting the opponent first. Jumping the gun, however, resulted in punishment. If participants counter-attacked when the man was (still) innocent (i.e., when he was holding a phone or had not drawn the gun yet), they were shot by a policeman standing in the background of the screen.

The authors reported reductions in body sway and heart rate during the anticipation of the trial outcome. Importantly though, these effects were only present when participants were equipped with a weapon (vs. when they were helpless), irrespective of whether it was a Safe or Threat opponent. It was hence suggested that reduced postural sway and heart rate serve an action preparatory purpose in the face of avoidable threat, which was supported by follow-up studies showing stronger reductions in heart rate and body sway to predict faster shooting reactions (Hashemi et al., 2019, 2021). A similar response was corroborated in several experiments that included an option to actively avert an aversive outcome (e.g., Löw et al., 2015; Rösler & Gamer, 2019). These studies did not assess postural sway but showed bradycardia along with an increase in skin conductance (Löw et al., 2015) and focused gaze (Rösler & Gamer, 2019) during the preparatory period for a motor response. However, individual differences may perturb the seeming adaptiveness of this state. Specifically, more pronounced reductions in body sway, but not heart rate, have been associated with vulnerability markers of anxiety-related psychopathologies (i.e., lower hair cortisol concentrations and higher trait anxiety, Hashemi et al., 2021).

IMMOBILITY UNDER ATTACK. Immobility under Attack occurs upon approaching but inescapable threat. Such scenario may be reflected in experimental designs simulating a threatening situation without providing an option to actively avoid it (e.g., Azevedo et al., 2005; Hagenaars et al., 2012; Lopes et al., 2009; Roelofs et al., 2010). In these experiments, participants typically stand on a stabilometric platform while watching aversive pictures (e.g., Azevedo et al., 2005) or films (Hagenaars et al., 2012). Stimulus sets thereby usually consist of unpleasant aversive scenes (e.g., showing mutilated bodies and corpses), positive (e.g., pictures of sports) and/or neutral ones (e.g., neutral objects; Azevedo et al., 2005; Hagenaars et al., 2012). Importantly, participants cannot actively influence the sequence of events.

Experiments employing such and similar paradigms have shown reduced body sway when participants watched the aversive stimuli as compared to pleasant (e.g., Facchinetti et al., 2006; Hagenaars et al., 2012) and/or neutral ones (e.g., Lopes et al., 2009; Niermann et al., 2015; Roelofs et al., 2010; but see Stins & Beek, 2007) along with bradycardia (Facchinetti et al., 2006; for a review see Hagenaars, Oitzl, et al., 2014). Reductions in postural sway thereby unfolded on either the medio-lateral axis (e.g., Facchinetti et al., 2006) or the anterior-posterior axis of the balance board (e.g., Niermann et al., 2015; Roelofs et al., 2010).

Such freezing-like behaviors in humans have been linked to a variety of clinical constructs (Hagenaars et al., 2012; Lopes et al., 2009; Niermann et al., 2015; Roelofs et al., 2010). For example, reduced body sway has been shown to be more pronounced in participants with a history of aversive life events (vs. without; Hagenaars et al., 2012), adolescents with insecure (vs. secure) attachment styles (Niermann et al., 2015), panic disorder patients (vs. healthy controls), although this was only generally visible as a function of anticipatory anxiety but not of picture type (Lopes et al., 2009), as well as in highly anxious adults (vs. low anxious; Roelofs et al, 2010). In other (sub)clinical samples, this relationship was reversed, demonstrating posttraumatic stress disorder patients (vs. healthy controls, Fragkaki et al., 2017) and adolescents with relatively low (vs. high) basal cortisol levels to exhibit reduced expressions of freezing behavior (Niermann et al., 2017).

TONIC IMMOBILITY. Due to ethical restrictions, Tonic Immobility as a response to extreme life-threatening situations has mostly been investigated in retrospective studies or procedures resorting to a traumatic event in the past. For example, Volchan and colleagues (2011) exposed participants with and without PTSD to a script of their autobiographical trauma while recording their postural sway. Those reporting intense immobility after listening to these scripts also displayed measurably lower amplitudes of body sway along with tachycardia and lower heart rate variability. These responses seemed stronger in those with PTSD than without. PTSD symptom severity also seems to be affected by freezing during the traumatic event. Rizvi and colleagues (2008) asked women who had been sexually or physically assaulted within the previous 2 months about whether they had experienced freeze responses during the event, defined as bodily immobility or paralysis. Their findings indicated that those who had frozen developed more severe symptomatology afterward than those who had not. Generally, however, signs of Tonic Immobility in humans remain relatively unexplored.

Together, the findings suggest resemblances between rodent and human behavioral responding to different levels of threat. Similar to what has been reported in rodents, a specific autonomic pattern consisting of a concomitant activation of the parasympathetic (i.e., indicated by bradycardia; Gladwin et al., 2016; Hashemi et al., 2021) and sympathetic autonomic nervous system (i.e., indicated by skin conductance increase; Löw et al., 2015; Rösler & Gamer, 2019) has been linked to a decrease of motor activity. Bradycardia under threat of shock has thereby been associated with several adaptive functions such as optimized perceptual processing (Lojowska et al., 2015, 2019), decision-making (de Voogd et al., 2022) and action preparation (Roelofs & Dayan, 2022). Similar to rodents, the concomitant decrease in motor and heart rate activity may thereby be indicative of both Attentive Immobility and Immobility under Attack. To differentiate these states, experimental designs are required that stimulate orienting responses, as these may elicit behaviors comparable with those in respective rodent work where alternations between orienting and freezing were suggested to be reflective of Attentive Immobility (Blanchard & Blanchard, 1989, 1990). Tonic Immobility may be accompanied by tachycardia in humans but, due to the retrospective nature of studies investigating this effect, this remains an outstanding question (Volchan et al., 2011, 2017).

In addition to the reported homologies in cardio-behavioral defensive responding in rodents and humans, there is evidence for similar neural underpinnings of these states. Both human and animal studies suggest that a shift from forebrain to midbrain activation marks the transition from Post-encounter to Circa-strike defenses. The periaqueductal grey is thereby thought to play a crucial role in mediating this switch (McTeague, 2016; Mobbs et al., 2007, 2009; Signoret-Genest et al., 2022). However, due to movement restrictions in brain imaging environments in human research, the neural substrates of defensive states are largely predicated on animal models. Human researchers have tried to circumvent this issue by approximating freezing with bradycardia (Wendt et al., 2017) or by conducting the same experiment twice in and outside the scanner (Hashemi et al., 2019), but both methods have limitations: First, though bradycardia has been reported to be positively related to freezing (Hashemi et al., 2021; Roelofs et al., 2010), it seems to occur in a wide range of contexts and may thus lack threat-specificity (e.g., Jennings et al., 2009; Ribeiro & Castelo-Branco, 2019), which would be pertinent to crystallize fear-related circuits. Second, conducting the same experiment twice is time-consuming and the conditions of each run are not equal, limiting their comparability.

1.4. Summary and Research Questions

Taken together, translational research on immobility-related defensive behaviors has shown similarities between rodents and humans but several limitations and questions about these states remain. First, there is no standardized way to analyze freezing behaviors in neither rodent, nor human research. Moreover, the most common measure to infer freezing in humans (i.e., reductions in postural sway) as well as its experimental investigation lack ecological validity and comparability with respective rodent work. Second, similar cardio-behavioral phenomena have been associated with Attentive Immobility and Immobility under Attack, rendering their conceptual distinction inconclusive. Third, as brain scanning environments are restricted and impede the evaluation of postural sway, much of the knowledge regarding the neural foundations of immobility-related defensive states in humans is derived from animal studies.

The current thesis thus aimed at addressing these short-comings by 1) investigating a potential movement metric (i.e., decrease of oculomotor activity) that may help in deducing fear-related motion reductions in brain imaging environments and 2) employing an ecologically valid design in virtual reality that may a) contribute to deciphering the states of Attentive Immobility and Immobility under Attack and b) provide a more suitable, holistic measure of defensive behaviors and their concomitant autonomic dynamics in humans.

To address the first issue, we built on prior work by Rösler and Gamer (2019) who observed reductions in gaze dispersion that were accompanied by a concomitant activation of parasympathetic (resulting in heart rate decrease) and sympathetic (instigating skin conductance increase) activity when participants awaited a potential shock (as compared to an inevitable and no shock) that was avoidable by a quick button press. Stronger reductions in gaze dispersion and heart rate thereby predicted faster motor reactions on a trial-wise basis. Reminiscent of an immobility-related defense state upon Post-encounter threat (i.e., Attentive Immobility), the inhibition of oculomotor activity has been discussed as a marker for freezing-like motion in humans. As eye-tracking technology is implementable in a variety of field and laboratory contexts (e.g., brain imaging environments), this oculomotor effect seemed particularly potent to resolve movement constraints in brain scanning studies. However, several questions about the suitability of this potential marker of freezing-like behaviors in humans remain unanswered. We therefore embarked on four studies modifying and extending the study by Rösler and Gamer (2019). The experimental designs range from movement-restricted laboratory (Study 1-3) to highly ecologically valid designs (i.e., virtual reality; Study 4).

Study 1 includes two experiments investigating the robustness (Experiment 1) and threat-specificity (Experiment 2) of reduced gaze dispersion and concomitant autonomic dynamics (bradycardia and skin conductance increase) as well as their action-preparatory function in anticipation of an avoidable shock. As the original experimental design by Rösler and Gamer (2019) required a narrow attentional focus to prepare for shock avoidance, Experiment 1 tests whether the observed centralization of gaze persists when threat-avoidance requires spatially distributed attention. Experiment 2 sets out to elucidate whether the observed oculomotor and autonomic dynamics are threat-specific.

Based on these findings, Study 2 and 3 further explore the robustness of reduced gaze dispersion (Study 2) and its relationship with reduced body sway (Study 3). Specifically, Study 2 tests whether and how reduced oculomotor activity as a presumed action-preparatory mechanism interacts with other notorious gaze patterns such as attentional preferences for social (Experiment 1) and threatening (Experiment 2) stimuli. As both reductions in gaze and body movements have been discussed as translational indicators for freezing, Study 3 explores their relationship, elaborating on potential limitations of previous assessments of immobility-related defensive behaviors in humans.

Finally, Study 4 aims at addressing shortcomings of previous human studies that mainly employed movement-restricted experimental designs with low ecological validity and assessed complex defensive behaviors with single measures. We therefore measured multiple behavioral metrics including both body and gaze movements (i.e., movement speed, body, head, and eye rotations) as well as autonomic dynamics (heart rate and skin conductance) and individual differences (subjectively perceived dread, trait anxiety, and trait aggression) in an immersive virtual reality environment. By simulating increasing levels of threat and prompting orienting responses, we seek to elucidate the validity of the TICM and proposedly different immobility-related defensive states (i.e., Attentive Immobility and Immobility under Attack).

2. Study 1:

Centralized gaze as an adaptive component of defensive states in humans

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Abstract

Adequate defensive responding is crucial for mental health but scientifically not well understood. Specifically, it seems difficult to dissociate defense and approach states based on autonomic response patterns. We thus explored the robustness and threat-specificity of recently described oculomotor dynamics upon threat in anticipation of either threatening or rewarding stimuli in humans. While visually exploring naturalistic images, participants (50 per experiment) expected an inevitable, no, or avoidable shock (Experiment 1) or a guaranteed, no or achievable reward (Experiment 2) that could be averted or gained by a quick behavioral response. We observed reduced heart rate (bradycardia), increased skin conductance, pupil dilation, and globally centralized gaze when shocks were inevitable but, more pronouncedly, when they were avoidable. Reward trials were not associated with globally narrowed visual exploration, but autonomic responses resembled characteristics of the threat condition. While bradycardia and concomitant sympathetic activation reflect not only threat-related but also action-preparatory states independent of valence, global centralization of gaze seems a robust phenomenon during the anticipation of avoidable threat. Thus, instead of relying on single readouts, translational research in animals and humans should consider the multi-dimensionality of states in aversive and rewarding contexts, especially when investigating ambivalent, conflicting situations.

Keywords: Defensive States, Threat, Reward, Fear, Gaze, Freezing

Introduction

Various forms of defensive behaviors have evolved to protect an organism from potential harm in threatening situations (Anderson & Adolphs, 2014; LeDoux, 2012). Depending on the context (e.g., availability of escape routes; temporal and spatial distance of a threat), they occur in a cascade-like fashion ranging from hard-wired automatic, initial reactions to deliberative goal-directed behaviors (Blanchard et al., 2011; Blanchard & Blanchard, 1989; LeDoux & Daw, 2018; Mobbs et al., 2015; Roelofs, 2017).

An evolutionarily conserved response in the face of real or perceived threat, that was extensively investigated in rodents, is a defensive behavioral pattern eventually termed freezing (Fanselow, 1980). It is characterized by movement cessation, accompanied by a transient decrease in heart rate, i.e. bradycardia (Fanselow, 1984; Tovote et al., 2005; Vianna & Carrive, 2005b; Walker & Carrive, 2003). This defensive mode of simultaneous behavioral and cardiovascular inhibition has been suggested to help avoiding predator detection (Whishaw et al., 1991) optimizing perceptual and attentional processing (Kapp et al., 1992; Lang & Davis, 2000), and to prepare fast responses to approaching threat (Butler et al., 2007; Griebel et al., 1996).

Upon distal yet inevitable threat, humans seem to engage in similar defensive responding denoted by reduced body sway (i.e., freezing), as measured by stabilometric platforms, and a co-activation of sympathetic (e.g., heightened skin conductance) and parasympathetic (e.g., bradycardia) branches of the autonomic nervous system (Gladwin et al., 2016; Roelofs, 2017; Rösler & Gamer, 2019). This integrated defense state has been referred to as attentive immobility/freezing, supposedly preparing the individual for further defensive actions, if eventually the threat becomes imminent or escape options appear (Hamm, 2020; Szeska et al., 2021; Volchan et al., 2017). Confusingly, attentive immobility in humans has been discussed as both a vulnerability factor for psychopathologies (e.g., Hagenaars et al., 2012; Lopes et al., 2009; Roelofs et al., 2010) and an adaptive action preparatory mechanism (Gladwin et al., 2016; Hashemi et al., 2021; Rösler & Gamer, 2019). To reconcile these divergent results, it has been suggested that freezing tends to be associated with clinical constructs when participants cannot escape the threat, while it adaptively facilitates action preparation when subsequent harm can be actively avoided (Rösler & Gamer, 2019). Experimental context thus seems to constitute a main determinant of the behavioral defense state (Bastos et al., 2016; Blanchard & Blanchard, 2008). While attentive immobility describes a state of heightened vigilance and action preparation when escape might still be an option, a lack of escape routes upon imminent threat elicits a more desperate defense state that has also been termed ‘immobility under attack’ (Volchan et al., 2017).

However, this explanation does not hold for all studies in this domain. For example, reduced body sway was found to be related to both, vulnerability markers of psychopathologies and faster threat-reactions when participants were able to actively avoid aversive stimulation. Heart rate, in contrast, was only associated with faster motor responses in this study (Hashemi et al., 2021). Diverging from the widespread idea that due to its robust co-occurrence (Roelofs, 2017), bradycardia might be adduced as a proxy for freezing (e.g., Löw et al., 2015; Wendt et al., 2017), cardiac and motor inhibition seem to reflect different aspects of a defense state. Previous studies using active contexts suggested that transient bradycardia indeed also occurs independently of threat, constituting a more general action-preparatory mechanism (Jennings et al., 2009; Löw et al., 2008, 2015; Obrist et al., 1970). This goes along with the notion that appetitive and defensive responding bear fundamental similarities in that they require the organism to anticipate and prepare for subsequent actions (Lang et al., 2013; Löw et al., 2008). Responses that specifically index and discriminate between threat-induced, defensive and reward-related, appetitive states in humans are thus necessary to understand fear-associated neural circuitries and behaviors. Oculomotor dynamics may function as such a marker: Rösler and Gamer (2019) were the first to report reduced visual scanning along with bradycardia and increases in skin conductance during the expectation of an avoidable aversive electrotactile stimulation. Specifically, participants showed less and longer fixations that were closer to the center of the screen when anticipating an evitable shock they could avoid by pressing the space bar as compared to an inevitable or no shock. Corroborating the idea of an action preparatory mechanism, such narrowed overt attention as well as enhanced bradycardia predicted the speed of threat-escapes on a trial-wise basis. These results align with previous findings indicating a narrowed focus of attention (i.e., lower accuracy when responding to peripheral vs. central stimuli in a maze) while participants had to actively flee from a predator chasing them as compared to when they were not actively haunted (Vaughn & Brasier, 2019). However, as previous research suggests that eye movements follow task-specific predictions of expected events (Anderson et al., 1997), it remains unclear whether these findings relied on the specific experimental situation that required a narrow attentional focus to prepare for the upcoming flight response.

We thus conducted two experiments to investigate whether oculomotor changes upon threat can also be observed in situations requiring a wider focus of attention and whether they are threat-specific. Using adapted versions of said paradigm by Rösler and Gamer (2019), we assessed gaze dynamics and autonomic responses during the expectation of an avoidable, no or inevitable threat (Experiment 1) or an achievable, no or guaranteed reward (Experiment 2). In Experiment 1, we modified the task to avert the threat such that it required more distributed spatial attention. Instead of a simple button press, subjects could now avoid the aversive electrotactile stimulation by quickly reacting upon peripherally presented response prompts. In Experiment 2, we transferred the paradigm into a new context to test whether the concomitant inhibition of oculomotor behavior and cardiac output, resembling the previously described freezing-associated bradycardic defense state, represents a threat-specific phenomenon: Instead of shocks, participants could earn a guaranteed, no or an achievable financial reward that could be won by quickly responding to a peripherally presented stimulus.

Methods

Participants

The current study was based on a similar design as Rösler and Gamer (2019). In this study, 50 participants were examined which allowed for detecting medium effect sizes in repeated measures analyses of variance (ANOVAs, $f = 0.25$) at an alpha level of .05 with a statistical power greater than .95 when assuming a correlation of $r = .50$ between factor levels (Faul et al., 2007). We therefore decided to acquire a similar number of valid datasets for both experiments in the current study.

Subjects (Experiment 1: $N = 58$; Experiment 2: $N = 60$) signed up for the study via an online platform and thus formed a sample of convenience. All participants had normal or corrected-to-normal vision (contact lenses). In Experiment 1, seven participants were excluded due to problematic eye tracking data (i.e., more than 30% of eye-tracking trials with baseline outliers or missing baseline position data, or a range of baseline coordinates exceeding 5° of visual angle after trial exclusion) and another one because of frequent extrasystoles in the heart rate data resulting in a total of 50 participants (40 women, age: $M = 28.00$ years, $SD = 9.78$ years). In Experiment 2, six participants were excluded due to problematic eye tracking data and four due to technical errors during data recording, also forming a final sample of 50 participants (40 women, age: $M = 28.13$ years, $SD = 10.79$ years). All participants provided written informed consent and were reimbursed with 10€ (Experiment 1) or 4€ plus a variable bonus according to their performance in the study (additional 4€ max; Experiment 2).

The experiments were conducted according to the declaration of Helsinki and have been approved by the ethics committee of the University of Würzburg. All datasets generated and analyzed in the current study are available on the Open Science Framework at https://osf.io/whpt5/?view_only=34f40d237f234c24b91f3a06e2c81a24.

Experimental Design

Based on Rösler and Gamer (2019), participants were presented with a screen depicting naturalistic pictures against a grey background during which eye movements, pupil width, heart rate (HR) and skin conductance (SC) were measured. Experiment 1 included a threat context where participants could receive an individually calibrated, aversive electrotactile stimulation using a Digitimer Constant Current Stimulator DS7A (Hertfordshire, United Kingdom; see electronic supplementary material for further details) while Experiment 2 involved a reward context where participants could earn money (fixed amount of 0.10 € per trial).

For visual stimulation, we used 60 affectively neutral images depicting naturalistic scenes (768 × 576 pixels, visual angle of 24.00° × 18.11° at a viewing distance of 50 cm) from the McGill Calibrated Colour Image Database (Olmos & Kingdom, 2004) presented in a random order. Although the pictures did not include particularly salient features, half of them (randomly determined within each participant) were horizontally flipped to prevent biases in eye movements provoked by an incidental imbalance in visually stimulating features on one side. The experiment was programmed with Presentation® (Neurobehavioral Systems Inc., Version 18.1) and run on a 24’’ Asus VG248QE display (53.126 × 29.889 cm, 1920 × 1080 pixels, refresh rate 60 Hz). Naturalistic images (8 s) were preceded by a white fixation cross (6.5-8.5 s) turning red, green or yellow (2 seconds). Depending on the color, participants were instructed whether to expect an inevitable shock or no reward (red), no shock or guaranteed reward (green) or an avoidable shock or achievable reward (yellow) after disappearance of the naturalistic picture. Afterwards, the screen turned blank (1 s) in no shock and shock or no reward or reward trials while the shock and reward trials were accompanied by a shock or a reward delivery. Differing from Rösler and Gamer (2019) who used a verbal prompt to press the space bar as fast as possible, an open and a closed door (Experiment 1) or treasure chest (Experiment 2) appeared on either side of the screen in avoidable shock/achievable reward trials (225 × 388 pixels, distance between the center of the screen and the center of the object in visual angle: 19.88°). The position of the open door or open treasure chest was counterbalanced within each participant. Participants were told they could avoid the electrotactile stimulation or gain the reward by a quick joystick movement into the direction of the open door or treasure chest (for an illustration of the experimental design see Fig. 1). In order to ensure that participants would receive a shock or win a reward in approximately 50% of the trials, they had to be faster than 600 ms in the first five trials. Afterwards, the threshold was individually adjusted to the median of the first five reaction times. Both experiments comprised 60 trials with 20 in each condition (see electronic supplementary material for more information on experimental setup and procedure).

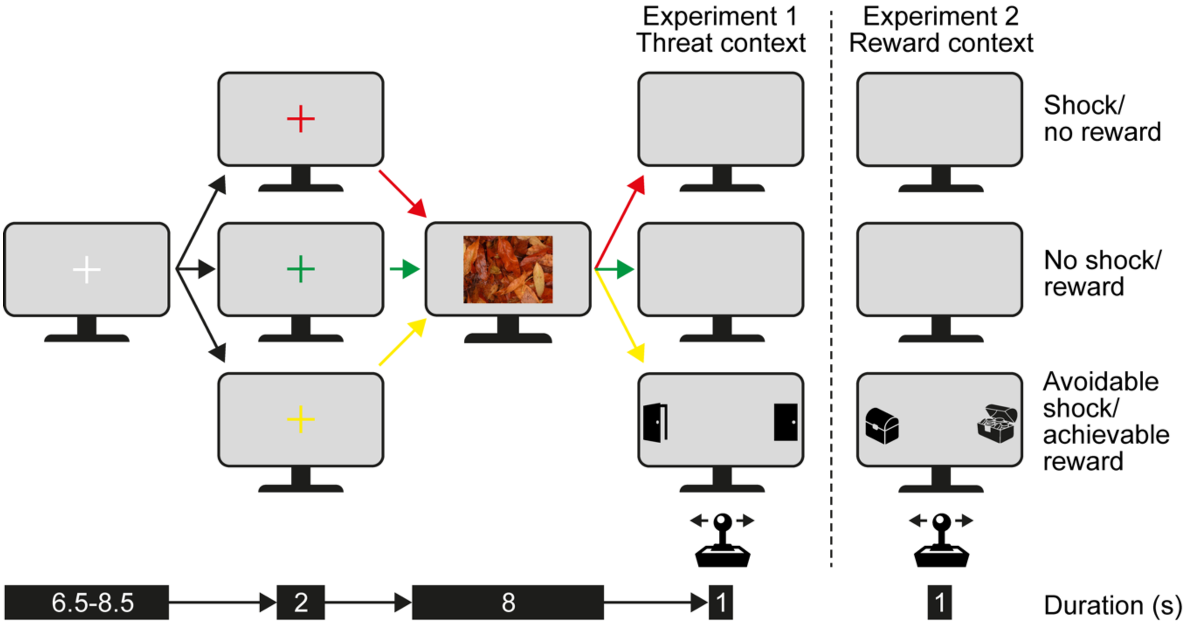

Figure 1. Study design of Experiment 1 (Threat context) and Experiment 2 (Reward context) adapted from Rösler and Gamer (2019). Participants were told that a colored fixation cross would signal whether to expect an inevitable, no, or an avoidable shock (Experiment 1) or no, a guaranteed or achievable reward (Experiment 2) after an anticipation phase during which naturalistic images were presented. Avoidable shocks and achievable rewards could be averted or gained by a quick joystick movement towards an indicated side (an open door or an open treasure chest, respectively). Note that the size of color cues and response prompts are not drawn to scale.

Data recording

Eye-Tracking. Movements of the right eye were measured using an EyeLink 1000Plus system (SR Research Ltd., Ottawa, Canada) in the tower mount configuration with a sampling rate of 1000 Hz. Gaze position data were parsed into fixations and saccades using EyeLink's default configuration. Saccades were defined as fast eye movements with a velocity exceeding 30°/s or an acceleration exceeding 8,000°/s^2. The last 300 ms before stimulus onset were defined as baseline and used for an offline drift correction. To ensure that participants fixated the center of the screen during the baseline, we used our lab's established iterative outlier detection algorithm (End & Gamer, 2017; Rösler & Gamer, 2019). Therefore, we temporarily removed the highest and lowest values of baseline data, separately for x and y coordinates, from the distribution and inspected whether they deviated more than three standard deviations from the mean of the remaining data. If so, one or both of these values were labeled as outliers and permanently removed, otherwise they were returned to the data set. This procedure was iteratively applied to the remaining distribution until no further x- and y-values met the removal criterion. Individual gaze drift was finally corrected by subtracting the x- and y- coordinates of the baseline from the fixation coordinates during stimulus exploration. Trials with baseline outliers or missing baseline position data (Experiment 1: 8.20%, Experiment 2: 7.90%) as well as trials with premature behavioral responses during the anticipation phase (Experiment 1: 0.13%, Experiment 2: 0.07%) were excluded from all further analyses. We then computed three oculomotor metrics on a second-by-second basis: the average distance of fixations from the center of the screen in pixels (center bias), the duration of individual fixations, and the number of fixations. For the analyses, these three metrics were averaged into 8 one-second bins covering the entire period of picture viewing.

Pupil width. From the eye-tracking data, we also extracted the recorded pupil diameter. In a first step, we linearly interpolated blink periods and downsampled the data to 100 Hz. Subsequently, we applied a 2 Hz low-pass filter and converted the values from arbitrary units to mm according to (Hayes & Petrov, 2016). We then calculated changes in pupil diameter relative to a 1 s baseline period preceding cue onset. These values were then averaged into 20 bins of 0.5 s each spanning the whole cue and anticipation period. Note that we used a smaller bin duration for pupil size than for the other oculomotor and physiological measures since the pupil responds more quickly to external and internal events.

Electrodermal activity. Skin conductance was recorded continuously using a BIOPAC MP160 system (BIOPAC Systems, Inc.) at a sampling rate of 500 Hz from two Ag/AgC1 electrodes filled with 0.05 ml NaCL electrolyte placed on the thenar and hypothenar eminences of the non-dominant hand. For the analyses, SC data were downsampled to 20 Hz and values were averaged into 10 one-second bins for each condition (5 additional one-second bins for the post-stimulus phase were added for data visualization, see Fig. 4). The 10 s interval started two seconds prior to image onset to include the cue phase. The last second prior cue onset served as baseline and was subtracted from all subsequent data points of each trial.

Heart rate. An electrocardiogram (ECG) was recorded using the same BIOPAC system with disposable Ag/AgC1 electrodes placed on the right clavicle and the lower left ribcage with the reference electrode placed on the right lower ribcage. Sampling rate was 500 Hz. ECG data were filtered using a 2 Hz high-pass filter to remove slow signal drifts. Afterwards, R-peaks were detected semi-automatically using in-house software and manually edited in case of detection errors. R-R-intervals were converted to HR in beats per minute and a real-time scaling procedure (Velden & Wölk, 1987) was implemented to calculate mean heart rate for 10 one-second time bins (plus 5 bins for data visualization) spanning the same time window as for SC. The HR in the last second prior to cue onset served as baseline and was subtracted from all following time bins.

Statistical analyses

Data preprocessing and analyses were performed using R (Version 3.3.3, Core Team, 2018) on a significance level of 5%. For all dependent variables, we calculated repeated-measures analyses of variance (rmANOVA) with trial type (Experiment 1: inevitable shock, no shock, avoidable shock; Experiment 2: guaranteed reward, no reward, achievable reward) and second (10 or 20 bins including the cue period for skin conductance, heart rate and pupil width and 8 bins restricted to the picture presentation phase for all three metrics of visual exploration) as within-subject factors for each study. Degrees of freedom were adjusted according to Greenhouse-Geisser to compensate for potential violations of the sphericity assumption. To specifically compare the respective active condition (Experiment 1: avoidable shock; Experiment 2: achievable reward) with the remaining two conditions, post-hoc t-tests were performed using false discovery rate correction (FDR, Benjamini & Hochberg, 1995) to adjust for alpha-error accumulation (comparisons between the two passive conditions in each experiment are reported in Figures S3-S5).

We additionally computed a generalized linear mixed model (GLMM) for each study using both heart rate and centralization of gaze during the second half of the picture viewing period separately as predictors for reaction times in the avoidable shock and achievable reward trials, respectively. Subject ID was added as a random intercept into the GLMM. Further details on the calculations, the internal consistency (Table S9) and correlations between autonomic and oculomotor measures (Fig. S2) are included in the supplementary material.

Results

Oculomotor behavior during the anticipation of threat and reward

Using a 3 × 8 rmANOVA, we first compared the average distances of fixations from the center of the screen (global center bias) during image presentation between inevitable, no and avoidable shock trials. Visual exploration decreased markedly towards the end of the anticipation period when participants awaited an avertable shock as compared to both other conditions (Fig. 2a; interaction trial type × second, $F_{(14,686)} = 13.64$, $\varepsilon = .31$, $p < .001$, $\eta^2_g = .03$; main effects for this and the following analyses are reported in Table S1 and Table S2). No robust differences were observed between the inevitable and the no shock condition (see Fig. S3). In avoidable shock trials, global center bias predicted faster response times on a trial-wise basis ($\beta = 0.21$, $SE = 0.05$, $t_{(772.73)} = 3.66$, $p < .001$).

Contrastingly, we found differential effects in the reward-context: There were no statistically significant differences in the temporal progression of the global center bias between reward, no reward, and achievable reward trials (Fig. 2d; interaction trial type × second, $F_{(14,686)} = 0.89$, $\varepsilon = .19$, $p = .438$, $\eta^2_g < .01$) and global center bias did not predict faster response times in achievable reward trials on a trial-wise basis ($\beta = -0.01$, $SE = 0.04$, $t_{(720.66)} = -.27$, $p = .788$).

To get a more nuanced picture of the visual scanning patterns in threat and reward contexts, we generated fixation density maps. These maps show a pronounced centralization of fixations in avoidable shock trials but a more strategic gaze pattern of horizontal exploration in the reward context (Fig. 3). To statistically confirm this impression, we calculated distinct center biases for the horizontal and vertical coordinates of fixations, respectively. Separate 3 × 8 rmANOVAs showed a center bias to be evident towards the end of the anticipation phase when participants expected a potential aversive stimulation on both the horizontal axis (Fig. 2b, interaction trial type × second: $F_{(14,686)} = 6.71$, $p < .001$, $\eta^2_g = .02$) as well as the vertical axis (Fig. 2c, interaction trial type × second: $F_{(14,686)} = 19.57$, $p < .001$, $\eta^2_g = .04$). Comparable analyses for the reward context revealed an absent horizontal center bias (Fig. 2e, interaction trial type × second, $F_{(2,98)} = 2.23$, $p = .097$, $\eta^2_g = .01$), but a strong vertical center bias in achievable reward trials (Fig. 2f, interaction trial type × second, $F_{(2,98)} = 6.35$, $p < .001$, $\eta^2_g = .01$).

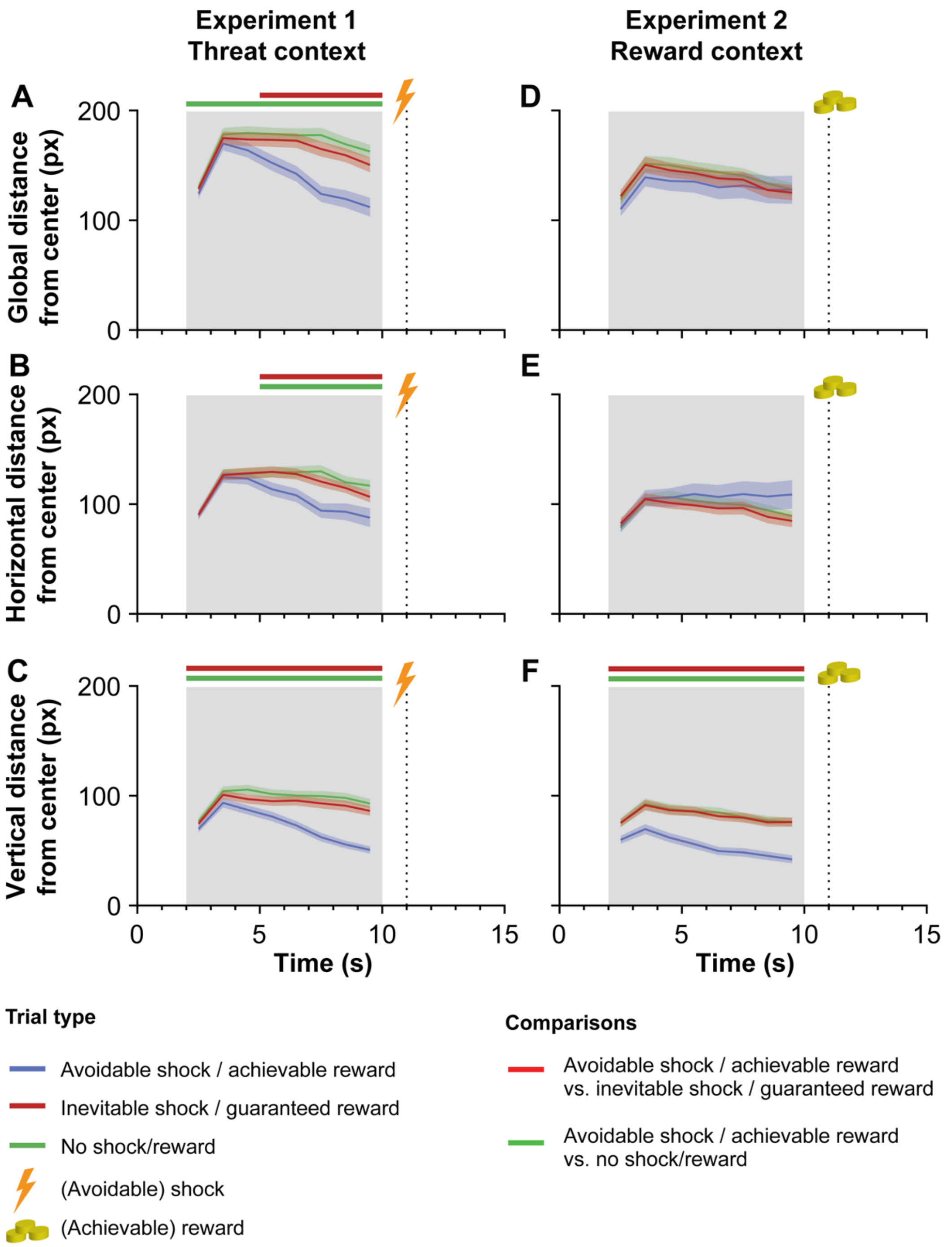

Figure 2. Changes in center bias during the anticipation of an inevitable, no or avoidable shock in Experiment 1 (global center bias: A, horizontal center bias: B, vertical center bias: C) or a guaranteed, no or achievable reward in Experiment 2 (global center bias: D, horizontal center bias: E, vertical center bias: F). Shaded ribbons denote standard errors of the mean. Horizontal lines at the top of each figure indicate significant differences between avoidable shock (A, B, and C) or achievable reward (D, E and F) trials and the other two trial types (after false discovery rate correction). Shading in grey denotes the phase between onset and offset of picture presentation with the offset prompting quick responses in the avoidable shock and achievable reward trials, respectively.

A direct comparison of the response patterns between both experiments using a 2 × 3 × 8 ANOVA now including the between-subjects factor experiment confirmed significant differences in the observed interaction effect between experiments for general reductions in visual scanning (Table S3; three-way interaction, $F_{(14,1372)} = 8.31$, $\varepsilon = .25$, $p < .001$, $\eta^2_g = .01$). This was also evident for both horizontal center bias (Table S4; three-way interaction, $F_{(14,1372)} = 6.80$, $\varepsilon = .25$, $p < .001$, $\eta^2_g < .01$) and vertical center bias (Table S5, three-way interaction, $F_{(14,1372)} = 10.82$, $\varepsilon = .24$, $p < .001$, $\eta^2_g = .01$). Moreover, the predictive value of globally reduced image exploration for reaction times differed significantly between shock and reward contexts in the GLMM (interaction of experiment and amount of center bias, $\beta = -0.22$, $SE = 0.07$, $t_{(1513.10)} = -3.25$, $p = .001$). We also compared fixation numbers and durations between trial types within each experiment but failed to find substantial effects in both Experiments (see Table S1 and Fig. S1).

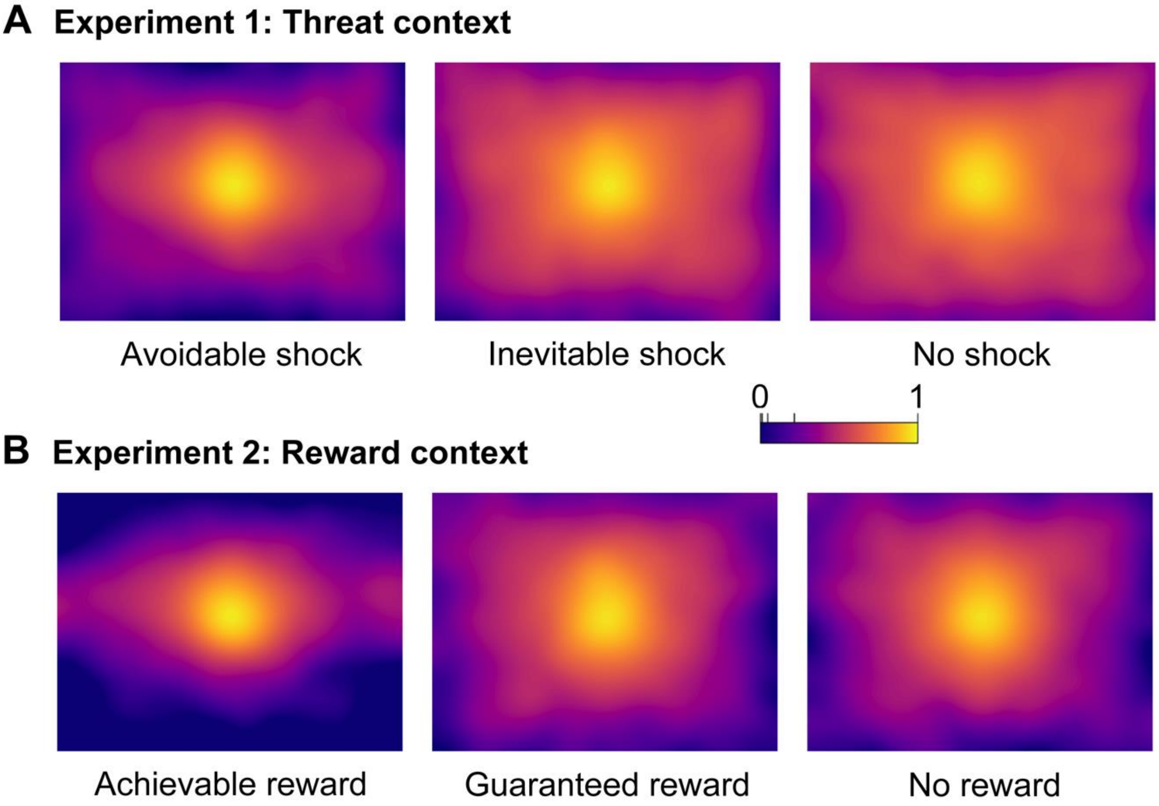

Figure 3. Normalized fixation density maps reflecting the distribution of fixations on the naturalistic images during the anticipation phase in Experiment 1 (a) and Experiment 2 (b). Fixation densities are depicted on a logarithmic scale.

Autonomic responses during the anticipation of threat and reward

To compare heart rate changes between conditions, we performed a 3 × 10 rmANOVA (trial type by seconds, now also including the 2s cue period) for each experiment. In the aversive context (Experiment 1), average heart rate increased right after cue onset, decreased over the anticipation period and increased again after picture offset across all conditions. This dynamic became gradually more pronounced from no to inevitable to avoidable shock trials but statistically significant differences only emerged between the avoidable shock and the other two conditions (Fig. 4a and Fig. S4, interaction trial type × second, $F_{(18,882)} = 13.07$, $\varepsilon = .27$, $p < .001$, $\eta^2_g = .04$). In the reward context, achievable reward trials but not guaranteed or no reward trials showed similar heart rate trends with a marked increase after cue onset followed by a decrease and another increase after picture offset (Fig. 4d; interaction trial type × second, $F_{(18,882)} = 23.38$, $\varepsilon = .28$, $p < .001$, $\eta^2_g = .09$).

Whereas heart rate deceleration during the second half of picture viewing did not significantly predict faster response times in avoidable shock trials on a trial-wise basis in a GLMM ($\beta = 0.19$, $SE = 0.58$, $t_{(819.59)} = 0.32$, $p = .745$), it did so in achievable reward trials ($\beta = 1.60$, $SE = 0.57$, $t_{(803.59)} = 2.81$, $p = .005$). However, this apparent difference between studies was not statistically significant when contrasting both experiments within one GLMM (interaction of experiment and mean heart rate, $\beta = 1.41$, $SE = 0.82$, $t_{(1625.76)} = 1.73$, $p = .085$; main effect of mean heart rate, $\beta = 0.19$, $SE = 0.56$, $t_{(1611.69)} = 0.34$, $p = .735$). A direct comparison of heart rate changes during the anticipation phase using a 2 × 3 × 10 ANOVA with the additional between-subjects factor experiment revealed a significant three-way interaction, underlining that despite some similarities, heart rate changes also differed between threat and reward contexts (Table S6, $F_{(18,1764)} = 3.45$, $\varepsilon = .305$, $p = .003$, $\eta^2_g < .01$)

Skin conductance levels increased during the anticipation of both an inevitable and an avoidable shock in comparison to no shock trials (Fig. 4b; interaction trial type × second, $F_{(18,882)} = 12.50$, $\varepsilon = .16$, $p < .001$, $\eta^2_g = .04$), whereas in a reward context SC levels only increased in achievable reward trials while they remained stable in guaranteed and no reward trials (Fig. 4e; interaction trial type × second, $F_{(18,882)} = 38.68$, $\varepsilon = .09$, $p < .001$, $\eta^2_g = .27$). A direct comparison between experiments using a 2 × 3 × 10 ANOVA including the between-subjects factor experiment confirmed a different temporal progression of skin conductance changes as a function of trial type in both experiment (Table S7; three-way interaction, $F_{(18,1764)} = 17.59$, $\varepsilon = .14$, $p < .001$, $\eta^2_g = .03$).

Changes in pupil size revealed a similar pattern: In the shock context, pupil width increased more strongly over time in both inevitable shock and avoidable shock trials as compared to no shock trials, with the strongest increase in avoidable shock trials (Fig. 4c; interaction trial type × second, $F_{(38,1862)} = 24.47$, $\varepsilon = .15$, $p < .001$, $\eta^2_g = .03$). In the reward context, only achievable reward trials contrasted with the other two trial types were associated with a strong increase in pupil width over the course of the whole trial (Fig. 4f; interaction trial type × second, $F_{(38,1862)} = 35.63$, $\varepsilon = .08$, $p < .001$, $\eta^2_g = .06$). Comparing these interaction effects between experiments confirmed a significant difference between shock and reward contexts regarding differential changes in pupil width between trial types over time (Table S8; three-way interaction, $F_{(38,3724)} = 2.39$, $\varepsilon = .13$, $p = .039$, $\eta^2_g < .01$).

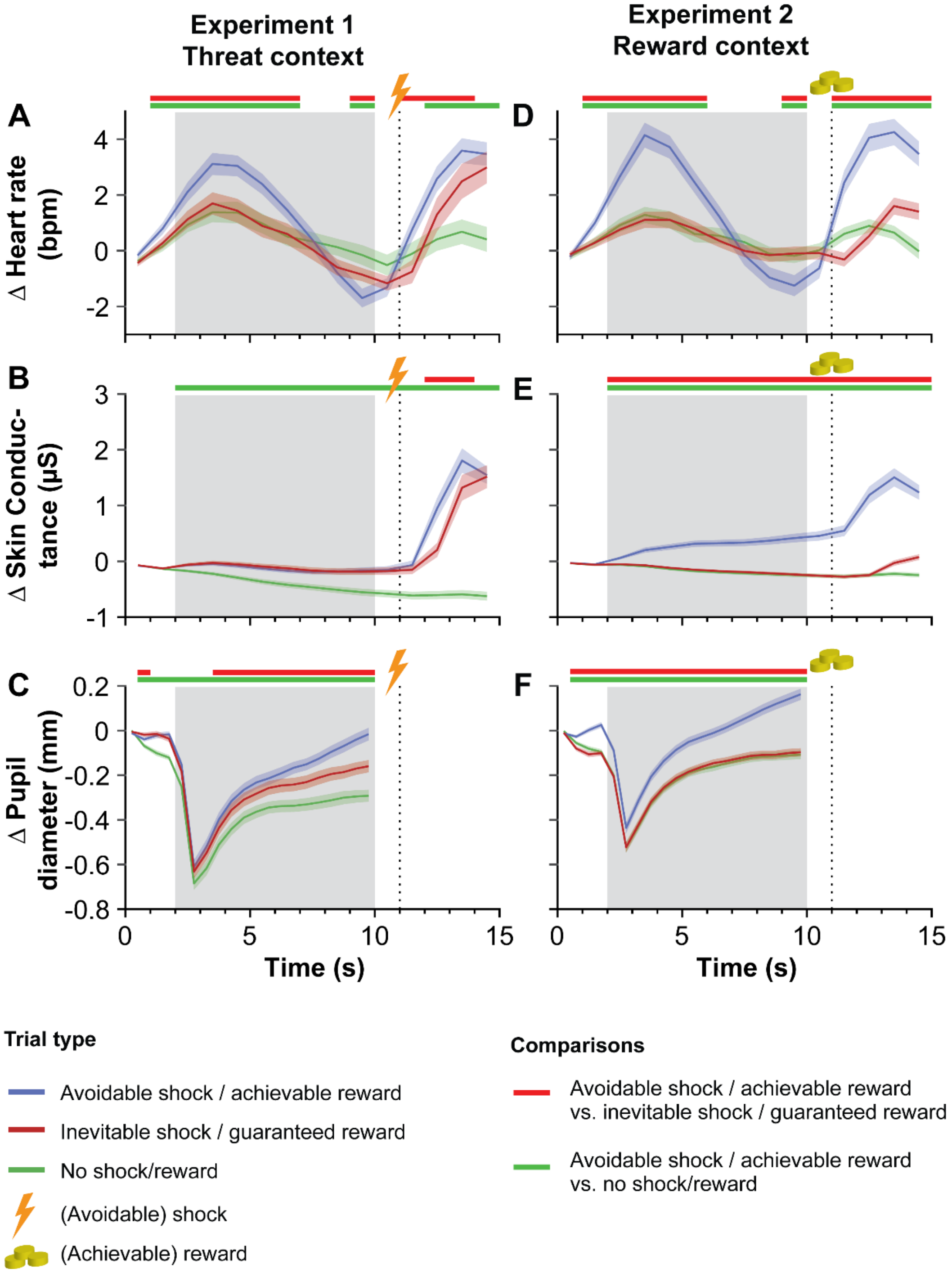

Figure 4. Autonomic responses during the anticipation of an inevitable, no or avoidable shock in Experiment 1 (heart rate: A, skin conductance: B, pupil width: C) or a guaranteed, no or achievable reward in Experiment 2 (heart rate: D, skin conductance: E, pupil width: F). Shaded ribbons denote standard errors of the mean. Horizontal lines at the top of each figure indicate significant differences between avoidable shock (A, B, and C) or achievable reward (D, E and F) trials and the other two trial types (after false discovery rate correction). Shading in grey denotes the phase between onset and offset of picture presentation with the offset prompting quick responses in the avoidable shock and achievable reward trials, respectively.

Discussion

The current two experiments set out to elucidate behavioral and autonomic components of defensive states by means of oculomotor, cardiovascular and electrodermal dynamics. Specifically, we pursued two major aims: first, to test whether reduced visual scanning is a stable phenomenon during the anticipation of an approaching avoidable threat and second, to examine whether changes in gaze behavior are suitable to discriminate between defensive and appetitive responding and whether they are adaptive for preparing subsequent threat-responses.

Supporting our first hypothesis, we replicated previously found reductions in visual scanning – denoted by decreasing average fixation distances from the center of the screen (i.e., increased center bias) – when participants expected an avoidable aversive stimulation (vs. inevitable or no stimulation) even though threat-escape required more distributed spatial attention (Rösler & Gamer, 2019). This pattern of reduced visual exploration upon avoidable threat was evident in horizontal and vertical components of fixations even though a broad horizontal scanning of the display – as observed in the reward context of Experiment 2 – might have been advantageous to quickly detect peripherally presented action cues. With respect to our second hypothesis, we failed to find comparable changes in gaze behavior in a rewarding context when looking at global distances of fixations from the center of the screen. Notably though, we revealed a more nuanced picture by dissecting the distribution of fixations into horizontal and vertical components. Whereas a reduction in visual exploration was evident on the vertical axis, we observed no such effect for horizontal eye movements when participants prepared a quick movement towards an indicated side (left or right) to win a reward.

While increased skin conductance levels and enhanced pupil dilation were observed during the anticipation of inevitable and avoidable shocks as well as when participants expected an achievable reward (vs. guaranteed or no reward), heart rate changes mainly differed between the active condition that required a response and the other two conditions across both experiments. Although the observed heart rate pattern consisting of an initial increase followed by a decrease and another increase starting shortly before trial outcome in Experiment 1 is consistent with the so-called cardiac defense (Vila et al., 2007) and lines up with previous findings on specific fear states in both rodents (Swiercz et al., 2018) and humans (Gladwin et al., 2016; Hashemi et al., 2019, 2021; Szeska et al., 2021; but see Löw et al., 2015), it was also evident on achievable reward trials in Experiment 2. Thus, contrasting the idea that transient bradycardia might be more sensitive to threat than reward processing (Löw et al., 2008), heart rate deceleration during anticipation of a motor response seems to be an important element of a more general action-preparatory mechanism independent of contextual valence which may support processes of attentional orienting and motor preparation (Jennings et al., 2009; Obrist et al., 1970). The overall pattern of skin conductance changes and pupil dilation in conjunction with heart rate deceleration indicates a co-activation of sympathetic and parasympathetic branches of the autonomic nervous system in threat and reward contexts which suggests some resemblance of physiological responses in prey preparing to avoid harm and predators preparing for approach (Löw et al., 2008). However, the current findings also indicate a shift towards sympathetic activity in the threat context. In this regard, it seems important to note that we did not explicitly match the negative valence of aversive shocks with the positive valence of financial rewards. Ensuring such comparability is extremely difficult (Andreatta & Pauli, 2015) but should be a matter of future research. Nevertheless, we believe that the motivational value of negative outcomes in Experiment 1 and positive outcomes in Experiment 2 was somehow comparable given that autonomic as well as behavioral responses were very similar between avoidable shock and achievable reward trials in the current study.

Importantly, the unique occurrence of globally reduced visual scanning (i.e., on both the horizontal and the vertical axis) in a threatening context, that did not merely reflect task-specific demands (Anderson et al., 1997) and predicted the speed of avoidance response, highlights the adaptive and defensive nature of this oculomotor component. Whether this centralization of gaze is indeed a threat-specific component of the defensive state itself or an attentional shift as part of an anticipatory state has yet to be conclusively addressed. As oculomotor responding has previously been shown to differ between gaining rewards and risking losses (Muhammed et al., 2020), a direct comparison comparison between (monetary) losses and shocks in future studies could be one way of further exploring the threat-specificity and defensive nature of this effect. Importantly, similar to reductions in body sway, the currently observed decrease of oculomotor activity can be characterized as inhibition of motion, which, due to similar temporal dynamics, allows an initial classification of these responses to reflect freezing-like behavioral states that promote fast subsequent defensive actions (Gladwin et al., 2016; Löw et al., 2015).

In the current study, fixation durations and numbers did just marginally differ between trial types and thereby failed to replicate previous findings (Rösler & Gamer, 2019). Whether this was due to changes in the experimental design, low reliability of corresponding measurements or genuinely absent effects on these fixation metrics remains to be an important question for future research. In general, centralization of gaze may best reflect oculomotor changes during the expectation of avoidable threat and future studies should explore whether these reductions in visual exploration correlate with reduced bodily movement (Roelofs, 2017). Moreover, it remains unclear whether and how reduced visual scanning interacts with gaze preferences evoked by more heterogeneous or dynamic visual material such as social scenes, video clips or three-dimensional virtual environments (e.g., End & Gamer, 2017; Rubo & Gamer, 2018). Finally, although we are not aware of gender differences in autonomic or oculomotor dynamics during the anticipation of avoidable threat in healthy individuals, the gender imbalance in our sample might limit the generalizability of our findings to men.

In conclusion, the current study offers some important insights and implications for future research on defensive states in humans. First, we showed that a global centralization of gaze is a stable and specific phenomenon during the anticipation of avertable threat. In contrast, bradycardia alone seems to be less threat-specific but instead reflects a more general action-preparatory mechanism when a motor response is required (regarding inevitable threat see Szeska et al., 2021). These findings confirm that defensive responding, which integrates behavioral and autonomic functions (among other components, such as endocrine responses) bear complex temporal dynamics that need to be considered when using them as indicators of fear. Instead of relying on single output measures, integrated analyses of multiple readouts appear more appropriate to define such defense states, which seems relevant to both animal and human research. Future studies should hence consider the multifaceted nature of heart rate deceleration when adducing it as a proxy for fear-related behavior (e.g., in neuroimaging environments, Wendt et al., 2017), particularly when creating ambivalent experimental conditions involving conflicts between threat and reward possibilities. Eye-tracking, on the other hand, seems to be more suitable to discriminate between defensive and appetitive states (see also Xia et al., 2021). As a whole, the current study contributes to a more comprehensive and nuanced picture of defensive states by elucidating the integrated nature of their autonomic and oculomotor components.

3. Study 2:

Fear lies in the eyes of the beholder – robust evidence for reduced gaze dispersion upon avoidable threat

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Abstract

A rapid detection and processing of relevant information in our environment is crucial for survival. The human eyes are drawn to social or threatening stimuli as they may carry essential information on how to behave appropriately in a given context. Recent studies further showed a centralization of gaze that reminded of freezing behaviors in rodents. Probably constituting a component of an adaptive defense mode, centralized eye movements predicted the speed of motor actions. Here we conducted two experiments to examine if and how these presumably survival-relevant gaze patterns interact. Subjects viewed images including social, i.e., faces (Experiment 1, *N*=50) or threatening stimuli, i.e., snakes or spiders (Experiment 2, *N*=50) while awaiting an inevitable (shock), no (safety), or an avoidable shock (flight) they could escape from by a fast button press. The social and threatening cues within the scenes differed in their distance from the image center and we acquired eye-tracking and autonomic physiological data. Although we observed an initial orienting towards social and threatening stimulus aspects, this exploration pattern vanished towards the end of flight trials when a pronounced centralization of gaze emerged. Replicating previous findings, the amount of this center bias predicted the speed of motor reactions, and we observed a concurrent activation of the sympathetic and parasympathetic nervous system. Taken together, exploration of potentially relevant cues seems to be part of a reflexive orienting response regardless of contextual valence. However, centralization of gaze may be a threat-specific action-preparatory response that occurs across a wide range of stimulus contexts.

Keywords: Defensive States, Orienting, Attentional bias, Threat, Fear, Gaze, Freezing

Introduction

Survival of any organism critically depends on the adequate detection of and responding to relevant information in the environment. This is particularly true for life-threatening events that have created an evolutionary pressure to produce appropriate behavior to flexibly adapt to danger (Blanchard et al., 2011; Blanchard & Blanchard, 1989; LeDoux & Daw, 2018; Mobbs et al., 2015; Roelofs, 2017). Which of these responses is most effective in a given situation depends on species-specific, individual as well as contextual factors such as the spatiotemporal proximity of a threat (Blanchard, 2017; Fanselow, 1994). Although principally adapted to an organisms advantage, exaggerated or reduced threat-responding may become maladaptive and contribute to the development and maintenance of various mental disorders (Grupe & Nitschke, 2013; LeDoux, 2012). For certain defensive behaviors, such potentially dysfunctional deviations have not been thoroughly understood, highlighting the importance to elucidate their phenomenology more rigorously to ultimately support novel therapeutic approaches towards maintaining or restoring mental health (Hashemi et al., 2021; Niermann et al., 2017; Rösler & Gamer, 2019).

The Threat Imminence Continuum Model (TICM) offers a compelling theoretical basis to study modes of defensive behavior in non-human and human animals without neglecting the complexity of individual and contextual factors (Fanselow & Lester, 1988; Hamm, 2020; Mobbs et al., 2020). It illustrates four contexts with increasing threat imminence, each of which is associated with typical defensive strategies: safety, pre-encounter, post-encounter, and circa-strike. In the safety context, the organism believes itself in no danger and can thus invest in long-term protection strategies such as building burrows or houses. If it becomes more likely to encounter a threat (pre-encounter phase), survival strategies usually entail heightened vigilance, careful appraisal of the environment, as well as the development of strategies about how to adapt to quick changes in the surroundings (Mobbs et al., 2020). As soon as a threat is present but still relatively distant (post-encounter phase), a state of attentive immobility typically helps in avoiding predator detection and gathering information about options to escape from or fight the threat (Volchan et al., 2017; Whishaw et al., 1991). As a last resort to imminent danger (circa-strike phase), fight-flight-freeze behaviors are engaged (Bolles, 1970; Fanselow, 1994; Mobbs et al., 2020).

These different defensive modes come along with evolutionary conserved physiological dynamics. Attentive immobility during post-encounter threat is usually accompanied by a co-activation of sympathetic (e.g., resulting in increased skin conductance and pupil width) and parasympathetic branches (e.g., provoking heart rate deceleration/bradycardia) of the autonomic nervous system (Volchan et al., 2017), which then switches to a sympathetically driven heart rate acceleration when fight-flight reactions are necessary (Fanselow & Lester, 1988; Mobbs et al., 2020). Thereby, bradycardia seems to function as an action-preparatory mechanism that facilitates fast subsequent defensive actions (Gladwin et al., 2016; Merscher et al., 2022; Rösler & Gamer, 2019). A recently discovered oculomotor pattern in humans during post-encounter threat seems to fulfil a similar purpose. Rösler and Gamer (2019) observed that participants showed fewer and longer fixations that were closer to the center of a screen (centralization of gaze) when they awaited a shock, they could avoid by quickly reacting to a response cue. Accompanied by heart rate deceleration and an increase in skin conductance, centralization of gaze predicted the speed of motor reactions on a trial-wise basis. This oculomotor pattern was hence also suggested to support action preparation. It has since been replicated and, as compared to heart rate deceleration, shown to not only serve action-preparatory functions independent of valence but also to specifically occur upon threat (vs. reward; Merscher et al., 2022). Implementable in neuroimaging environments in contrast to reduced body sway, which is usually adduced to indicate attentive immobility, it has thus been proposed to serve as a potent marker for said fear-related state and its associated neural circuitries. Given the outstanding questions revolving around this defensive state, for example concerning its relationship with clinical constructs (e.g., Hashemi et al., 2021), robust and specifically fear-related read-outs such as centralization of gaze seem pertinent to better understand adaptive and maladaptive characteristics of defensive behaviors and their neural underpinnings.

Although promising, centralization of gaze as a presumably adaptive component of the defense state of attentive immobility has only been examined with visual stimulus material characterized by a relatively homogeneous distribution of image features to prevent other gaze dynamics to interfere. Thus, we have yet to understand whether such reduced visual exploration remains when competing against other notorious gaze preferences or attentional biases. As such, humans seem to preferentially attend to social (i.e., other humans, End & Gamer, 2017; Fletcher-Watson et al., 2008) and threatening stimuli (e.g., spiders and snakes, Brosch & Sharma, 2005; Öhman, Flykt, et al., 2001; Öhman, Lundqvist, et al., 2001). Revealing important information on the emotional and attentional states of others, humans have a particular tendency to reflexively fixate on faces (Baron-Cohen et al., 1997; Driver et al., 1999; Emery, 2000; End & Gamer, 2017; Rösler et al., 2017). This attentional bias persists even when the faces compete against other visually salient aspects in a picture (Birmingham et al., 2008, 2009a, 2009b; Suda & Kitazawa, 2015). Similarly, phylogenetically fear-relevant stimuli such as spiders and snakes draw our attention. We detect them more quickly than neutrally or positively-valenced stimuli such as mushrooms, flowers or rather fear-irrelevant animals like birds, fish, and frogs (Lobue & Deloache, 2011; Masataka & Shibasaki, 2012; Öhman, Flykt, et al., 2001; Öhman, Lundqvist, et al., 2001, but see Wiemer et al., 2013). Both viewing dynamics (i.e., attentional biases for informative visual stimuli such as faces or spiders and snakes as well as centralization of gaze) thus seem to serve adaptive purposes facilitating well-being and survival, but it remains unclear whether and how they interact.

To examine this question, we carried out two experiments using adapted versions of the paradigm originally used by Rösler and Gamer (2019). In Experiment 1, we replaced the naturalistic images with pictures showing social stimuli (i.e., faces). In Experiment 2, we used pictures including threatening stimuli (i.e., spiders and snakes). Importantly, the respective stimulus content used in both experiments similarly differed in its distance from the image center. Thus, faces, spiders, and snakes could either be located near the image center but also appeared at various distances in the periphery. We acquired eye-tracking and autonomic data (i.e., skin conductance, heart rate, and pupil width). Based on previous studies, we expected that faces or spiders and snakes, respectively, will be initially fixated as part of a reflexive orienting response (e.g., Rösler et al., 2017; Wiemer et al., 2013). Towards the end of the anticipation phase, we assumed to observe fewer and longer fixations that are closer to the center of the screen in trials where participants could actively avoid a potential subsequent aversive shock as compared to no shock or certain shock trials. In line with previous findings, we further hypothesized centralization of gaze to predict faster responses in both experiments (Merscher et al., 2022; Rösler & Gamer, 2019). Regarding autonomic measures, we expected an increase in sympathetic arousal (i.e., skin conductance and pupil width) when participants anticipated both avoidable and inevitable shocks and increased parasympathetic activation (i.e., bradycardia) to be present only when participants prepare for shock avoidance, irrespective of whether social or threatening stimuli are shown (Merscher et al., 2022; Rösler & Gamer, 2019).

Methods

Participants

Based on previous studies (Merscher et al., 2022; Rösler & Gamer, 2019), it was planned to include fifty subjects per Experiment, allowing for the detection of medium effect sizes in repeated measures analyses of variance (ANOVAs, Cohen's $f = .25$) at an alpha level of .05 with a statistical power greater than .95 when assuming a correlation of $r = .50$ between factor levels.

Participants were recruited via an online portal hosted by the University of Wuerzburg forming a sample of convenience (Experiment 1; $N = 62$, age: $M = 24.9$, $SD = 4.23$ years, 40 females; Experiment 2; $N = 57$, age $M = 23.02$, $SD = 2.92$ years; 39 females). Subjects were compensated with 10€. In Experiment 1, twelve participants were excluded from the analyses: Six did not follow the instructions (i.e., they hit the space bar in inevitable shock or safety trials), three did not have a valid eye-tracking baseline (see below), and three were excluded due to technical problems with the autonomic recordings. In Experiment 2, seven participants were excluded from the analyses, six of which did not follow the instructions and another one's data were not correctly saved. Each final sample thus consisted of 50 participants (Experiment 1; age: $M = 24.88$, $SD = 4.36$ years, 31 females; Experiment 2; age: $M = 22.96$, $SD = 2.85$ years, 34 females) who were characterized based on their trait anxiety and anxiety sensitivity in both experiments and additionally regarding their fear of spiders and snakes in Experiment 2 (Table 1). Both studies were approved by the ethics committee of the University of Würzburg, and all participants provided written informed consent according to the declaration of Helsinki. Experiments were formally preregistered on aspredicted.org and the preregistration documents can be retrieved from https://aspredicted.org/ZMX_RVD (Experiment 1) and https://aspredicted.org/blind.php?x=689_VRX (Experiment 2). Data and analysis scripts are publicly available on the OSF (https://osf.io/7dutb/).

Table 1. Sample characteristics (*N* = 50 in both Experiments).

	Experiment 1 social Scenes	Experiment 2 threatening scenes
Age (years)	24.88 (4.36)	32.12 (2.83)
Gender (m/f/d)	19/31/0	16/34/0
STAI-T	44.32 (4.95)	37.04 (8.05)
ASI-3	14.68 (8.28)	18.74 (8.69)
SNAQ	-	19.46 (2.17)
FSQ	-	11.32 (16.16)

Note. Values indicate *M* (*SD*) unless otherwise noted. STAI-T = State-Trait Anxiety Inventory, trait version (Laux et al., 1981), ASI = Anxiety Sensitivity Index (Kemper et al., 2009), SNAQ = Snake Questionnaire (Klorman et al., 1974), FSQ = Fear of Spiders Questionnaire (Rinck et al., 2002).

Stimuli

Stimulus sets consisted of 60 color photographs of naturalistic scenes in both experiments (Figure 1). The images were obtained using search engines (e.g., Google picture search) or drawn from existing image databases (Nencki Affective Picture System, NAPS; Marchewka et al., 2014). All pictures were cropped and resized to achieve a similar resolution of 1200 x 900 pixels. In Experiment 1, images depicted various indoor and outdoor scenes including one or multiple human beings. Their valence ranged from neutral to slightly positive. The photographs were chosen such that the stimuli of interest (i.e., faces) differed in their distance from the image center. The distance of heads from the center of the screen (calculated by averaging the distance of all pixels that were part of a head) ranged from 128.20 to 576.01 pixels (see supplemental Figure S1 for an illustration of the distribution). In Experiment 2, pictures contained threatening instead of social stimuli. Half of them included spiders and the other half snakes. During stimulus selection, we tried to match the spatial distribution of spiders and snakes on the image to the social cues used in Experiment 1. As a result, the distance of spiders and snakes (quantified as the average distance of all pixels belonging to the animal) to the image center ranged from 105.35 to 588.46 pixels (see supplemental Figure S1 for an illustration of the distribution).

Figure 1. Exemplary pictures used in a previous study with the same design by Rösler and Gamer (2019, A), Experiment 1 (B), and Experiment 2 (C). Naturalistic pictures (A) were characterized by image features that were distributed relatively homogeneously in each picture. In contrast, images showing social (B, i.e., faces) and threatening stimuli (C, i.e., spiders/snakes), respectively, were selected such that the stimuli differed in their distance from the center of the screen. One image thereby contained one or multiple stimuli of interest. Please note that since we did not obtain permission for publishing the original stimuli used in Experiment 1 and 2, this Figure shows examples to illustrate general image properties.

Experimental Design

Based on Rösler and Gamer (2019), participants watched images depicting social (Experiment 1) or threatening stimuli (Experiment 2) against a grey background while their eye movements, pupil width (PW), heart rate (HR) and skin conductance (SC) were measured. Images were presented in random order. Each image (8 s) was preceded by a white fixation cross (6.5-8.5 s) turning red, green or yellow (2 s). Participants were instructed that an inevitable shock would follow on the red fixation cross (shock trials), no shock on the green one (safety trials) and an avoidable shock on the yellow one after disappearance of the picture (flight trials). Following image presentation, the screen turned blank (1 s) in safety and shock trials while participants received a shock after this phase in shock trials. In flight trials, participants were verbally prompted to avoid the electrotactile stimulation by hitting the space bar as fast as possible. The response prompt was shown for a maximum of 1 s and disappeared after the keypress. If participants were not fast enough, a shock was delivered after this phase. In order to ensure that participants would receive a shock in approximately 50% of the trials, they had to be faster than

240 ms in the first five flight trials. Afterwards, the threshold was individually adjusted to the median of the first five reaction times. Both experiments comprised 60 trials with 20 in each condition. Trial order was pseudo-randomized to ensure that no more than three trials of the same type appeared after one another (see Figure 2 for an illustration of the trial design).

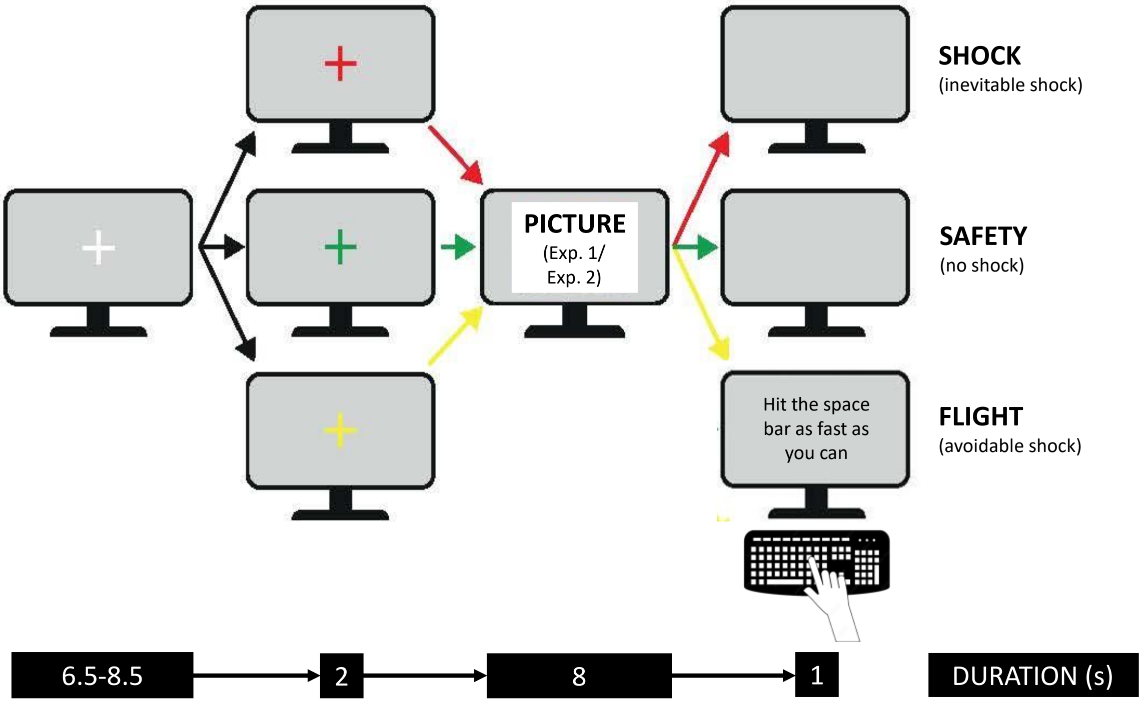

Figure 2. Illustration of the trial design. Participants were instructed that a colored fixation cross would signal them whether to expect an inevitable (red, shock), no (green, safety), or an avoidable shock (yellow, flight) they could prevent by hitting the space bar as fast as possible. Between the fixation cross and the trial outcome they viewed images that differed between the experiments. In Experiment 1, images showed social stimuli (i.e., faces) and Experiment 2 entailed images depicting threatening stimuli (i.e., spiders and snakes).

Apparatus

The experiment was programmed with Presentation® (Neurobehavioral Systems Inc., Version 18.1) and run on a 24’’ Asus VG248QE display (53.126 × 29.889 cm, 1920 × 1080 pixels, refresh rate 144 Hz). Eye movements were tracked using an EyeLink 1000 Plus system (SR Research Ltd., Ottawa, Canada) in the tower mount configuration with a sampling rate of 1000 Hz. Thereby, only the movement of the right eye was tracked at a viewing distance of 50 cm from the monitor. Shocks consisted of a train of three 2 ms square-wave pulses (separated by 50 ms) with alternating polarity that were individually calibrated and delivered via an electrode attached to the non-dominant calf using a Digitimer Constant Current Stimulator DS7A (Hertfordshire, United Kingdom). Heart rate and skin conductance were continuously measured using a BIOPAC MP160 system (BIOPAC Systems, Inc.) at a sampling rate of 500 Hz. For heart rate, disposable Ag/AgCl electrodes were placed on the right clavicle, the lower left and right ribcage (reference). For skin conductance, two Ag/AgCl electrodes filled with 0.05 ml NaCL electrolyte were placed on the thenar and hypothenar eminences of the non-dominant hand.

Procedure

After providing written informed consent, participants were given instructions about the different trial types. We explained that the image content was of no relevance to accomplish shock avoidance in flight-trials. Moreover, participants were asked to fixate a centrally displayed fixation cross whenever it was shown but they were allowed to freely explore the images. Subsequently, all electrodes for the physiological recording and for the shock delivery were attached and the participant was sat in a dimly lit sound-proof cabin while the experimenter stayed outside of the cabin where communication with the subject was still possible. We then individually calibrated the shock intensity on a visual analogue scale (VAS) from 0 = not painful at all to 10 = unbearably painful, aiming at reaching a rating of 4, which we defined as the threshold between an unpleasant and a slightly painful sensation. Shock intensity was incrementally increased by steps of 0.1 mA until reaching a VAS rating of 4. This procedure was repeated 3 times and the final amperages were averaged and increased by 50 %, which then formed the final shock intensity (Experiment 1: *M* = 0.66 mA, *SD* = 0.55 mA, Experiment 2: *M* = 0.49 mA, *SD* = 0.32 mA).

We started the experiment when participants were successfully calibrated and validated on the eye tracker using a nine-point grid. After finishing the experiment, participants filled in questionnaires. In both experiments, participants were asked to complete the German versions of the trait version of the State-Trait Anxiety Inventory (STAI-T; Laux et al., 1981) and the Anxiety Sensitivity Index (ASI-3; Kemper et al., 2009). With 20 items in total, the STAI-T asks participants for an assessment of their regular level of anxiety on a 4-point Likert scale from 1 (almost never) to 4 (almost always). Sum scores range from 20 up to 80 points and higher values indicate more pronounced trait anxiety. Internal consistency in the current sample as assessed by Cronbach's α was acceptable to good (Experiment 1: α = .76; Experiment 2: α = .83). The ASI-3 consists of 16 items measuring the participants' regular level of anxiety sensitivity on a 5-point Likert scale from 0 (very little) to 4 (very much). Sum scores thus range from 0 to 64.

Similar to the STAI-T, Cronbach's α was acceptable to good (Experiment 1: α = .86; Experiment 2: α = .77).

In Experiment 2, participants additionally completed the German versions of the fear of spiders questionnaires (FSQ; Rinck et al., 2002) as well as the snake questionnaire (SNAQ; Klorman et al., 1974). The FSQ consists of 18 items that ask participants to rate statements that are related to their fear of spiders on a 7-point Likert scale from 0 (not true at all) to 6 (exactly true). Sum scores can range from 0 to 108, with higher values indicating higher fear of spiders. Cronbach's α was .95 in our sample. The SNAQ counts 30 items in total that dichotomously ask whether participants would rate snake-phobia related statements as rather true or false, resulting in a maximum sum score of 30. Cronbach's α was .82 in the current sample.

Data processing

Eye Tracking. Eye movements were parsed into rapid eye movements exceeding a velocity of 30°/s or an acceleration of $8000°/s^2$ (saccades) and relatively stable gaze periods between the saccades (fixations). We used the 300 ms before cue onset as baseline for an offline drift correction. As a baseline quality check (cf. Merscher et al., 2022), we applied an iterative outlier detection algorithm that temporarily removed the highest and lowest values of baseline data for both x and y coordinates from the distribution and tested whether these data deviated more than three standard deviations from the mean of the remaining data. If one or both of these values met this criterion, they were marked as outliers and excluded from the data set, if not, they were returned. This was iteratively repeated until no further x- and y-values had to be removed. Gaze drifts were then corrected by subtracting the x- and y-coordinates of the baseline from the fixation coordinates during image viewing. For the analyses, we excluded all trials with baseline outliers as well as those where participants hit the space bar when no button press was required (Experiment 1: 9.07%, Experiment 2: 8.37 %). Participants were completely excluded when less than 50% of valid trials remained for further analyses (3 participants in Experiment 1, 0 in Experiment 2). Regarding the eye-tracking data, we extracted four different metrics: the average distance of fixations from the center of the screen (centralization of gaze), fixation number and duration overall as well as the fixation duration on specific regions of interest (ROI) on a second-by-second basis throughout the anticipation phase, resulting in 8 one-second bins. As regions of interest, we determined human heads (Experiment 1) and entire animals (Experiment 2) by manually delineating the respective image parts using GIMP (Version 2.10.32, GNU Image Manipulation Program). Fixations that started before stimulus onset or fell outside of the presentation screen were not considered and fixations spanning multiple bins were weighted according their relative duration within each individual bin. We first determined the number of fixations per second, considering the relative proportion in the respective bin in case of fixations spanning several bins. Afterwards, the total fixation duration per bin was calculated and divided by the respective number of fixations to yield average fixation durations.

Pupil width. Pupil diameter was also derived from the eye-tracking data. First, we linearly interpolated blinks and periods of missing data. Afterwards, data were downsampled to 100 Hz, filtered using a 2 Hz low-pass filter, and converted from arbitrary units to mm (Hayes & Petrov, 2016). We then subtracted baseline values (1 s prior to cue onset) from all subsequent values spanning the cue and picture viewing phase and averaged them into 20 bins of 0.5 s (5 additional seconds for the post-stimulus phase were added for data visualization, see Figure 5). Note that we used a smaller bin duration for pupil width than for the other oculomotor and physiological measures since the pupil responds more quickly to external and internal events (cf. Merscher et al., 2022).

Electrodermal activity. Data were downsampled to 20 Hz for further processing. We averaged the values into 10 one-second bins for each trial type, starting two seconds before stimulus onset to include the cue phase (plus 5 bins for data visualization). The last second prior cue onset served as baseline and was subtracted from all subsequent data points of each trial.

Heart rate. ECG data were filtered using a 2 Hz high-pass filter to remove slow signal drifts. Afterwards, R-peaks were detected semi-automatically using in-house software and manually edited in case of detection errors. R-R-intervals were converted to HR in beats per minute. Using a real-time scaling procedure (Velden & Wölk, 1987), we calculated the mean heart rate in bins per second for the same 10 s time frame (plus 5 bins for data visualization) as for SC. Equally matching the SC procedure, the last second preceding the cue onset was used as baseline and subtracted from all further values.

Statistical analyses

All data preprocessing and statistical analyses were performed using the statistical programming language R (Version 4.0.3, R Core Team, 2020) on a significance level of 5%. To compare the time course (10 bins for skin conductance and heart rate, 20 bins for pupil width, 8 bins for all metrics of visual exploration) of all dependent variables between the trial types (shock, safety, flight), we calculated repeated-measures analyses of variance (rmANOVA). Degrees of freedom were adjusted according to Greenhouse-Geisser to compensate for potential violations of the sphericity assumption. Post-hoc t-tests were then performed to test whether flight trials differed from the other two trial types. We used the false discovery rate correction to account for alpha-error accumulation (FDR, Benjamini & Hochberg, 1995).

To investigate whether centralization of gaze during the anticipation of an avoidable approaching threat interacts with gaze patterns evoked by social or threatening pictures, we performed a general linear mixed model (GLMM) with trial type (flight, shock, safety) and distance of social or threatening stimulus elements, respectively, from the image center as predictors for the distance of fixations from the center of the screen. Subject ID was inserted as a random intercept. To this end, we used the lmer4 package in R (Version 1.1-15; Bates et al., 2015). Model estimates were chosen to optimize the restricted maximum likelihood criterion and *p*-values for each predictor were calculated using Satterthwaite's approximation of degrees of freedom with the lmerTest package (version 2.0-25, Kuznetsova et al., 2017).

We computed further GLMMs to test whether heart rate and distance of fixations from the center of the screen predicted the speed of motor responses on a trial-by-trial basis. Therefore, we used both dependent variables as predictors for reaction times in flight trials and added the subject ID again as a random intercept into the GLMM. We focused on heart rate and centralization of gaze during the second half of the anticipation phase as previous studies found the strongest differences between trial types right before the trial outcome (Merscher et al., 2022; Rösler & Gamer, 2019). Thus, second 5-8 of picture viewing were considered and averaged on a trial-wise basis. Mean reaction times were similarly fast (Experiment 1: *M* = 282.42 ms, *SD* = 64.47 ms; Experiment 2: *M* = 303.49 ms, *SD* = 83.81 ms) and participants managed to avoid the shock comparably often in both experiments (Experiment 1: 59.8 %; Experiment 2: 59.9 %).

Results

Oculomotor data

To test whether visual scanning decreased when participants awaited an avoidable shock irrespective of the distribution of social or threatening stimulus elements in the pictures, respectively, we compared the distance of fixations from the center of the screen between the three trial types within each experiment. A 3 (trial types) × 8 (bins) rmANOVA showed that gaze dispersion reduced towards the end of the anticipation phase in flight as compared to safety and shock trials in both experiments (Experiment 1: interaction trial type × bin, $F_{(14,686)} = 32.49$, $\varepsilon = .43$, $p < .001$, $\eta^2_g = .13$; Experiment 2: interaction trial type × bin, $F_{(14,686)} = 20.56$, $\varepsilon = .37$, $p < .001$, $\eta^2_g = .06$; main effects for these and all further analyses can be found in Tables S1 and S2 in the supplementary material). This pattern was evident for trials showing images depicting the stimuli of interest near the center as well as in the periphery (Figure 3). Although visual exploration seemed to reduce less strongly in flight trials when threatening as compared to social stimuli were presented in the image periphery (Figure 3E and F), a formal analysis did not yield significant differences between experiments (interaction experiment × trial type × bin, $F_{(14,1372)} = 1.44$, $\varepsilon = .43$, $p = .196$, $\eta^2_g < .01$, supplemental Table S3).

To test whether participants dwelled longer on threatening than on social stimulus elements during the anticipation phase, we analyzed how long fixations remained on the stimuli of interest in both experiments. Shortly after image onset, social as well as threatening stimuli were fixated around 50% of the time, but these values declined sharply over the anticipation period irrespective of the trial type (Figure 4A and B; Experiment 1: main effect bin, $F_{(7,343)} = 187.45$, $\varepsilon = .50$, $p < .001$, $\eta^2_g = .45$; main effect trial type, $F_{(2,98)} = 1.20$, $\varepsilon = .86$, $p = .303$, $\eta^2_g < .01$; interaction trial type × bin, $F_{(14,686)} = 1.02$, $\varepsilon = .61$, $p = .420$, $\eta^2_g < .01$; Experiment 2: main effect bin, $F_{(7,343)} = 38.89.42$, $\varepsilon = .59$, $p < .001$, $\eta^2_g = .11$; main effect trial type, $F_{(2,98)} = 4.13$, $\varepsilon = .90$, $p = .023$, $\eta^2_g = 01$; interaction trial type × bin, $F_{(14,686)} = 1.17$, $\varepsilon = .61$, $p = .314$, $\eta^2_g < .01$). Contrasting both experiments in one 2 × 3 × 8 ANOVA using the between subjects factor experiment revealed a faster and steeper decline of fixation proportions for the social as compared to the threatening stimuli, underlining that participants indeed explored spiders and snakes longer than human heads or faces (interaction experiment × bin, $F_{(7,686)} = 27.16$, $\varepsilon = .57$, $p < .001$, $\eta^2_g = .05$, supplemental Table S6).

Finally, we investigated the influence of the spatial positions of social or threatening stimuli on the observed reduced gaze dispersion during the anticipation of an avoidable approaching threat. In Experiment 1, a GLMM with trial type (flight, shock, safety) and distance of social stimuli from the image center on gaze dispersion showed a significant interaction between the predictors (interaction distance of social stimuli × shock vs. flight trials, $\beta = 0.02$, $SE < 0.01$, $t_{(21680)} = 2.23$, $p = .026$; interaction distance of social stimuli × safety vs. flight trials, $\beta = 0.02$, $SE < 0.01$, $t_{(21680)} = 2.23$, $p = .025$). In Experiment 2, however, an equally constructed GLMM did not yield a significant interaction between the distance of threatening stimuli and shock (vs. flight) trials ($\beta = 0.01$, $SE < .001$, $t_{(21900)} = 0.88$, $p = .380$) or safety (vs. flight) trials ($\beta = 0.01$, $SE < .001$, $t_{(21900)} = 1.24$, $p = .214$). Examining these effects in more detail by separately analyzing each second of the anticipation period, the predictive power of the distance of the stimuli of interest for the distance of fixations from the center of the screen declined towards the end of the picture presentation in both experiments, becoming statistically insignificant in bin 8 for social but not for threatening stimuli (results for bin-wise GLMMs can be found in supplemental Table S7). Thereby, the interaction between the distance of the social stimuli in Experiment 1 and safety or shock (vs. flight) trials increased towards the end of the anticipation phase. This effect was less pronounced in Experiment 2 and the respective interaction only reached statistical significance for safety but not for shock (vs. flight) trials.

Generally, reduced gaze dispersion predicted the speed of motor reaction in flight trials on a trial-wise basis in both experiments (Experiment 1: $\beta = 2.83$, $SE = .39$, $t_{(828.69)} = 7.24$, $p < .001$; Experiment 2: $\beta = .27$, $SE = .05$, $t_{(805.96)} = 5.52$, $p < .001$). However, this effect was stronger when participants viewed social as compared to threatening stimuli (interaction of experiment and mean gaze dispersion: $\beta = -2.56$, $SE = .37$, $t_{(1671.15)} = -6.94$, $p < .001$).

The temporal profile of fixation durations and numbers also differed between the trial types, with fewer and longer fixations in flight trials towards the end of the anticipation phase as compared to shock and safety trials (see supplemental Figure S2, Table S4 and S5).

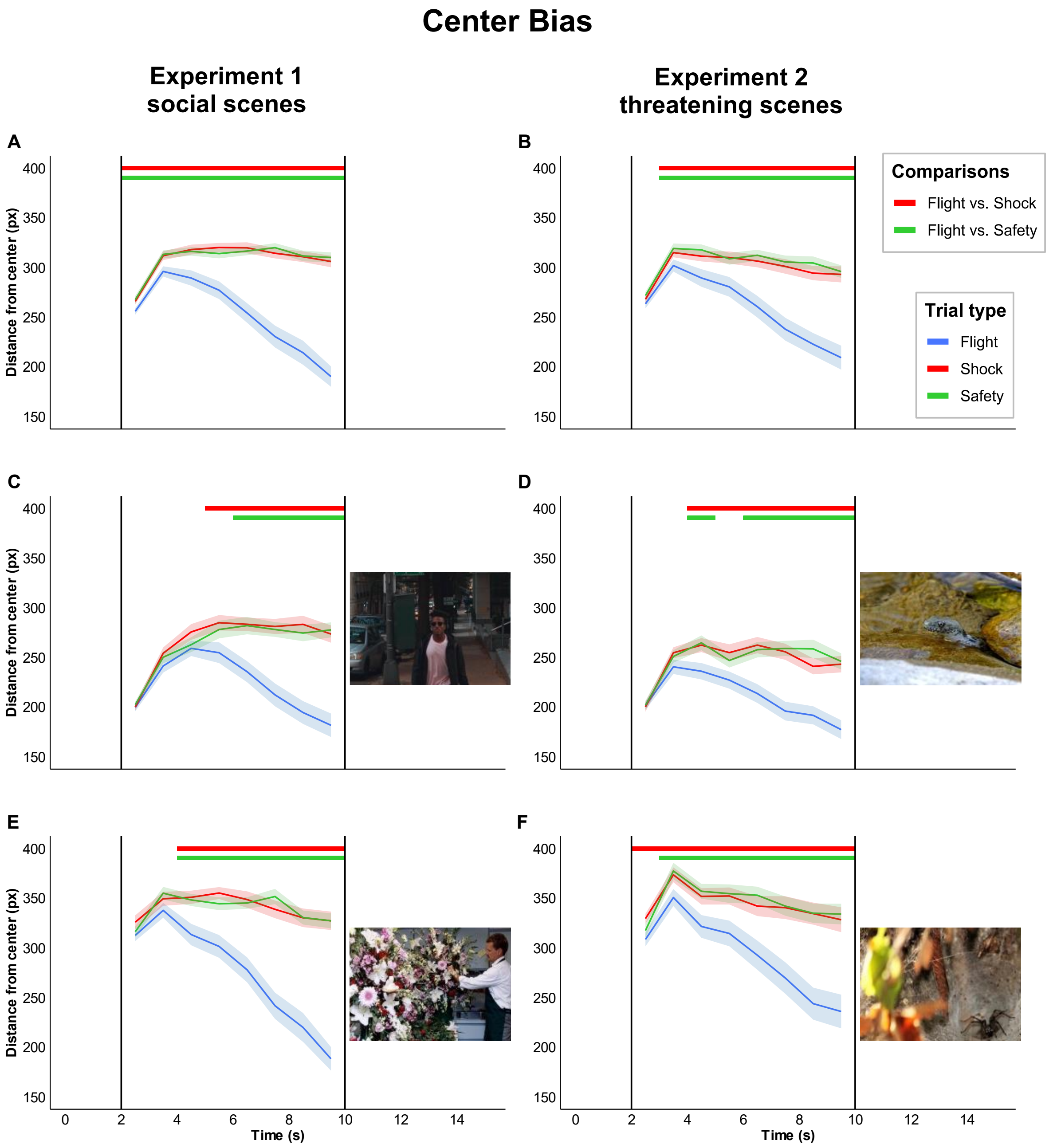

Figure 3. Distance of fixations from the center of the screen across all stimuli (Experiment 1: A, Experiment 2: B) as well as for images where stimuli were located near the center (20 images with smallest distances; Experiment 1: C, Experiment 2: D) or in the periphery of the pictures (20 images with largest distances; Experiment 1: E, Experiment 2: F). Vertical lines denote the onset and offset of the anticipation (i.e., picture viewing) phase. Horizontal lines on top of the graphs illustrate significant pairwise comparisons between flight trials and the other two trial types after false discovery rate correction. Fixations were initially predicted by the distance of social or threatening stimuli, respectively, irrespective of the trial type, but decreased in their distance from the center of the screen towards the end of the anticipation phase when participants awaited an avoidable threat (flight trial) as compared to shock and safety trials. The embedded pictures illustrate the relevant image properties.

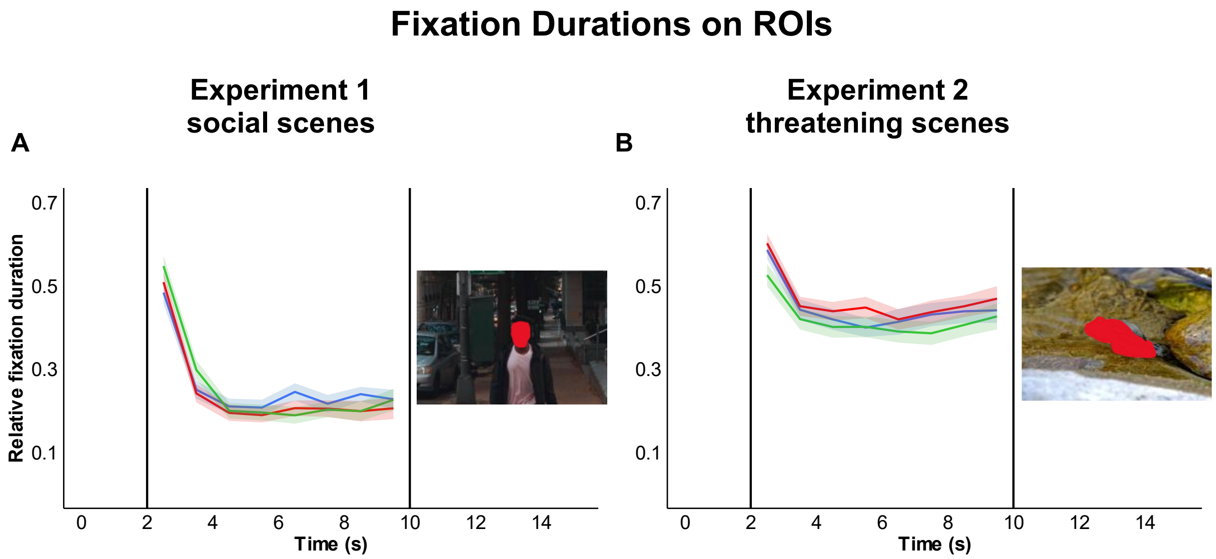

Figure 4. Fixation durations on heads in Experiment 1 (A) and spiders or snakes in Experiment 2, respectively (B). Vertical lines denote the onset and offset of the anticipation (i.e., picture viewing) phase. The embedded pictures illustrate the definitions of regions of interest for social and threatening scenes, respectively.

Autonomic data

To assess differences in heart rate dynamics between the three trial types, we first conducted a 3 (trial types) × 10 (bins) rmANOVA. When participants anticipated an avoidable shock, their heart rate increased after cue onset and decreased most strongly towards the end of the anticipation phase as compared to shock and safety trials in both Experiments (Figure 5A and B; Experiment 1: interaction trial type × bin, $F_{(18,882)} = 10.50$, $\varepsilon = .29$, $p < .001$, $\eta^2_g = .04$; Experiment 2: interaction trial type × bin, $F_{(18,882)} = 14.57$, $\varepsilon = .30$, $p < .001$, $\eta^2_g = .06$). A direct comparison of heart rate changes during the anticipation phase between both experiments using a 2 × 3 × 10 ANOVA did not reveal significant differences between Experiment 1 and 2 (interaction experiment × trial type × bin, $F_{18,1764} = .99$, $\varepsilon = .31$, $p = .431$, $\eta^2_g < 0.01$, supplemental Table S8). In none of the experiments did heart rate deceleration predict the speed of motor responses on a trial-wise basis (Experiment 1: $\beta = 10.90$, $SE = 6.36$, $t_{(857.12)} = 1.71$, $p = .087$; Experiment 2: $\beta = -1.14$, $SE = .94$, $t_{(849.09)} = -1.22$, $p = .223$).

Skin conductance mainly increased in shock trials in both Experiments (Figure 5C and D, Experiment 1: interaction trial type × bin, $F_{(18,882)} = 18.37$, $\varepsilon = .19$, $p < .001$, $\eta^2_g = .05$; Experiment 2: interaction trial type × bin, $F_{(18,882)} = 13.04$, $\varepsilon = .14$, $p < .001$, $\eta^2_g = .05$). Pupil dilation revealed a similar pattern: After the initial pupil constriction following picture onset, it increased when participants awaited an inevitable or an avoidable as compared to no shock in both Experiments (Figure 5E and F, Experiment 1: interaction trial type × bin, $F_{(38,882)} = 24.06$, $\varepsilon = .17$, $p < .001$, $\eta^2_g < .001$; Experiment 2: interaction trial type × bin, $F_{(38,882)} = 10.70$, $\varepsilon = .14$, $p < .001$, $\eta^2_g < .001$, see supplemental Tables S9 and S10 for comparisons between Experiments).

None of the dependent variables correlated significantly with any of the questionnaires across participants in flight trials except for center bias and fixation duration during the second half of the picture presentation, which yielded a significant association with fear of spiders in Experiment 2 (center bias: $r = -.33$, $p = .020$; fixation duration: $r = .32$, $p = .002$; see supplemental Table S11 for all correlations).

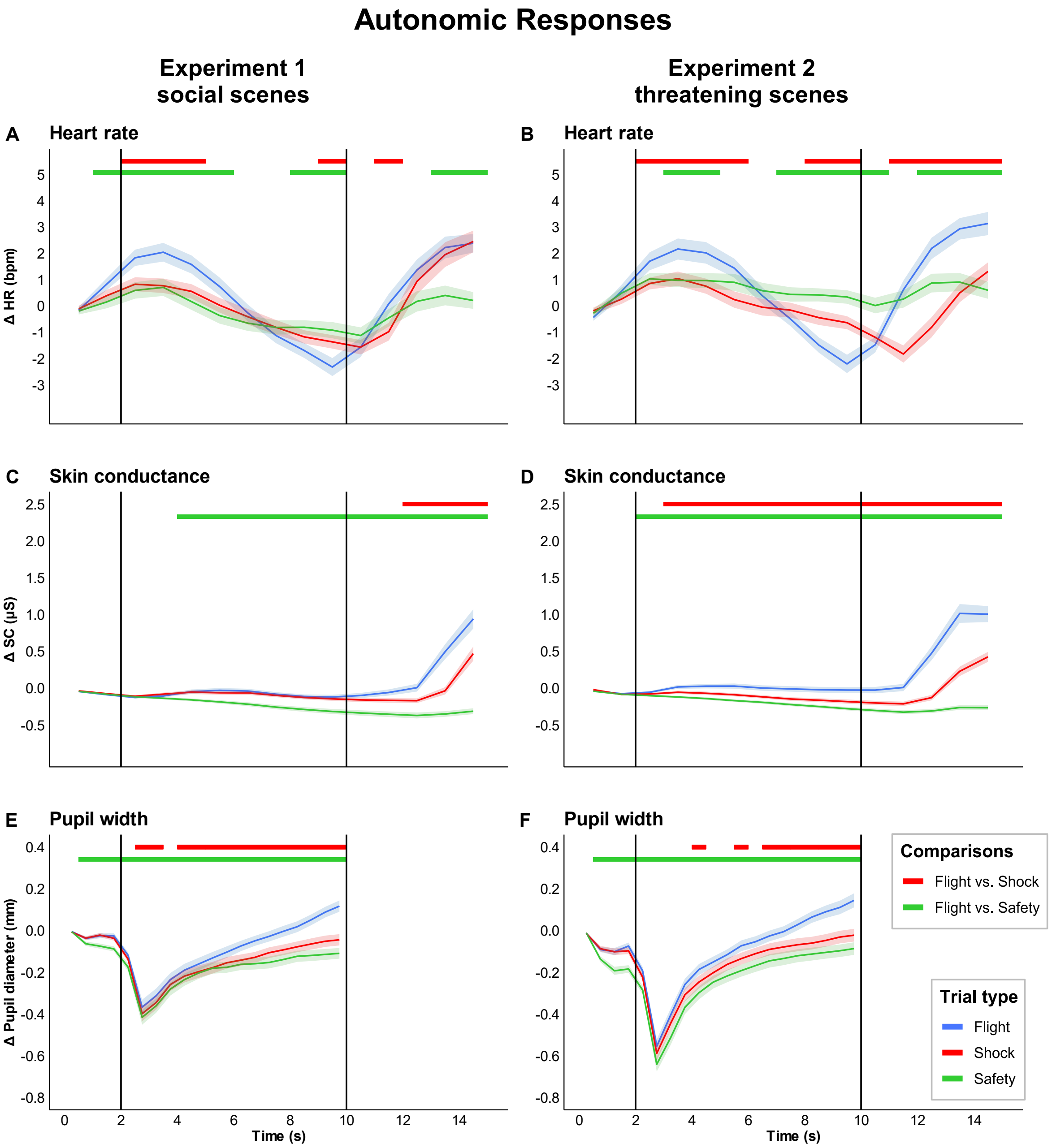

Figure 5. Changes in heart rate (Experiment 1: A; Experiment 2: B), skin conductance (Experiment 1: C; Experiment 2: D) and pupil width (Experiment 1: E; Experiment 2: F) over the course of the anticipation (i.e., picture viewing) phase. Vertical lines denote the onset and offset of the anticipation phase. Horizontal lines on top of the graphs illustrate significant pairwise comparisons between flight trials and the other two trial types after false discovery rate correction.

Discussion

In the current study, we examined the interplay between different survival-relevant oculomotor patterns and concomitant autonomic dynamics during threat of shock. Specifically, we tested whether and how previously shown gaze patterns, namely attentional biases for social (Experiment 1) and threatening stimuli (Experiment 2) as well as reduced gaze dispersion as an action preparatory component of a defensive state, interact. Moreover, we sought to replicate autonomic dynamics during the anticipation of avoidable (vs. inevitable vs. no) threat and examine whether they differed depending on whether neutrally to positively valenced (Experiment 1) or negatively valenced scenes (Experiment 2) were viewed.

In line with our first hypothesis, we found that fixations were initially predicted by the location of social or threatening stimulus elements, respectively. Both types of stimuli were strongly explored after image appearance, which then declined over the viewing period in all trial types. Towards the end of the anticipation phase, however, we specifically observed reduced visual exploration when participants could avoid a shock as compared to shock or safety trials irrespective of the location of the stimuli of interest. At the same time, fixation durations increased while their number and distance from the center of the screen decreased. More centralized fixations predicted faster motor responses on a trial-wise basis.

Upon closer examination however, visual exploration reduced slightly less in anticipation of avoidable threat when threatening instead of social stimuli were located in the periphery of the image. Along these lines, the distance of threatening stimuli predicted the distance of fixations throughout the entire anticipation period whereas this effect vanished towards the end of the anticipation phase for social stimuli. Seconding this finding, we observed that participants' exploration of threatening stimuli declined less over the anticipation period than that of the social stimuli, suggesting longer dwelling on the former.

Further in line with our hypotheses, we observed decreased heart rate when participants anticipated an avoidable shock and increased skin conductance and pupil width in both shock trials irrespective of whether threatening or social stimuli were viewed.

The observation that participants directed their attention towards social or threatening stimuli in the beginning of the anticipation phase, respectively, corroborates a wealth of previous findings on attentional preferences in humans for faces (e.g., Rösler et al., 2017) and phylogenetically fear-relevant stimuli such as snakes and spiders (e.g., Wiemer et al., 2013). These dynamics have been suggested to support survival and well-being by directing our attention to stimuli that may convey important information about emotional states of others as well as potential sources of danger (Driver et al., 1999; Öhman, Flykt, et al., 2001). Moreover, the observed reflexive exploration of social or threatening stimuli, respectively, followed by a quick habituation has been proposed to be characteristic of an orienting response, which supposedly distinguishes it from other phenomenologically similar states such as attentive immobility that are not subject to habituation (Barry et al., 2012; Roelofs, 2017).

Attentive immobility, in contrast, supposedly increases as a function of action preparation (Gladwin et al., 2016; Merscher et al., 2022). Along these lines, we observed previously described characteristics of attentive immobility to become more pronounced towards the end of the anticipation phase when participants awaited a shock they could avoid by a quick button press (Merscher et al., 2022; Rösler & Gamer, 2019; Volchan et al., 2017). Accordingly, motor preparation in these trials was accompanied by a reduction of gaze dispersion along with a co-activation of parasympathetic (bradycardia) and sympathetic (increase in skin conductance and pupil width) branches of the autonomic nervous system. Matching with the notion that attentive immobility adaptively supports action preparation, the reductions in visual scanning were associated with faster motor-reactions on a trial-wise basis. Similarly, the observed transient bradycardia in flight as compared to no or shock trials, although not significantly related to faster threat reactions in the current study, seconds that heart rate deceleration also serves this function (Hashemi et al., 2021; Jennings et al., 2009; Obrist et al., 1970; Rösler & Gamer, 2019; Swiercz et al., 2018).

When comparing Experiment 1 and 2, relatively similar patterns of gaze as well as autonomic dynamics were evident regardless of whether subjects viewed images depicting social or threatening stimuli. However, participants dwelled longer on threatening than social stimuli, which corroborates the previous notion that healthy individuals attend more to negatively-valenced stimuli than to neutral or positive ones (Lobue & Deloache, 2011; Veerapa et al., 2020). Whether increases (e.g., in phobic individuals; Gremsl et al., 2018) or decreases of this bias (e.g., in psychopathy; Baskin-Sommers & Brazil, 2022), could perturb the adaptiveness of attentive immobility as an action-preparatory defense state remains an important question for future research.

Taken together, initial fixations on faces or spiders and snakes, respectively, seem to reflect a reflexive orienting process while reduced gaze dispersion along with bradycardia and increases in skin conductance and pupil width as part of the state of attentive immobility seems to indicate an adaptive component of a defense state preparing the organism for fight or flight (Merscher et al., 2022; Öhman, Flykt, et al., 2001; Öhman, Lundqvist, et al., 2001; Rösler & Gamer, 2019). Both viewing patterns thereby depend on the proximity of threat, which is in line with the assumptions of the TICM claiming survival related strategies to change with decreasing spatiotemporal distance of a threat (Mobbs et al., 2020). While it seemed that the anticipation period of 8 seconds provided enough time to execute both survival-relevant strategies (i.e., orienting and action preparation), which, in the TICM, would most closely resemble the post-encounter context, it remains unclear whether and how their interaction would change during a shorter period of time. Moreover, future studies should investigate whether and how this interaction unfolds when shock avoidance requires selective attention for specific cues that need to be detected during the anticipation of a trial outcome. Finally, the manifestation of reduced gaze dispersion upon avoidable threat in more complex and realistic scenarios (e.g., dynamic scenes or 3-dimensional virtual environments, e.g., Rubo & Gamer, 2018, 2021) as well as its relationship with reduced body sway, which is typically assessed to measure attentive immobility (Roelofs, 2017), should be tested in future studies.

In conclusion, the current study highlights the robustness of reduced gaze dispersion as an action preparatory component of a defensive state even when competing against other notorious gaze preferences. As this oculomotor pattern has previously been shown to be threat-specific and robust, even when threat-avoidance requires more spatially distributed attention, the current findings stress its usability in neuroimaging environments as an additional marker for fear-associated states and their related neural circuitries in humans (Merscher et al., 2022). This is especially pertinent to the literature, as reduced body sway, also referred to as freezing-like behavior in humans (Roelofs, 2017), cannot be measured in magnetic resonance imaging environments (e.g., Schipper et al., 2019). Given the confusion regarding the relationship between attentive immobility and clinical constructs (Hashemi et al., 2021), it seems even more important to thoroughly examine all components of this integrated defense state to facilitate translational research on the underlying neurocircuitry and to further our understanding of factors contributing to the development and maintenance of various mental disorders.

4. Study 3:

Can I see it in the eyes? An investigation of freezing-like motion patterns in response to avoidable threat

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Abstract

Freezing is one of the most extensively studied defensive behaviors in rodents. Both, reduced body and gaze movements during anticipation of threat also occur in humans and have been discussed as translational indicators of freezing but their relationship remains unclear. We thus set out to elucidate body and eye movements and concomitant autonomic dynamics in anticipation of avoidable threat. Specifically, 50 participants viewed naturalistic pictures that were preceded by a colored fixation cross, signaling them whether to expect an inevitable (shock), no (safety), or a potential shock (flight) that could be avoided by a quick button press. Body sway, eye movements, heart rate and skin conductance were recorded. We replicated previously described reductions in body sway, gaze dispersion, and heart rate, and a skin conductance increase in flight trials. Stronger reductions in gaze and heart rate but not in body sway predicted faster motor reactions on a trial-wise basis, highlighting their functional role in action preparation. We failed to find a trait-like relationship between body and gaze movements across participants, but their temporal profiles were positively related within individuals, suggesting that both metrics reflect the same construct. However, future research is needed as assessments of both measures might lack ecological validity. A more ethological examination of different movement dynamics upon threat would not only warrant better comparability between rodent and human research but also help determine whether and how eye-tracking could be implemented as a proxy for fear-related movements in restricted brain imaging environments.

Keywords: Translational Neuroscience, Defensive Behaviors, Body Sway, Threat, Fear, Gaze, Freezing

Introduction

Translating findings from animal research to humans has contributed significantly to our knowledge and treatment of human mental health disorders (Hamm, 2020; McTeague, 2016). However, some areas of translational research lack the necessary conceptual clarity and methodological rigor, which hinders effective interdisciplinary collaboration and scientific progress. This is particularly true for research on fear- and anxiety-related defensive states, which are still poorly understood (Signoret-Genest et al., 2022; Volchan et al., 2017). As defensive behaviors are believed to play a significant role in the development of anxiety-related disorders (e.g., Pittig et al., 2020), it is essential to discuss and address the shortcomings in this field.

Freezing in response to threats is one of the most extensively studied defensive behaviors in rodents (Trott et al., 2022). It is defined as a complete cessation of movement except for respiration (Bolles & Collier, 1976), which is commonly assessed using video tracking or force transducers that record location changes of the animal within a chamber (Chang et al., 2009; Trott et al., 2022). Rodent freezing has been observed in response to potential (Blanchard & Blanchard, 1989) and inescapable threats (Blanchard et al., 1991). Although varying autonomic dynamics have been reported to accompany this somatomotor response, a particular cardio-behavioral pattern has been suggested to consist of transient episodes of freezing and heart rate deceleration (e.g., Vianna & Carrive, 2005).

In contrast to a measure of full body motion as in rodents, the freezing response in humans is commonly measured by calculating the standard deviation of weight displacements on a stabilometric force platform during bipedal stance (Roelofs, 2017; Roelofs & Dayan, 2022). Similar to what has been observed in rodents, this reduction in body sway has been observed upon approaching inevitable (e.g., while passively viewing aversive pictures, Hagenaars et al., 2012) and avoidable threat (e.g., while preparing for active threat avoidance; Gladwin et al., 2016; Hashemi et al., 2021; Niermann, 2018) and was found to be associated with bradycardia and concomitant sympathetic activation (Roelofs, 2017). This seemingly integrated autonomic and behavioral pattern has been linked to adaptive functions such as optimized perceptual processing and action preparation in both animals and humans (Roelofs & Dayan, 2022).

Together, these findings indicate a homology in defensive responding between rodents and humans. Supporting this notion, it has been proposed that similar neural defense circuits involving the central amygdala and the periaqueductal gray play a crucial role in orchestrating different somatomotor and autonomic defense patterns in both species (Fadok et al., 2017;

Roelofs & Dayan, 2022; Terburg et al., 2018). However, our understanding of these circuits remains limited. This may be due to different interpretations and assessments of freezing behaviors, divergent experimental designs across and within disciplines, and constraints in human brain imaging studies (McTeague, 2016).

Regarding the latter, it is not feasible to assess body sway in brain scanners, which would be a critical measure for linking freezing behaviors to their neuroanatomical profile. Human researchers have tried to circumvent this issue by approximating somatomotor activity with heart rate dynamics (Wendt et al., 2017) or running the same experiment twice in and outside the brain scanner (Hashemi et al., 2019) but both methods have limitations. 1) Despite robust associations between bradycardia and reductions in motor activity (e.g., Hashemi et al., 2021; Roelofs et al., 2010), bradycardia has been shown to be elicited in a wide range of stimulus contexts and may thus lack the necessary threat-specificity (Jennings et al., 2009; Merscher et al., 2022). Moreover, reports of seemingly paradoxical heart rate dynamics along with freezing in rodents hamper reliance on bradycardia alone to infer freezing-like states (Signoret-Genest et al., 2022). 2) When running the same experiment twice in and outside the scanner, reliability may be compromised due to differences in context.

A suitable measure of motion cessation or reduction therefore seems crucial to further elucidate the neuroanatomical profiles of motor activity in response to threats in humans. It has recently been suggested that oculomotor activity may serve as such a measure (Merscher et al., 2022; Rösler & Gamer, 2019). Specifically, Rösler & Gamer (2019) found inhibitions of oculomotor activity indicated by less dispersed, fewer, and longer fixations, when participants awaited an avoidable aversive stimulation (see also Xia et al., 2021). This was accompanied by heart rate deceleration and an increase in skin conductance levels, bearing similarity with the above-mentioned cardio-behavioral defense state. In line with that, reduced gaze dispersion significantly predicted the speed of motor reactions on a trial-wise basis, suggesting it to support action preparation under threat (Roelofs & Dayan, 2022). Merscher and colleagues (2022) corroborated these findings with slightly modified experimental designs, indicating that this gaze effect may be a threat-specific and robust response to avoidable threat that promotes fast subsequent defensive actions.

Reminded of inhibitions in somatomotor activity, the authors cautiously classified this decrease of oculomotor activity as freezing-like behaviors in humans but the relationship between the two motion metrics remained unclear. In the current study, we therefore elucidated changes in gaze and body movements as well as concomitant heart rate and skin conductance dynamics in anticipation of avoidable threat. Thereby, we reproduced the study by Rösler and

Gamer (2019) with an additional measure of posturography to assess changes in body sway. Specifically, participants stood on a stabilometric platform while awaiting an inevitable shock (shock), no shock (safety) or an avoidable shock (flight) they could avert by a quick joystick button press in response to a verbal prompt.

Based on previous studies, we expected to observe reduced oculomotor (i.e., reduced gaze dispersion, fewer and longer fixations) and somatomotor activity (i.e., reductions in postural sway), accompanied by heart rate decelerations and an increase in skin conductance in flight trials (Gladwin et al., 2016; Rösler & Gamer, 2019). In line with the notion of an action preparatory state, we further hypothesized that the presumed reductions in oculomotor activity, body sway, and heart rate during the second half of the anticipation phase would predict the speed of motor reactions on a trial-wise basis (Gladwin et al., 2016; Löw et al., 2015; Merscher et al., 2022; Rösler & Gamer, 2019). Lastly, to examine whether the expected changes in movement would reflect a broader, underlying defensive state, we explored their relationship.

Methods

Participants

Sixty adults (35 identifying as female and 25 as male) aged 18 to 35 years (*M* = 24.07 years; *SD* = 4.91 years) were recruited via an online portal of the University of Wuerzburg. Inclusion criteria were age between 18 and 35 years and no glasses. Three participants had to be excluded due to insufficient eye-tracking baseline quality detected by our data-driven exclusion criterion detailed below, two because the joystick lost its Bluetooth connection during the experiment, one due to dizziness, and four due to movement artifacts in the heart rate data. The final sample thus consisted of 50 participants (25 females and males, age *M* = 24.10 years; *SD* = 3.72 years) This sample size is consistent with previous studies using comparable experimental designs (Merscher et al., 2022; Rösler & Gamer, 2019) and yields a power greater than .95 for the detection of medium effect sizes in repeated measures analyses of variance (ANOVAs, $f = 0.25$) at a significance level of .05 under the assumption of a correlation of $r = 0.50$ between factor levels (Faul et al., 2007). Participants were screened for trait anxiety and anxiety sensitivity (Table 1). The current experiment was preregistered on aspredicted.org (https://aspredicted.org/MIP_EEL) and all scripts and data are made available on the Open Science Framework (https://osf.io/rjd8t/?view_only=ed93e41bf8b04615a23a3850d064b16e). According to the declaration of Helsinki, we received ethical approval by the ethics committee of the University of Wuerzburg.

Table 1. Characteristics of the final sample ($N = 50$).

	Participants
Age (years)	24.1 (3.72)
Gender (female/male/nonbinary)	25/25/0
STAI	38.62 (9.69)
ASI	20.54 (10.45)

Note. Values indicate *M* (*SD*) unless otherwise noted. STAI = State-Trait Anxiety Inventory, trait version (Laux et al., 1981). ASI = Anxiety Sensitivity Index (Kemper et al., 2009).

Experimental Design

The experimental design remained largely the same as in a previous study by Rösler and Gamer (2019) with the exception that participants were standing on a stabilometric platform during the task (Fig. 1). It was programmed with Presentation (Neurobehavioral Systems Inc., v., 18.1) and stimuli were depicted on a wall-mounted 27'' Lenovo ThinkVision T27h-2L screen (596.74 × 335.66 cm, 2560 x 1440 pixels, refresh rate 144 Hz). For visual stimulation we used 60 affectively neutral images showing nature sceneries (1600 x 1200 pixels, visual angle of 86.06° × 50.54° at a viewing distance of ca 65 cm) from the McGill Calibrated Colour Image Database (Olmos & Kingdom, 2004) presented in random order. Half of the pictures were horizontally mirrored to avoid any biases in eye movements that might be caused by an unintentional difference in visually appealing features on one side. Each participant accomplished three different trial types that were cued by a colored fixation cross: shock (red), safety (green), and flight (yellow). In the beginning of each trial, a white fixation cross was presented in the center of the screen (6.5-8.5 s) that changed its color to either red, green or yellow (2 s). This was followed by a presentation of a naturalistic image depicted against a grey background. After image disappearance, the trial outcome was delivered depending on the trial type. In shock trials, the screen turned blank for 1 s after which participants received a shock. Safety trials never resulted in a shock. In flight trials, participants were instructed to react to a verbal prompt that appeared after stimulus offset with a quick joystick button press to avoid aversive electrotactile stimulation. The prompt disappeared after the button press and was displayed for a maximum duration of 1 s. The response time threshold was individually adjusted such that participants had to react faster than 240 ms in the first five flight trials and, subsequently, faster than the median of their response times in the first five trials (cf. Löw et al., 2015). This procedure guarantees that participants need to respond quickly but have a realistic chance of escaping the shock. Across participants, 51.40 % of flight trials terminated with a shock (*SD* = 14.00 %), that was presented 1 s after the offset of the naturalistic image. Shocks were delivered by a Digitimer Constant Current Stimulator DS7A (Hertfordshire, United Kingdom) as a train of three 2 ms square-wave pulses with alternating polarity with 50 ms breaks in between. This stimulation is noticed as a single sensation. Participants were instructed to fixate on the fixation cross whenever it was shown but to freely explore the naturalistic images. The whole experiment consisted of 60 trials (20 for each trial type). Instead of running it in one session as in Rösler and Gamer (2019), we divided it into two blocks comprising 30 trials each to avoid circulation problems due to prolonged standing. The trial order was pseudo-randomized such that no more than three trials of the same type followed one another.

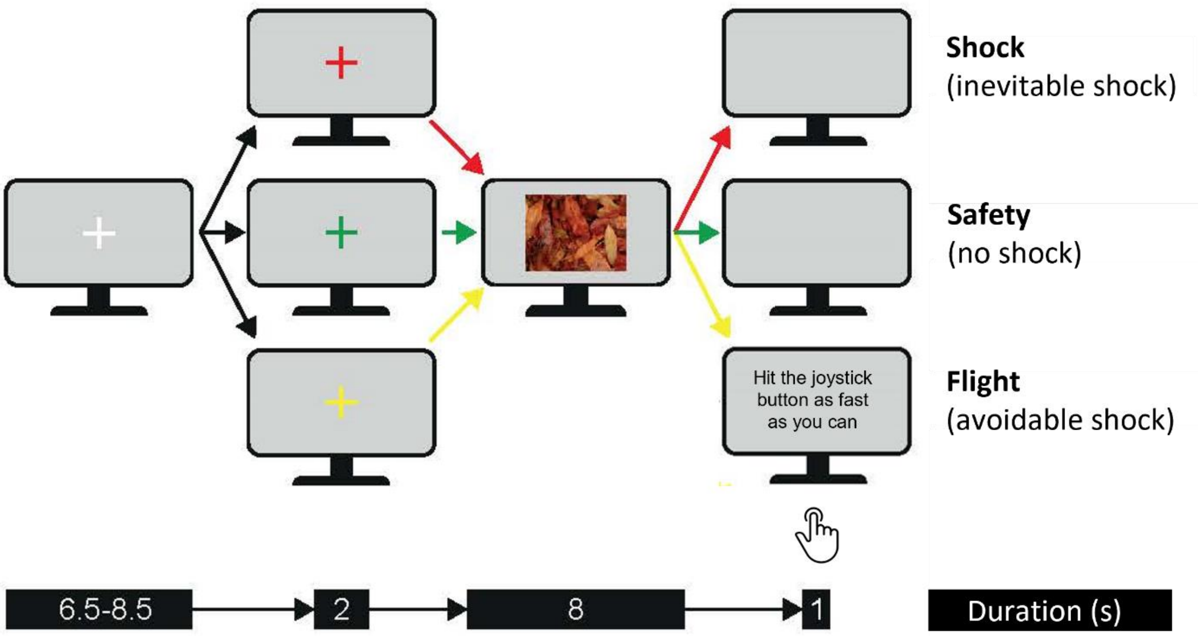

Figure 1. Illustration of the trial design based on Rösler and Gamer (2019). Participants viewed naturalistic images that were preceded by a colored fixation cross signaling them whether to expect an inevitable (red, shock trials), no (green, safety trials), or an avoidable shock (yellow, flight trials) they could prevent by pressing a joystick button as fast as possible.

Procedure

After providing written informed consent, participants were asked to complete the German versions of two questionnaires: the trait version of the State-Trait Anxiety Inventory (STAI-T; Laux et al., 1981) and the Anxiety Sensitivity Index (ASI-3; Kemper et al., 2009). With 20 items in total, the STAI-T measures each participant’s level of trait-anxiety on a 4-point Likert scale from 1 (almost never) to 4 (almost always). Sum scores range from 20 up to 80 points.

Internal consistency as assessed by Cronbach's α in the current sample was excellent (α = .91). The ASI-3 comprises 16 items assessing how sensitive to anxiety participants are on a 5-point Likert scale from 0 (very little) to 4 (very much) with sum scores ranging from 0 to 64. Cronbach's alpha for the current sample was very good (α = .88). After completing the questionnaires, subjects read instructions about which color would signal each of the three different outcomes. They were told that the naturalistic pictures appearing after the color cues were irrelevant for the task but could be freely explored. Participants were additionally instructed to fixate the center of the screen when a fixation cross was visible. Subsequently, electrodermal and electrocardiographic electrodes as well as the electrode for shock delivery were attached. Participants then stepped on a stabilometric platform and placed their feed on markers horizontally aligned around the center of the platform in a shoulder-wide comfortable stance. They were given a wireless joystick, which they held in their dominant hand. We asked them to bend their arm such that the joystick head would point towards the screen. Subjects then underwent a shock calibration procedure. In line with Rösler and Gamer (2019), they were asked to rate the shock intensity on a visual analogue scale from 0 to 10 (VAS; 0 = "Not painful at all" to 10 = "unbearably painful") with 4 marking the point where the perceived intensity turns from very unpleasant to slightly painful. Shock intensity was incremented from 0 mA in steps of 0.1 mA until reaching a rating of 4. This procedure was repeated three times. The amperages of every repetition that were rated at a VAS level of 4 were then averaged and the result amplified by 50% to counteract habituation effects during the experiment. The eye tracker was then calibrated and validated using a nine-point grid. The calibration procedure was repeated until the average deviation between fixation points during calibration and validation was smaller than 1° visual angle. Finally, the experiment was started, and each participant completed 60 trials in two blocks with a five-minute break in between the blocks. At the start of the second block, the eye-tracker was calibrated again.

Data processing

Body Sway. Our primary behavioral outcome was postural sway, which is typically used as a translational readout to indicate freezing behaviors in humans (Roelofs, 2017). To this aim, we constructed a stabilometric platform using two strain gauge load cells (PSD-S1) mounted below a wooden plate (72 x 57 cm). One cell was positioned on the right and one on the frontal side of the plate. Flexible spacers were attached to the left and rear sides. The signal of the load cells was fed into two analog input channels of a BIOPAC MP160 device and recorded with a sampling rate of 1000 Hz using Acq*Knowledge* 5.0 (Biopac Systems Inc., Goleta, CA, USA). An online low-pass filter (infinite impulse response filter) with a cutoff frequency of 1 Hz and a Q-value of 0.707 was applied to remove high frequency noise. This setup allowed us to continuously measure weight displacements of the participant along the mediolateral (ML) and anterior-posterior (AP) axis. Since previous work on freezing behaviors in humans demonstrated that movement along the AP axis is more sensitive to threat-related changes (Klaassen et al., 2021; Roelofs et al., 2010), we concentrated on this measure.

For the analysis, we calculated a second-wise standard deviation of excursions in the AP direction relative to the last second prior to cue onset, spanning 10 one-second bins including a 2s cue phase and the 8s anticipation period. For data visualization, we considered additional 5s involving the trial outcome.

Oculomotor activity. Eye movements were recorded using a video-based eye-tracking system from SensoMotoric Instruments (SMI RED250mobile; Teltow, Germany) and its corresponding software SMI iView RED250 mobile (version 4.4) at a sampling rate of 250 Hz. Raw gaze positions were parsed into fixations and saccades using an algorithm comparable to our previous studies (Merscher et al., 2022; Rösler & Gamer, 2019). In detail, eye movements exceeding a velocity of 30°/s or an acceleration greater than $8000°/s^2$ were classified as saccades. Periods of stable gaze positions between saccades were defined as fixations. To ensure that participants were fixating the centrally presented fixation cross at trial start, an iterative outlier detection algorithm was applied to exclude trials with large eye movements within a 300 ms interval before cue onset, which later served as our baseline. Specifically, and in line with previous studies (Rösler & Gamer, 2019; Merscher et al., 2022), we removed the most extreme values of data on the x- and y-axis during this interval temporarily to test whether one or both points were more than 3 standard deviations away from the average of the remaining data. Data points fulfilling this condition were permanently removed from the data set and labelled as outliers. Otherwise, they were returned to the dataset. This procedure was repeated until no more data points were labeled as outliers. Single trials with baseline outliers or missing baseline position data were excluded from all further analyses (13.8%). Similarly, trials where participants reacted prematurely or pressed the joystick button in trials that did not require a response were excluded (< .01 %). If less than 50% of the trials remained in at least one experimental condition, participants were removed entirely from the sample. This applied to three participants of the whole sample.

Gaze dispersion. Based on previous studies, gaze dispersion was calculated as the main oculomotor indicator of threat-related reductions in visual exploration (Rösler & Gamer, 2019; Merscher et al., 2022). Therefore, fixation locations were extracted for each trial and corrected for potential gaze drifts by subtracting the x- and y-values of the baseline from the fixation coordinates during picture presentation. Afterwards, we calculated second-wise averages of the Euclidian distance of fixations from the center of the screen spanning the 8 seconds of picture viewing. Fixations that started before stimulus onset or fell outside of the presentation screen were not considered and fixations spanning multiple bins were weighted according their relative duration within each individual bin.

Fixation numbers and durations. As additional visual metrics, we calculated second-wise averages of fixation numbers and durations for the 8 seconds of picture viewing. The same criteria for excluding individual fixations as described above were implemented. In detail, we first determined the number of fixations per second, considering the relative proportion in the respective bin in case of fixations spanning several bins. Afterwards, the total fixation duration per bin was calculated and divided by the respective number of fixations to yield average fixation durations.

Autonomic measures. Both autonomic measures (electrocardiogram (ECG), electrodermal activity (EDA)) were continuously recorded using a wireless BIOPAC MP160 device (BIOPAC Systems Inc., Goleta, CA, USA) at a sampling rate of 1000 Hz. For the analysis of each measure, we considered 10 seconds (plus five additional seconds for data illustration) after the onset of the colored fixation cross. The second prior to that served as baseline and was subtracted from all subsequent values on a trial-wise basis.

ECG. Disposable Ag/AgCl electrodes were attached to the right sternum as well as to the lower left and right ribcage (reference electrode). The transducer was tied around the chest of the participant. For data processing, we first removed slow signal drifts using a 2 Hz high-pass filter. Afterwards, we used a semi-automatic R-peak detection algorithm that allowed for manual edits in case of detection errors. Finally, we converted R-R periods to HR in beats per minute and calculated the weighted HR for each of 10 one-second bins (plus 5 for data illustration) using a real-time scaling procedure (Velden & Wölk, 1987).

EDA. Two Ag/AgCl electrodes were filled with .05 NaCl electrolyte paste and placed on the thenar and hypothenar eminences of each participant's non-dominant hand. Data were down-sampled to 20 Hz and subsequently averaged into 10 one-second bins (15 for data visualization).

Statistical analyses

We preprocessed and analyzed the data using R (v. 4.0.3, R Core Team, 2018) on a significance level of 5%. To test our hypotheses, we performed comparable analyses as Rösler and Gamer (2019). Specifically, we calculated rmANOVAs with trial type (shock, safety, flight) and bin (8 one-second bins for all oculomotor variables and 10 one-second bins for postural sway and all autonomic measures) as within-subject factors for each dependent variable. Degrees of freedom were adjusted according to Greenhouse-Geisser to compensate for potential violations of the sphericity assumption. To specifically compare flight trials with the other two respective trial types, post-hoc t-tests were carried out in case of significant main or interaction effects involving this factor using the false discovery rate to adjust for alpha error accumulation (FDR, Benjamini & Hochberg, 1995). Note that we slightly changed our statistical analysis with regard to our preregistration by focusing on only 10 instead of 15 bins for postural sway and both autonomic measures after cue onset. This is due to the focus on the anticipation phase and in line with our previous studies (e.g., Merscher et al., 2022).

We additionally performed three separate generalized linear mixed models with gaze dispersion, heart rate, and body sway, respectively, as predictors for reaction times in flight trials. As in Merscher and colleagues (2022), these analyses focused on the respective responses during the second half of the picture viewing period. Participant ID was inserted as random intercept. Model estimates were chosen to optimize the restricted maximum likelihood criterion and *p*-values for each predictor were calculated using Satterthwaite's approximation of degrees of freedom with the lmerTest package (version 2.0-25, Kuznetsova et al., 2017).

In addition to our preregistered analyses, we calculated between-subject Pearson correlations between the respective averages of the dependent variable during the second half of picture viewing in flight trials. Additionally, we calculated repeated measures correlations coefficients using the rmcorr package to determine the within-individual relationship among all dependent variables over the whole anticipation phase (8 s) in flight trials (version 0.5.4, Bakdash & Marusich, 2019). Participant ID and trial number were inserted as random intercepts and we used the FDR correction to account for alpha-error accumulation (Benjamini & Hochberg, 1995).

Results

Body Sway

To elucidate whether body sway in the anterior-posterior direction decreased in anticipation of avoidable threat, we calculated a 3 (trial type) × 10 (bins in seconds) repeated measures ANOVA (rmANOVA). Body Sway did not generally differ between trial types (main effect trial type: $F_{2,98}$= 2.84, $\varepsilon = 0.90$, $p = .069$, $\eta^2_g = 0.01$) but changed significantly over time (main effect bin: $F_{9,441}$= 8.91, $\varepsilon = 0.57$, $p < .001$, $\eta^2_g = 0.02$; see Figure 2A). Specifically, participants swayed less towards the end of the anticipation phase when they awaited an avoidable shock as compared to an inevitable shock or no shock (interaction effect trial type × bin: $F_{18,882}$= 2.36, $\varepsilon = 0.52$, $p = .012$, $\eta^2_g = 0.02$). Body sway during the second half of picture viewing did not predict reaction times in flight trials on a trial-wise basis ($\beta = 983.915$, $SE = 6094.82$, $t_{743.95} = .15$, $p = .878$). An explorative analysis of body sway on the mediolateral axis as well as an integrated assessment of movement on both axes is reported in the supplementary material (see supplemental Figure S1).

Oculomotor activity

A 3 (trial type) × 8 (bin in seconds) rmANOVA revealed that gaze dispersion differed between trial types (main effect trial type: $F_{2,98}$= 47.03, $\varepsilon = 0.72$, $p < .001$, $\eta^2_g = 0.14$) as well as generally over time (main effect bin: $F_{7,343}$= 42.69, $\varepsilon = 0.39$, $p < .001$, $\eta^2_g = 0.08$; see Figure 2B). When anticipating a flight opportunity, fixation dispersion narrowed towards the center of the screen over time (interaction effect trial type × bin: $F_{14,686}$= 11.14, $\varepsilon = 0.35$, $p < .001$, $\eta^2_g = 0.02$). A GLMM demonstrated that gaze dispersion during the second half of picture presentation positively predicted the speed of motor reactions when participants could avert a shock on trial-wise basis ($\beta = 0.11$, $SE = 0.04$, $t_{442.45} = 2.653$, $p = .008$).

Fixation durations and numbers also differed between trial types (main effects trial type, durations: $F_{2,98}$= 20.28, $\varepsilon = 0.57$, $p < .001$, $\eta^2_g = 0.11$, numbers: $F_{2,98}$= 20.24, $\varepsilon = 0.78$, $p < .001$, $\eta^2_g = 0.07$) and over time (main effects bin, durations: $F_{7,343}$= 63.39, $\varepsilon = 0.21$, $p < .001$, $\eta^2_g = 0.14$; Fig. 2D, numbers: $F_{7,343}$= 35.32, $\varepsilon = 0.42$, $p < .001$, $\eta^2_g = 0.07$; see Figure 2C and D). Specifically, fixations increased in duration and decreased in number over time in flight trials as compared to the other two trial types (interaction effects trial type × bin, durations: $F_{14,686}$= 16.20, $\varepsilon = 0.16$, $p < .001$, $\eta^2_g = 0.04$, numbers: $F_{14,686}$= 9.18, $\varepsilon = 0.42$, $p < .001$, $\eta^2_g = 0.03$).

Autonomic measures

A 3 (trial type) × 10 (bins in seconds) rmANOVA showed that heart rate varied between trial types (main effect trial type: $F_{2,98}= 9.75$, $\varepsilon = 0.99$, $p < .001$, $\eta^2_g = 0.05$) and over time (main effect bin: $F_{9,441}= 37.40$, $\varepsilon = 0.31$, $p < .001$, $\eta^2_g = 0.12$; see Figure 2E). In flight trials, an initial increase after picture onset, a stark decrease over the anticipation period and another increase after trial outcome was visible in contrast to the other trial types (interaction effect trial type × bin: $F_{18,882}= 26.96$, $\varepsilon = 0.28$, $p < .001$, $\eta^2_g = 0.10$). Mean heart rate during the second half of the anticipation phase significantly predicted reaction times in flight trials ($\beta = 1.40$, $SE = .64$, $t_{749.01} = 2.17$, $p = .030$).

Skin conductance levels also differed between trial types (main effect trial type: $F_{2,98}= 8.54$, $\varepsilon = 0.77$, $p = .001$, $\eta^2_g = 0.03$) and varied over time (main effect bin: $F_{9,441}= 8.53$, $\varepsilon = 0.24$, $p < .001$, $\eta^2_g = 0.03$; Figure 2F). They similarly increased in both flight and shock trials over the anticipation period (interaction effect trial type × bin: $F_{18,882}= 9.22$, $\varepsilon = 0.18$, $p < .001$, $\eta^2_g = 0.03$).

None of the dependent variables was significantly related to any of the questionnaires across participants in flight trials (see supplemental Figure S2).

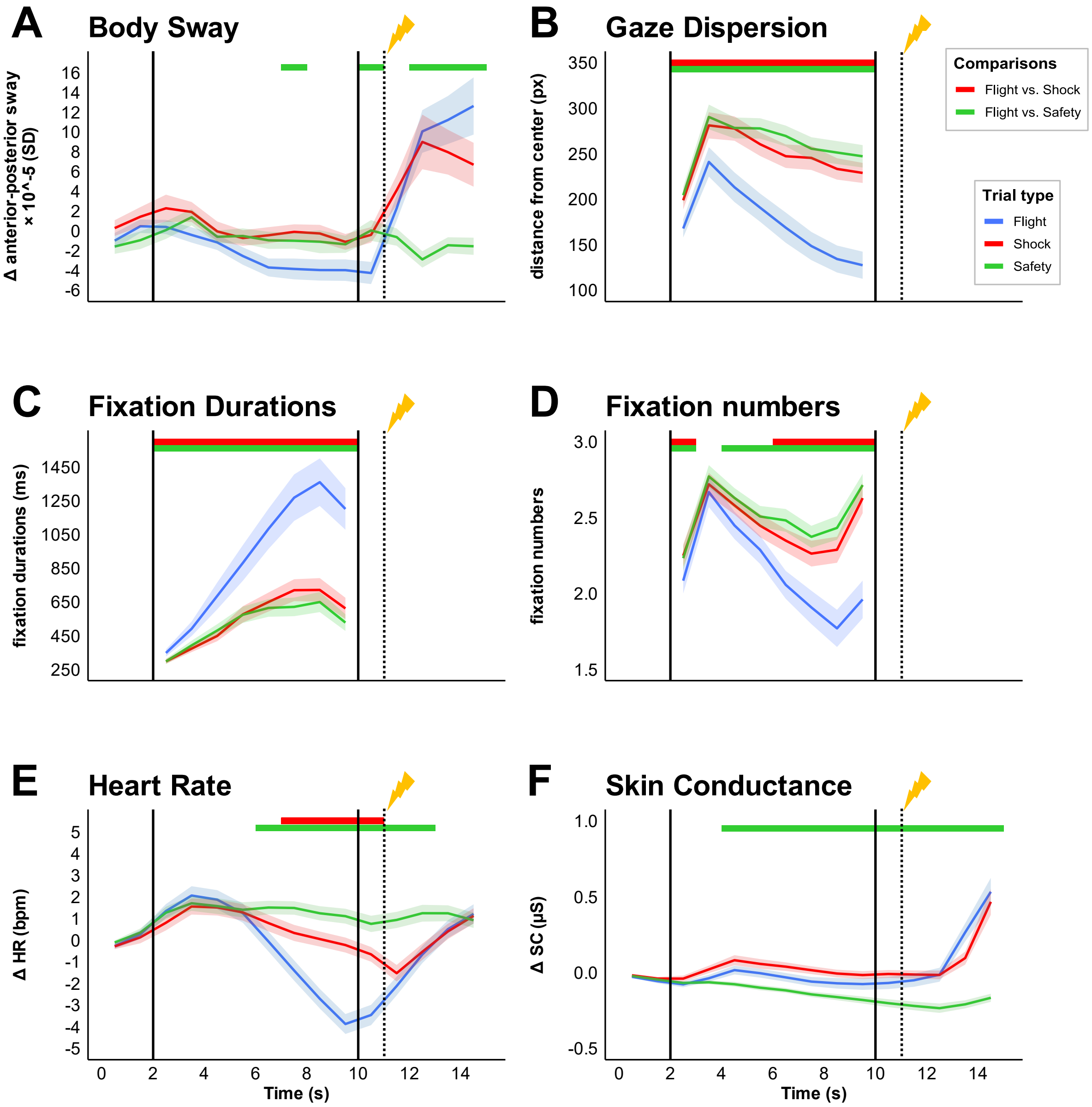

Figure 2. Temporal changes in body sway (A), gaze dispersion (B), fixation durations (C), fixation numbers (D), heart rate (E), and skin conductance (F) as a function of trial type starting at cue onset. The black vertical lines denote picture on- and offsets. The green and red horizontal bars on top of each plot indicate false-discovery rate corrected significant pair-wise comparisons between flight and shock (red) or safety trials (green), respectively. Note that average fixation durations might exceed bin size when fixations were spanning multiple bins and that body sway values are calculated to the power of ten for better visual illustration.

Relationship between (oculo)motor and autonomic measures

To test the relationship between the average values of all dependent variables during the second half of the anticipation phase, we first calculated Pearson correlations across participants. In this analysis, we failed to find significant relationships between any of the dependent variables except for that between fixation durations and numbers (Figure 3).

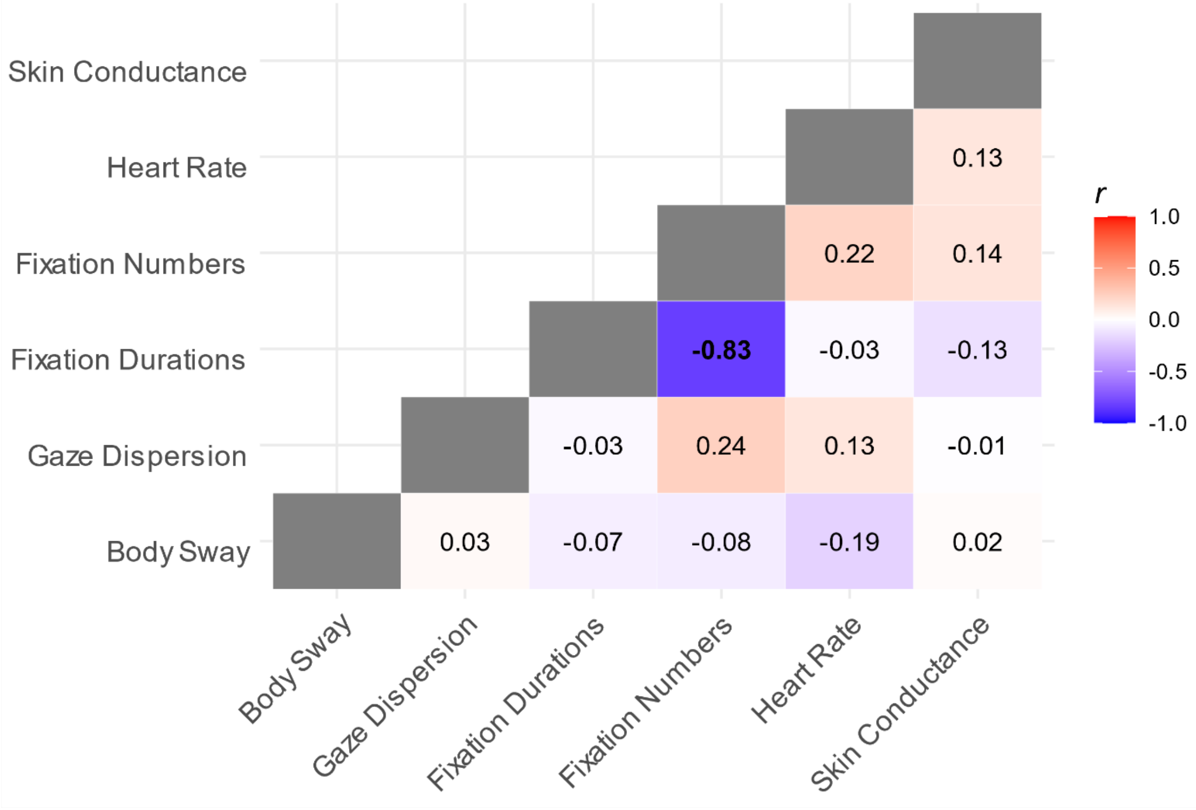

Figure 3. Pearson correlations across subjects ($N = 50$) between average values of all dependent variables during the second half of the anticipation phase in flight trials**.** Bold *r*-values denote significant relationships between two respective measures.

When considering the within-individual temporal profiles throughout the entire anticipation phase, however, we found mostly small but significant relationships between all dependent measures, respectively, except for the association between skin conductance and gaze dispersion. Importantly, the time course of gaze and body movements yielded a positive, albeit tiny correlation (*r* = .03, *p* = .001), and both movement metrics were positively associated with heart rate, respectively (body sway, *r* = .05, *p* < .001; gaze dispersion, *r* = .09, *p* < .001; Figure 4).

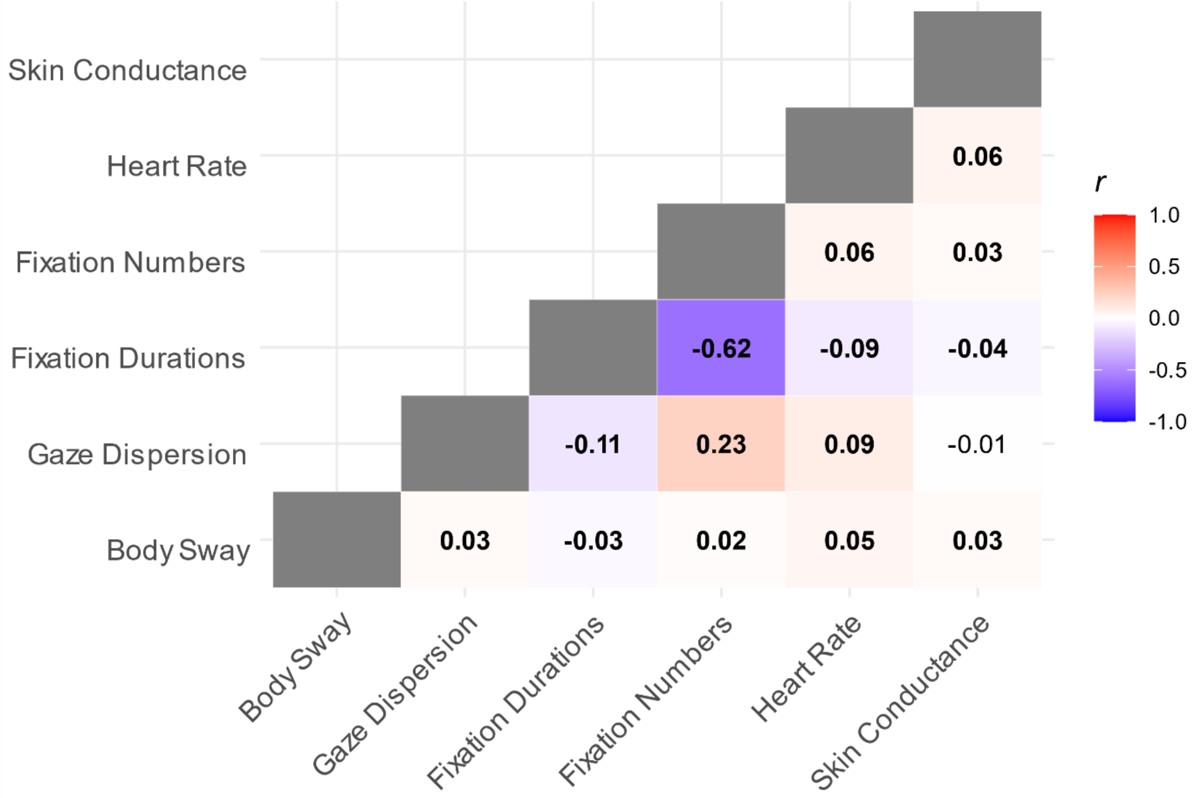

Figure 4. Within-individual relationship between the temporal profiles of all dependent variables during the entire anticipation phase in flight trials. Trial number and subject ID were inserted as random intercepts. Bold *r*-values denote significant relationships between the time course of two respective measures.

Discussion

The current study elucidated oculo(motor) and autonomic responses to avoidable threat. Of particular focus was the co-occurrence and relationship between gaze and body movements, which both have been discussed as potential markers for freezing responses in humans. In line with our hypotheses, we showed a decrease in body sway, gaze movements (denoted by less dispersed, longer, and fewer fixations), and heart rate, as well as an increase in skin conductance when participants awaited an avoidable shock (flight; vs. an inevitable (shock) or no shock (safety)). Thereby, reduced gaze dispersion and heart rate, but not body sway, predicted faster reaction times to the threat on a trial-wise basis. Body sway and gaze dispersion were not related across participants, but their temporal profiles seemed slightly, but significantly correlated during the anticipation phase in flight trials.

The findings of this study largely align with prior research, which has identified a defensive state that is characterized by movement inhibition and a simultaneous activation of the parasympathetic and sympathetic autonomic nervous systems when humans are confronted with an avoidable approaching threat (Gladwin et al., 2016; Hashemi et al., 2021; Merscher et al., 2022; Roelofs & Dayan, 2022; Rösler & Gamer, 2019; Volchan et al., 2017). Specifically, we replicated a previously shown, although only slight decrease in body sway along with bradycardia (Gladwin et al., 2016; Hashemi et al., 2019; Roelofs, 2017), an increase in skin conductance (Löw et al., 2015; Wendt et al., 2017), and a decrease in eye movements in flight trials (Merscher et al., 2022; Rösler & Gamer, 2019).

It has been demonstrated that components of this state aid in action preparation, which is seconded in our findings showing stronger reductions in gaze dispersion and heart rate to predict faster motor reactions on a trial-wise basis (Gladwin et al., 2016; Merscher et al., 2022; Rösler & Gamer, 2019). While cardiac deceleration has been proposed to constitute an action preparatory mechanism irrespective of contextual valence (Hashemi et al., 2019; Jennings et al., 2009; Merscher et al., 2022; Obrist et al., 1970), reduced gaze dispersion seems to be a threat-specific phenomenon (Merscher et al., 2022). Although reductions in body sway did not have significant predictive power for reaction times as previously shown (Hashemi et al., 2019), its unique occurrence in flight trials as compared to safety and shock trials, where no action was required, may hint at its preparatory purpose.

As reductions in gaze (Merscher et al., 2022; Rösler & Gamer, 2019) and body movements (Gladwin et al., 2016; Hashemi et al., 2021) have been observed in anticipation of avoidable threat, it has been suggested that both movement metrics may be indicative of freezing-like behavior in humans. Although we failed to find a relationship between gaze and body movements across participants, their temporal profiles were significantly related, suggesting a potentially broader, underlying defensive state. This was supported by our findings of a previously shown small but significantly positive association between the temporal profiles of both heart rate and gaze (Merscher et al., 2022) and body movements (Hashemi et al., 2021), respectively, which suggests an integrated cardio-behavioral condition. Although the correlation coefficients were small, their significance support the notion of a within-individual, integrated defensive state that has been observed in rodents (Signoret-Genest et al., 2022; Vianna & Carrive, 2005) and humans (Gladwin et al., 2016; Löw et al., 2015; Merscher et al., 2022).

However, this claim needs to be further elucidated given the limitations of the current as well as previous studies investigating freezing behaviors in humans. First, both motion measures (i.e., reduced body sway and gaze dispersion) as well as their experimental investigation lack ecological validity. Specifically, participants were instructed to stand in a stable bi-pedal stance and view two-dimensional pictures, which does neither reflect unrestricted movements and exploration in the real world, nor does it map to typical experimental conditions of animal studies, where rodents can freely explore their cage or even larger arenas (Anagnostaras et al., 2010; Blanchard & Blanchard, 1989; de Oca et al., 2007). This rather restricted laboratory setting is also reflected in the currently used stimulus material that was defined by a homogeneous distribution of pictorial features to prevent other notorious gaze dynamics to interfere (Merscher et al., 2022; Rösler & Gamer, 2019). A previous study indeed followed up on this limitation and showed that reductions of gaze dispersion robustly persisted even when more complex scenes were explored (i.e., social and threatening scenes; Merscher & Gamer, in prep). However, it remains an important question for future studies whether and how the observed gaze effects would manifest in more dynamic settings and 3-dimensional environments. This is also true for the metric of body sway, which does not consider the multidimensionality of full body movements and thus lacks comparability with the respective readout in rodents where freezing is usually indicated by a complete cessation of movements except respiration (Bolles, 1970).

Second, the methodological procedures to assess body sway lack standardization and comparability within human research. For example, human researchers inferred freezing based on changes in body sway on either the front-to-back (e.g., Niermann et al., 2017; Roelofs et al., 2010), side-to-side axis (e.g., Azevedo et al., 2005; Facchinetti et al., 2006), or both (Hagenaars et al., 2014) using different types of calculations. Some researchers computed the standard deviation of weight shifts in small time windows (e.g., Gladwin et al., 2016), while others used longer time windows and different filters (Niermann et al., 2015, 2017). In light of these methodological issues and mixed findings, it is hardly surprising that reductions in body sway during the anticipation of flight responses were relatively small in the current study and did not predict the speed of motor reactions as previously suggested (Roelofs & Dayan, 2022). Future studies should strive for the development and standardization of more rigorous methodological approaches to reliably measure defensive behaviors in humans.

Third, while both human and rodent studies have suggested that a particular defensive state consists of freezing and bradycardia, which we seconded in our findings, other autonomic patterns have also been reported to accompany freezing behaviors in rodents (i.e., tachycardia, Carrive, 2000; Iwata & LeDoux, 1988; LeDoux et al., 1984). Additionally, in both species, freezing behaviors seem to occur in different contexts (upon avoidable and inevitable threats), suggesting that this behavior may be part of distinct defensive states (Volchan et al., 2017). Indeed, it has been suggested that different types of freezing exist that can be linked to qualitatively different neuroanatomical profiles (Brandão et al., 2008). To avoid confusion and miscommunication, data-driven theoretical concepts should be developed when referring to these different defensive states (for a suggestion see Volchan et al., 2017).

Taken together, future studies should further elucidate defensive states in more ecologically valid scenarios using multiple movement and autonomic readouts that allow to disentangle their attentional, behavioral, and autonomic components. Thereby, it is crucial to ensure methodological and conceptual comparability within and between different disciplines to promote fruitful translational research on the neurocircuitry of these integrated defensive states and their pathological deviations.

Overall, the current study replicated a previously observed defensive state upon avoidable threat that promotes fast subsequent defensive actions. It is denoted by inhibitions of gaze and body motion as well as heart rate decrease and skin conductance increase. The reductions in both oculomotor and motor activity over time were positively related in the current study, suggesting them to reflect a broader, underlying construct, potentially reflecting freezing in humans. As previously suggested, the observed reductions in gaze activity as measured by eye-tracking technology may constitute threat-specific and robust components of a defensive state when threat is avoidable (Merscher et al., 2022). Although further research is necessary to investigate the relationship between somatomotor and oculomotor behaviors in more ecologically valid settings, eye-tracking technology seems to be a promising tool, among other measures, to identify fear-related neural circuits in neuroimaging studies, which should be explored in future research (Merscher et al., 2022; Rösler & Gamer, 2019).

5. Study 4:

Unraveling the (oculo)motor and autonomic patterns of defensive states in humans in an unconstrained, immersive virtual world

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Abstract

Exhibiting defensive states when facing threats is crucial for physical and mental health. Animal research has indicated that distinct defensive modes can be differentiated depending on the spatiotemporal distance of a threat. However, the dynamic nature of defensive states in humans remains poorly understood, as previous studies mainly a) employed movement-restricted experimental designs with low ecological validity, and b) assessed complex defensive behaviors with single measures. The current study thus assessed multiple behavioral (movement speed, body, head, and eye rotations), autonomic (heart rate and skin conductance), and individual (subjectively perceived dread, trait anxiety, trait aggression) defense patterns in humans in a virtual reality environment. One-hundred participants carried out a foraging task while the context increased in threat. Participants perceived more threatening contexts as more dreadful. Upon relatively distal, ambiguous threat, we observed reductions in gaze and body movements along with decreased heart rate, similar to freezing behaviors in rodents. When the threat became real, skin conductance levels reached their peak while heart rate unexpectedly decreased. We found positive cross-correlations among all movement metrics, among the autonomic measures as well as between heart rate and body, head, and gaze rotations, respectively, suggesting interrelated changes of these measures across the contexts. Heightened trait anxiety was associated with greater reductions in movement speed across all contexts. Overall, the current study provided important insights into defensive states in humans based on multiple measures in an ecologically valid design.

Introduction

Imagine walking down a deserted, eerie alley. Suddenly, you hear a cracking sound behind you. You turn around, freeze, and your senses sharpen. A hooded man emerges from the darkness. Your natural response might be to switch the sides of the road, increase your pace, and possibly even position your keys between your fingers as a weapon. Such defensive responses to real or potentially threatening situations are principally adapted to protect us from harm (Adolphs, 2013; Lang & Bradley, 2010). Failing to react with the appropriate defensive response, however, can result in physical injury, or, at worst, death, and is related to various mental health disorders (Pittig et al., 2020).

Fear and anxiety-related disorders place a large burden on those affected and, ranking among the most frequent disorders of our time, society at large (Bandelow & Michaelis, 2015). Although dysfunctional defensive reactions in response to threats are considered to contribute to the development of these disorders, their composition and overall phenomenology are poorly understood (Hashemi et al., 2021; Signoret-Genest et al., 2022). Deepening our understanding of defensive states and their pathological deviations is thus of utmost individual and societal importance. As the neural underpinnings of defensive reactions seem evolutionary well-conserved, translating animal findings to humans appears to be a promising avenue to pursue this endeavor (Blanchard, 2017; Hamm, 2020; McTeague, 2016).

Based on animal research, Fanselow and Lester's (1988) Threat Imminence Continuum Model (TICM) organizes different defensive states on a universal set of threat contexts (see Figure 1). The contexts thereby range from absent threat on one end to imminent threat on the other: During Safety, the animal believes to be in no prospect of danger and is free to engage in preferred activities such as mating and feeding. With a heightened risk of being caught by a potentially looming predator, however, it must modify its behaviors during the Pre-encounter stage. Here, a threat is not present, but it is increasingly likely to encounter one in the near future. This phase is typically accompanied by heightened alertness, vigilance, and arousal. During the Post-encounter phase, a potential threat is present - the prey has sensed the predator but not vice versa. To protect itself from harm, the prey usually freezes. This movement cessation, along with a transient decrease in heart rate, has been suggested to help in optimizing attentional and cognitive processing while being less visible to a predator. It has hence been subsumed under the term attentive immobility (Blanchard et al., 2011; Gallup & Rager, 1996; Marx et al., 2008; Mobbs et al., 2015; Roelofs, 2017; Roelofs & Dayan, 2022; Volchan et al., 2017). Should this reaction fail and the predator attack, the phase of the most imminent threat - Circa-strike - is initiated. Depending on whether escape routes are available, fight-or-flight responses may be the only chance for the prey to survive. The ultimate choice of defensive behavior is thus determined by the spatiotemporal probability of a threat as well as other potentially relevant contextual (e.g., the availability of escape routes; Blanchard & Blanchard, 1989) and individual factors (e.g., previous experiences; Maren, 2008).

Due to its compelling suitability to link defensive states to different contexts of threat, researchers have also applied this model to humans (Mobbs et al., 2007, 2009, 2010, 2020; Qi et al., 2018). Previous findings generally highlighted that, on a basal level, defensive states seem to be evolutionary well-preserved, allowing deliberate translations from animal research to humans. For example, similar changes in basal behavioral and autonomic defensive responses seem to occur in non-human and human animals: Specifically, human studies showed post-encounter threat to be accompanied by reductions in body sway, as measured by weight shifts on a stabilometric platform, and a parasympathetically-driven heart rate decrease (bradycardia) similar to attentive immobility reactions in rodents (Hashemi et al., 2021; Volchan et al., 2017). Reduced body sway (Gladwin et al., 2016), heart rate, and recently found reductions in eye movements during Post-encounter threat (Merscher et al., 2022; Rösler & Gamer, 2019) were suggested to serve as action preparatory mechanisms, enabling faster subsequent threat reactions (Roelofs, 2017). Note, though, that the relationship between these measures, particularly that between oculomotor and motor activity, remains an open question (Merscher et al., 2022). The presumed optimization of cognitive and attentional processes in rodents during this defensive state also seems evident in humans. Lojowska and colleagues (2015, 2019) showed heightened information processing of visually coarse information, presumably aiding rapid risk assessment and further defensive actions.

Similar patterns in humans and animals have also been found in the Circa-strike phase. For example, researchers demonstrated a shift from forebrain to midbrain activation between Post-encounter and Circa-strike threat in humans similar to that demonstrated in animals (Mobbs et al., 2007, 2009). This has been shown to be accompanied by a switch from parasympathetically dominated bradycardia to a sympathetically driven heart rate acceleration (manifesting in tachycardia) and fight-or-flight reactions (Gladwin et al., 2016; Roelofs, 2017).

Although these defensive responses are principally adapted to an organism's advantage, evidence points toward individual differences in defensive responding, potentially predisposing certain individuals to psychopathologies (Adenauer et al., 2010; Hashemi et al., 2021). For example, highly anxious and aggressive humans have been observed to display enhanced reductions in body sway upon approaching threat (Hashemi et al., 2021; Niermann et al., 2017). However, inconsistent findings in this regard render the relationship between individual differences and defensive responses elusive (Hashemi et al., 2021; Merscher et al., 2022; Niermann et al., 2017; Rösler & Gamer, 2019).

Overall, humans seem to show similar defensive patterns to rodents, suggesting that the TICM is a valid theoretical framework to decipher defensive states in both animals and humans. However, previous human studies mainly employed highly simplified laboratory computer experiments that may be ineffective at triggering naturalistic defensive responses (Balban et al., 2021). Specifically, participants usually watch stimuli on a screen, which reduces the immersive experience of the threat and limits movement. Furthermore, the integrated phenomenology of certain defensive behaviors, particularly those subsumed under the term attentive immobility during Post-encounter threat, is still not thoroughly understood (Hashemi et al., 2021; Merscher et al., 2022; Rösler & Gamer, 2019), which may partly stem from simplified assessments of complex defensive behaviors based on single measures (e.g., reduced body sway as an index for freezing behaviors in humans; Roelofs, 2017).

Scientists have therefore called for a more rigorous measurement and integration of different measures into broader defense patterns (Signoret-Genest et al., 2022) and a more ethological approach to studying defensive states (Mobbs et al., 2018). One novel development in psychological research is the use of virtual reality (VR) technology (McCall & Blascovich, 2009). VR enables what has been called a “virtual ethology” (McCall et al., 2016): Combining high ecological validity with high experimental control in the same study, VR seems to be a well-suited method to measure and differentiate integrated defensive states and their individual components in humans.

In the current study, we therefore used virtual reality to simulate the stages of the TICM (Safety, Pre-encounter, Post-encounter, Circa-strike). As an additional phase, we inserted an ‘Approach’ phase between the Post-encounter and Circa-threat stage to mimic a still distal but approaching threat. Threat levels were manipulated such that they increased continually throughout all stages, with a hooded man appearing during Post-encounter threat. The man ran towards the participants (Approach) and either attacked them during Circa-strike or veered off (canceled Circa-strike). While having to protect themselves from potential ‘harm’ (i.e., white noise and a loss of points), participants were carrying out a foraging task. Thereby, we aimed at simulating essential non-defensive behaviors in the wild that are similarly relevant for survival as defensive reactions (Mobbs et al., 2020). Specifically, participants were instructed to collect as many cherries as possible spread across an open field, which were counted as points.

Based on previous literature, we expected

i) Signs of attentive immobility during the Post-encounter phase. Specifically, we expected reduced body movements (speed, body and head rotations), eye movements, heart rate and an increase in skin conductance levels (Gladwin et al., 2016; Hashemi et al., 2021; Merscher et al., 2022; Rösler & Gamer, 2019).

ii) Circa-strike threat (i.e., real attacks) to evoke the highest sympathetic activity (i.e., increase in skin conductance levels and heart rate) as compared to all less imminent threat contexts (Lojowska et al., 2015; Löw et al., 2015; McCall et al., 2016; Rösler & Gamer, 2019).

We further explored

iii) whether and how the different measures would be related by calculating cross-correlations between them. We were especially interested in the relationship between subjective dread ratings and all behavioral and autonomic measures.

iv) the relationship between interindividual differences (perceived dread, trait anxiety, and trait aggression) and signs of attentive immobility during Post-encounter threat (decrease in body and eye movements and heart rate).

Note that we slightly modified our hypotheses with regard to our preregistration (https://doi.org/10.17605/OSF.IO/G8AUR). Specifically, we looked at rotations of the eyeball instead of saccadic amplitudes and fixation numbers as an indicator for eye movements, because this measure is closer to the gaze inhibition we were interested in. Additionally, instead of examining second-wise temporal profiles of our dependent variables, we used phase-wise means.

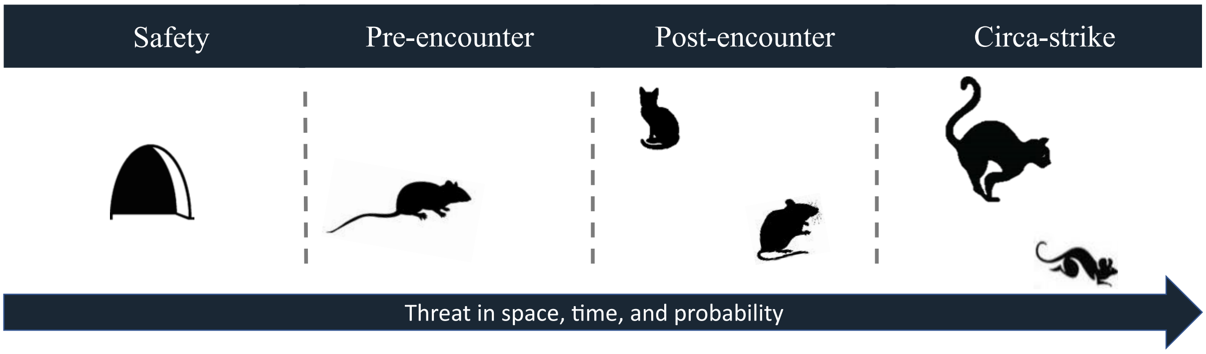

Figure 1. Threat Imminence Continuum Model (Fanselow & Lester, 1988; Mobbs et al., 2020).

Methods

Participants

Based on our preregistration, we collected data from 115 participants, nine of whom were pilot participants. We further excluded six additional participants for whom we had technical problems during data acquisition or who aborted due to motion sickness. The final sample consisted thus of $N = 100$ who were characterized based on age, gender, their trait anxiety, trait aggression, and previous experience with VR and gaming (Table 1). An a-priori power simulation using a linear mixed model resulted in a power of 90% to find a small effect (ES = 0.1) in the contrast between the safety phase and post-encounter/approach with $N = 100$. Participants, mainly students, were recruited via an online portal hosted by the University of Wuerzburg. Subjects could choose whether they wanted to receive course credits or be reimbursed with 10€ per hour for their participation. Exclusion criteria were epilepsy, pregnancy, constraints in physical fitness (incl. weight over 100 KG), susceptibility for motion sickness, as well as uncorrected defective vision. Participants had to be between 18 and 50 years old. Note that information of four participants about gaming and VR experience is missing. All participants provided written informed consent according to the ethical declaration of Helsinki and approved by the ethics committee of the University of Würzburg. The study was formally preregistered on the Open Science Framework, which, along with data and materials, can be retrieved from https://osf.io/e4gav/.

Table 1. Characteristics of the final sample (*N* = 102).

	Participants
Age (years)	24.4 (5.3)
Gender % (female/male/nonbinary)	75/25/0
STAI	44.38 (8.69)
STAXI-2	17.31 (3.90)
VR experience (*n* = 99)	2.27 (0.88)
Gaming experience (*n* = 99)	1.88 (1.15)

Note. Values indicate *M* (*SD*) unless otherwise noted. STAI = State-Trait Anxiety Inventory, trait version (Laux et al., 1981). STAXI-2 = State-Trait Anger Expression Inventory, trait version (Rohrmann et al., 2013).

Procedure

The study was conducted at the Virtual Reality Lab at the Institute of Psychology of the University of Würzburg. Before the participants came to the lab, they filled in an informed consent form and questionnaires online. Participants completed the demographic questions as well as the trait scale of the German versions of the State-Trait Anxiety Inventory (STAI-T; Laux et al., 1981) and the State-Trait Anger Expression Inventory (STAXI-2; Rohrmann et al., 2013) online. STAI-T comprises 20 items asking participants to rate their regular level of anxiety on a 4-point Likert scale from 1 (almost never) to 4 (almost always). Reaching from 20 up to 90 points, higher global scores indicate higher trait anxiety. The inventory has been reported to have good psychometric properties (internal consistency: α = .90; Laux et al., 1981), which we could replicate in our sample (α = .90). The trait scale of the STAXI-2 consists of 10 items on which respondents judge their general degree of aggressiveness from 1 (almost never) to 4 (almost always). Thereby, higher scores indicate higher trait aggressiveness. Internal consistency of this test has been shown to be acceptable up to excellent (α = .79 - .91, Rohrmann et al., 2013, and α = .72 in our sample).

In the lab, participants again read and signed an information sheet. Subsequently, electrodes for the physiological recordings were attached and a 2-minute baseline of heart rate and skin conductance was acquired. Afterward, we helped the participants into the VR treadmill, put on the HMD, and started a brief walking training in the virtual environment using the treadmill. We began the experiment when the eye tracker was successfully calibrated, which was conducted according to the manufacturer's calibration protocol. Participants were instructed to look at blue dots that were sequentially shown at the center, right, left, upper and lower side of the panel.

After finishing the experiment, subjective dread levels during the main task were assessed using an adapted version of the post-scan questionnaire created by Mobbs and colleagues (2009). The questionnaire asks participants to rate how anxious they felt during the different phases on a 10-points likert scale. As a reference point for their ratings, they were given three screenshots from each phase indicating the different levels of threat imminence according to the TICM (supplemental Figure S1).

Experimental Design

The VR environment was a wide, open field of approximately 50*50m with big cherries distributed across the lawn (Figure 2). Representing a non-defensive survival-relevant task in the wild (i.e., foraging), participants were asked to collect as many cherries as possible by walking over them. Feedback about the current number of collected cherries was given using a counter in the upper right top of the subject’s field of view. While participants walked around and collected the cherries, the environment changed. The participants started in the Safety phase which was accompanied by a bright and summery atmosphere (clear sky, sunshine, and the sound of birds). After a few seconds, the sky turned dark evoking an eery uncertain atmosphere accentuated with scary sounds such as thunder, representing the Pre-encounter phase. The Post-encounter phase was initiated when a hooded man appeared somewhere in the relatively distant surroundings of the participant. The appearance was accompanied by a muffling grunt. During the Approach stage, which we added to mimic a still relatively distal but approaching threat as a continuous transition between Post-encounter and Circa-strike, the man started running toward the participant. Here, it was still unclear whether he was dangerous. Subjects were pre-instructed that he was harmless as long as he did not carry a weapon. He then either veered off (canceled Circa-strike) or pulled out a baseball stick as a weapon, which signaled that he would attack (Circa-strike). During an attack, the opponent further approached the participants and, upon contact, started beating them, which was accompanied by a mildly aversive auditory stimulation (white noise) and a loss of points worth one cherry. The participants were thereby not helplessly exposed to the attacker but equipped with a weapon to shoot him. They could defy him and avoid negative repercussions 80% of the time when accurately targeting him as soon as he pulled out his baseball stick. When the counterattack was successful (61.23%), the opponent collapsed to the ground for which the participant was rewarded a point worth one cherry. If the participants missed the opponent (12.66 %), the shot was not counted as successful

(16.77%), or they pulled the trigger while it was still uncertain whether the attacker would attack (i.e., during Approach or canceled Circa-strike, 9.35%) they were punished with a point loss worth one cherry and white noise (see supplemental Table S1 for an overview of shots fired).

The entire experiment consisted of 15 regular trials. Each regular trial thereby encompassed all phases of the TICM played in the order of increasing threat-imminence. The duration of each phase thereby lasted approximately 10 seconds (randomly varying between 8s and 12s), except for the approach phase which lasted between 5 and 8 seconds. To counteract expectancy effects, we added four anti-habituation trials that followed a different sequence and duration of events than regular trials. Additionally, we randomly varied between real and canceled attacks so that either occurred in 50% of all regular trials.

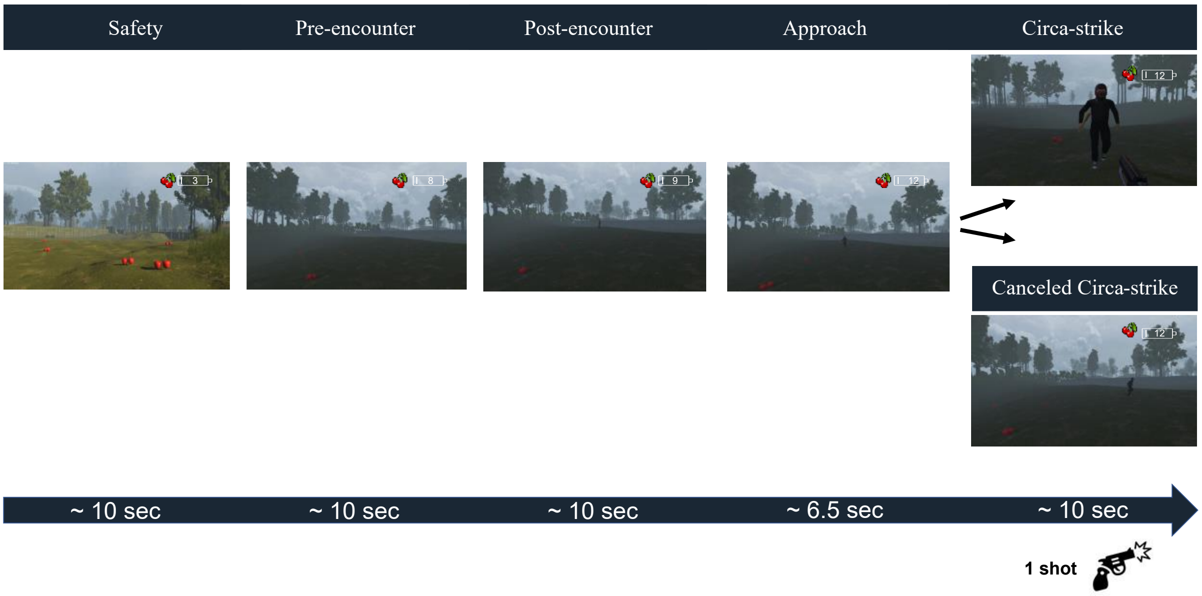

Figure 2. Illustration of the experimental design. The design was developed based on the Threat Imminence Continuum Model by Fanselow and Lester (1988) but comprised an additional phase between Post-encounter and Circa-strike to signal still distal but approaching threat (Approach). While carrying out a foraging task (i.e., collecting cherries distributed across the lawn), participants experienced a Safety, Pre-encounter, Post-encounter, and Approach context that either resulted in a Circa-strike attack or a canceled attack (canceled Circa-strike). The safety context was thereby characterized by a summery and bright atmosphere, which turned to an eerie ambiance in the Pre-encounter phase. Danger could now lurk at any moment. A hooded man entered the scene audible by a grunting noise in the distance initiating the Post-encounter context. He ran towards the participant during the Approach phase and then either turned away (canceled Circa-strike) or pulled a baseball stick to set out for an attack (Circa-strike). An attack was visually represented by the hooded man further approaching and, when close enough, beating the participant with his baseball stick. This went along with a point loss and a mildly aversive stimulation (white noise). Participants could fight him by accurately shooting him with a gun they were equipped with throughout the entire experiment. If they succeeded, they received points worth one cherry and the attacker collapsed to the ground. If they failed or pulled the trigger while it was still unclear whether the man would attack, however, they were also punished with point loss and white noise. 15 regular trials followed the sequence of increasing threat while four anti-habituation trials had a different phase sequence to counteract expectancy effects. 50% of all regular trials thereby ended in a real attack (Circa-strike).

Apparatus and stimulus presentation

The immersive virtual reality environment was presented using an HTC Vive Pro-Eye (HTC Corporation, Taoyuan, Taiwan) head-mounted display (HMD). The VR goggles included two OLED displays with an integrated eye tracker assessing eye movements of each eye (SensoMotoric Instruments; 3.5" screen diagonal, 1440*1600 pixel resolution, 90 Hz frame rate). Body movements and head rotation were measured using the “camera” in the virtual environment, which represented the participants’ head position and rotation. The HTC system also included a gyroscopic controller which was used to intuitively control a virtual gun within the experimental scenario. To enable subjects to move around in the virtual environment regardless of the spatial limitations of the real laboratory environment, we used an omnidirectional treadmill (Virtualizer ELITE 2, Cyberith GmbH, Vienna, Austria). The device allows a 360° rotation around the vertical axis and thus makes a virtual movement in any desired direction possible. A harness secures the user from falling while the walking motion is simulated by sliding over a smooth surface with the feet. To facilitate this, the platform tilts underneath the user in the direction of walking. Sensors in the platform capture the feet moving and translate it to the position of the participant’s avatar in virtual reality, allowing them to derive their movement speed (90 Hz). The virtual environment itself was created and developed using the game engine Unity3D (Unity Technologies, San Francisco, USA). The attacker's avatar was taken from the Autodesk Character Generator (Autodesk Inc., California, USA). Its behavior was programmed in Unity 3D using the C# programming language (Microsoft, New Mexico, USA). The entire experimental procedure and the recording of behavioral, and eye-tracking measures were also controlled using C# scripts within the game engine. Heart rate and skin conductance were continuously measured using a wireless Biopac BioNomadix system (BIOPAC Systems Inc., Santa Barbara, California) at a sampling rate of 2000 Hz. For heart rate, disposable Ag/AgC1 electrodes were placed on the right clavicle, and the lower left, and right rib cage. Skin conductance was measured using two Ag/AgC1 electrodes filled with 0.05 ml NaCl electrolyte placed on the thenar and hypothenar eminences of the non-dominant hand.

Data recording and preprocessing

Bodily Movements. As primary behavioral indicators for attentive immobility in humans, we looked at three movement metrics: movement speed, body rotation around the vertical axis (i.e., turning around), and 3-dimensional head rotation recorded throughout the entire experiment. Movement speed and head rotation were derived from the position of the participant in the virtual world, and body rotation from the direction on the treadmill. All three metrics were downsampled to 10 Hz. For movement speed, we used the 3D coordinates per sample and calculated the displacement using the Euclidean distance between these coordinates. For head rotation, we used the “straight gaze vector” (a vector perpendicular to the face) and calculated the angle of this vector between subsequent samples. Body rotation was preprocessed similarly: We took the rotation around the vertical axis and calculated the difference in direction between two samples. We averaged the data into phase-wise means.

Eye Movements. As our oculomotor readout, we focused on the rotation of the eyeball. The rotation angles were calculated using the Euclidean distance between three-dimensional unit vectors at a sampling rate of 90 Hz. To down-sample to 10Hz, the distances between samples were summed within 100 msec bins. Similar to bodily movement, we calculated means per phase of the TICM.

Skin conductance (SC). Raw SC data were used as representing skin conductance levels across phases. These data were z-transformed and baseline corrected by subtracting the first sample per trial from the remaining trial data. Subsequently, the data were averaged into means per phase of the TICM.

Heart rate (HR). A semi-automatic algorithm was used to detect R-peaks, which was supplemented by manual edits in case of detection errors. Periods between R-peaks were considered and transformed into HR in beats per minute. For statistical testing and data illustration, we used grand averages per phase. Similar to skin conductance, the first sample of the safety phase served as a trial-wise baseline, which was subtracted from all further HR data recorded during the trial.

Statistical Analysis

Data management and analyses were performed using R (Version 3.3.3, Core Team, 2018) with significance levels of 5%.

For each dependent variable (movement speed, body, head, and eye rotation, skin conductance, and heart rate) as well as subjective dread ratings to check our manipulation, we compared mean values of the four contexts of the TICM using linear mixed models (LMM). The six stages of the TICM (Safety, Pre-encounter, Post-encounter, Approach, Circa-Strike, Canceled Circa-Strike) were inserted as predictors. Random intercepts were fit per subject ID. Assumptions of LMM were met (checked using the performance package; Lüdecke et al., 2021) and planned contrasts between the stages of the TICM were calculated. Specifically, we fit the models using the lme4 package (Bates et al., 2015).

In addition, we calculated cross-correlations (at lag 0) between the different measures across phases for each participant and averaged those across participants. This analysis allowed us to test whether the pattern across phases is similar for the different measures, i.e. how the measures relate to each other. To assess whether these cross-correlations are significant, we performed a permutation analysis: We shuffled the order of the phases randomly, removing any relationship, and calculated the cross-correlation between two measures. We repeated this 1000 times to get a null distribution; the number of permuted cross-correlations that is as or more extreme than the true cross-correlation then determines the significance. Note that for this analysis, the canceled Circa-strike phase followed on the Circa-strike phase in our statistical model (for a visualization see Figure S1 in the Supplementary Material).

We also calculated the correlations between the dependent measures per phase and subjective dread ratings. These analyses ignore the temporal pattern within participants but indicate whether those participants who indicate a specific phase as particularly dreadful also tend to be the ones that score high on a different measure in that phase.

Finally, to examine the effect of trait anxiety and trait aggression on the dependent variables, especially movement speed, we included them and their interactions with phase in separate LMMs.

Results

Manipulation check

The experimental manipulation of increasing threat levels throughout the phases of the TICM was reflected in subjective dread ratings. As intended, they increased throughout the phases of the TICM up until Circa-Strike ($F_{(5,\ 506.92)}$ =153.75, $p < .001$, $R^2_{\text{conditional}} = .76$, $R^2_{\text{marginal}} = .30$; effect size of phase: $\eta^2_P = 0.60$), with significant contrasts between subsequent phases (Figure 3 and Table 2). Only when the opponent veered off after approaching (i.e., canceled circa-strike), dread ratings decreased in contrast to the Approach phase.

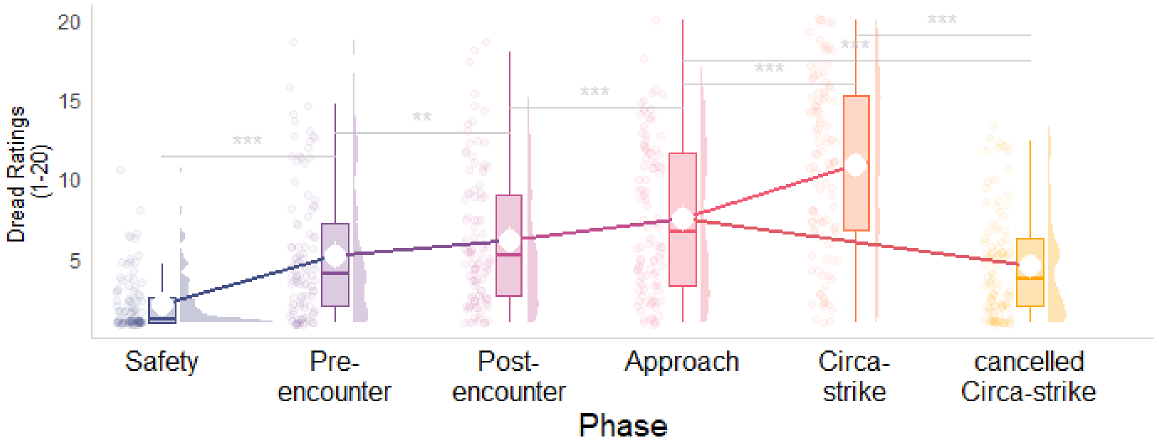

Figure 3. Phase averages of dread ratings throughout the simulated phases of the TICM.

Table 2: Custom contrasts between subsequent TICM phases for subjectively perceived dread levels and all (oculo)motor and autonomic dependent variables.

Dependent variable	**contrast**	**β**	***SE***	***df***	***t***	***p***
Perceived dread	Safety vs. Pre-Encounter	3.09	0.34	507.00	9.14	<.001
	Pre-Encounter vs. Post-Encounter	0.97	0.34	507.00	2.88	<.001
	Post-Encounter vs. Approach	1.36	0.34	507.00	4.01	<.001
	Approach vs. Circa-Strike	3.43	0.34	507.00	9.85	<.001
	Approach vs. Canceled Circa-Strike	-3.01	0.34	507.00	-8.91	<.001
	Circa-Strike vs. Canceled Circa-Strike	-6.34	0.34	507.00	-18.75	<.001
Movement Speed	Safety vs. Pre-Encounter	0.06	0.04	512.00	1.58	.114
	Pre-Encounter vs. Post-Encounter	-0.41	0.04	512.00	-11.19	<.001
	Post-Encounter vs. Approach	-0.23	0.04	512.15	-6.22	<.001
	Approach vs. Circa-Strike	0.02	0.04	512.00	0.42	.675
	Approach vs. Canceled Circa-Strike	0.17	0.04	512.00	4.50	<.001
	Circa-Strike vs. Canceled Circa-Strike	0.15	0.04	512.00	4.08	<.001
Body Rotations	Safety vs. Pre-Encounter	0.90	1.36	512.02	0.66	.508
	Pre-Encounter vs. Post-Encounter	6.89	1.36	512.02	5.06	<.001
	Post-Encounter vs. Approach	-13.95	1.37	512.38	-10.20	<.001
	Approach vs. Circa-Strike	2.13	1.37	512.02	1.56	.119

Table 2 continued

	Approach vs. Canceled Circa-Strike	6.50	1.37	512.02	4.74	<.001
	Circa-Strike vs. Canceled Circa-Strike	4.36	1.37	512.02	3.19	.002
Head Rotations	Safety vs. Pre-Encounter	19.72	8.41	512.00	2.34	.019
	Pre-Encounter vs. Post-Encounter	30.97	8.41	512.00	3.68	<.001
	Post-Encounter vs. Approach	-103.03	8.43	512.14	-12.22	<.001
	Approach vs. Circa-Strike	-2.56	8.45	512.00	-0.30	.762
	Approach vs. Canceled Circa-Strike	48.53	8.45	512.00	5.69	<.001
	Circa-Strike vs. Canceled Circa-Strike	50.62	8.45	512.00	5.99	<.001
Eye Rotations	Safety vs. Pre-Encounter	0.08	0.03	512.00	2.47	.014
	Pre-Encounter vs. Post-Encounter	0.12	0.03	512.00	3.52	<.001
	Post-Encounter vs. Approach	-0.73	0.03	512.05	-21.99	<.001
	Approach vs. Circa-Strike	0.29	0.03	512.00	8.61	<.001
	Approach vs. Canceled Circa-Strike	0.38	0.03	512.00	11.34	<.001
	Circa-Strike vs. Canceled Circa-Strike	0.09	0.02	512.00	2.73	.006

Table 2 continued

Heart Rate	Safety vs. Pre-Encounter	0.18	0.04	500.00	4.15	<.001
	Pre-Encounter vs. Post-Encounter	0.04	0.04	500.00	-0.94	.350
	Post-Encounter vs. Approach	-0.09	0.04	500.00	-1.97	.050
	Approach vs. Circa-Strike	-0.13	0.04	500.00	-3.06	.002
	Approach vs. Canceled Circa-Strike	-0.10	0.04	500.00	-2.36	.024
	Circa-Strike vs. Canceled Circa-Strike	0.03	0.04	500.00	0.80	.426
Skin Conductance	Safety vs. Pre-Encounter	0.02	0.04	505.00	0.61	.543
	Pre-Encounter vs. Post-Encounter	0.06	0.04	505.00	1.75	.080
	Post-Encounter vs. Approach	-0.02	0.04	505.00	-0.70	.485
	Approach vs. Circa-Strike	0.09	0.04	505.00	2.68	.008
	Approach vs. Canceled Circa-Strike	-0.01	0.04	505.00	-0.26	.793
	Circa-Strike vs. Canceled Circa-Strike	-0.10	0.04	505.00	-2.94	.003

(Oculo)motor measures

Movement speed significantly changed with increasing threat imminence (Figure 4A, $F_{(5,\ 511.77)} = 113.25$; $p < .001$, $R^2_{\text{conditional}} = .81$, $R^2_{\text{marginal}} = .18$; effect size of phase: $\eta^2_p = 0.53$). While participants became slightly but only descriptively faster from Safety to Pre-encounter they significantly slowed down towards Post-encounter to become even slower in the Approach phase (for all statistical parameters on contrasts between subsequent phases see Table 2). When the opponent attacked (Circa-strike), movement speed did not differ significantly compared to the Approach phase. When the attacker turned away (canceled Circa-strike), on the other hand, participants started to move faster again.

Body, head, and gaze rotations all yielded a similar phase profile that only partially resembled that of movement speed. All rotation metrics changed significantly throughout the phases of the TICM (Figure 4B, body: $F_{(5,511)} = 24.73$; $p < .001$, $R^2_{\text{conditional}} = .54$, $R^2_{\text{marginal}} = .09$, effect size of phase: $\eta^2_p = 0.19$; Figure 4C, head: $F_{(5,\ 511.69)} = 47.43$; $p < .001$, $R^2_{\text{conditional}} = .80$, $R^2_{\text{marginal}} = .08$, effect size of phase: $\eta^2_p = 0.32$; Figure 4D, eyes: $F_{(5,\ 511.96)} = 123.81$; $p < .001$, $R^2_{\text{conditional}} = .91$, $R^2_{\text{marginal}} = .09$, effect size of phase: $\eta^2_p = 0.55$), showing a slight increase from Safety to Pre-encounter (for all statistical parameters on contrasts between subsequent phases see Table 2). In contrast to the observed reductions in movement speed, however, rotations further increased from Pre- to Post-encounter threat. Only from Post-encounter to Approach, all forms of rotations sharply decreased. Rotations remained similarly low when the attacker set out to attack but increased again when he turned away. Eye rotations, in contrast, increased when the opponent attacked and also when he veered off.

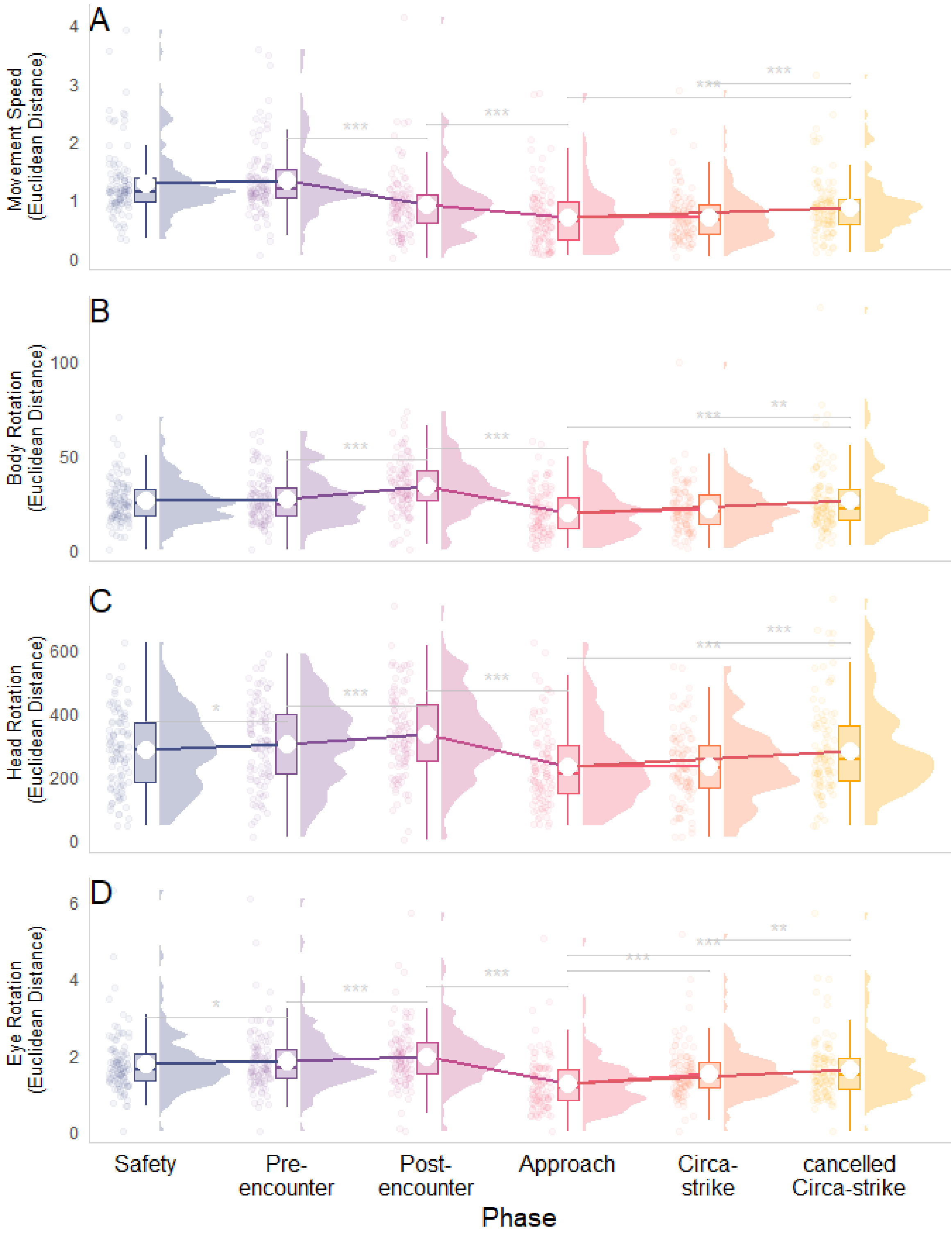

Figure 4. Phase averages of the movement-related dependent variables throughout the simulated phases of the TICM (A: Movement Speed, B: Body rotation, C: Head rotation, D: Eye rotation).

Autonomic measures

Heart rate changes were significantly predicted by the phases of the TICM (Figure 5A; $F_{(5,\,500)} = 9.68$; $p < .001$, $R^2_{\text{conditional}} = .36$, $R^2_{\text{marginal}} = .05$; effect size of phase: $\eta^2_p = 0.09$). HR first increased from Safety to the Pre-encounter phase, remained on a similar level during Post-encounter and slightly decreased towards Approach, which became marginally significant (for all statistical parameters on contrasts between subsequent phases see supplemental Table 2). It then decreased even stronger from Approach to Circa-strike regardless of an actual attack or no attack.

Skin conductance levels also changed significantly throughout the simulated phases of the TICM (Figure 5B; $F_{(5,\,505)} = 4.64$; $p < .001$, $R^2_{\text{conditional}} = .31$, $R^2_{\text{marginal}} = .02$; effect size of phase: $\eta^2_{\text{p}} = 0.04$). Comparing the sequential phases did not lead to significant differences between phases; however, overall there seemed to be an increase in SCL from Safety to Circa-strike (contrast Safety vs Circa-strike: $\beta = 0.21$, $SE = 0.04$, $t_{(540)} = 5.33$, $p < .001$; for all statistical parameters on contrasts between subsequent phases see supplemental Table 2).

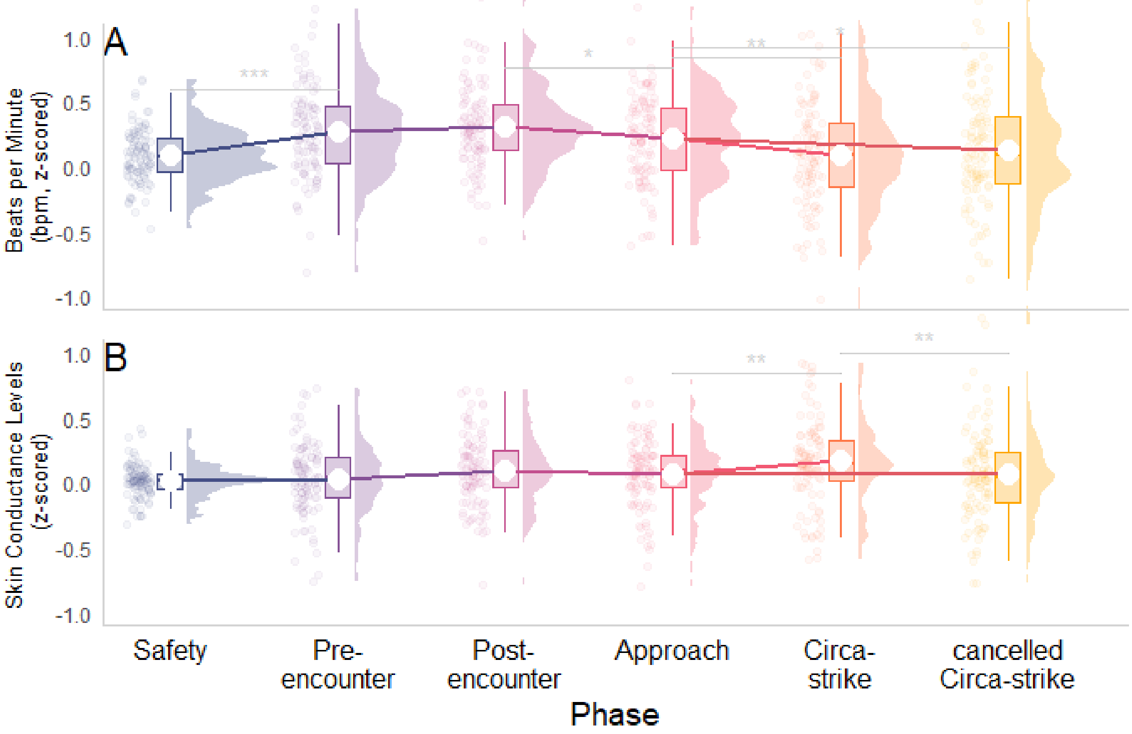

Figure 5. Phase averages of the autonomic measures throughout the simulated phases of the TICM (A: Heart Rate, B: Skin Conductance).

Relationship between (oculo)motor, autonomic, and subjective measures

In addition, we calculated pairwise cross-correlations between all dependent variables as well as subjective dread ratings across the phases of the TICM (Figure 6). This analysis allowed us to investigate whether the pattern over time is similar across variables within participants, i.e. whether the different measures increase or decrease together. The temporal profiles of all movement measures (movement speed, body, head, and eye rotation) were positively correlated. Heart rate changes throughout the course of the simulated TICM also yielded a small but significant positive relationship with all movement metrics except for movement speed. Skin conductance, in contrast, was negatively and only marginally correlated with movement speed and head rotation but not with the other movement indexes (body and gaze rotation).

Lastly, subjective dread ratings showed significant relationships with the time course of each dependent variable. Specifically, dread ratings were negatively correlated with all (oculo)motor measures and positively related to both autonomic measures.

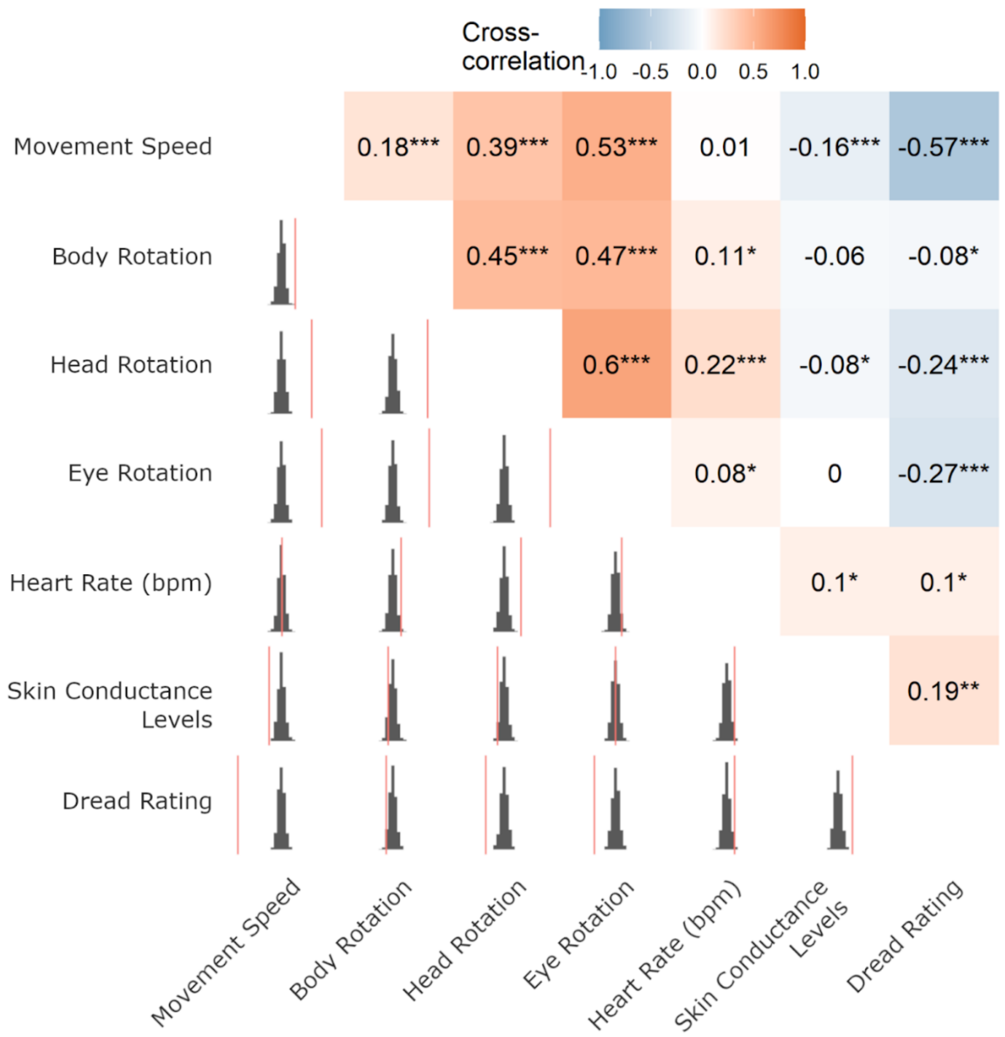

Figure 6. Pairwise Cross-Correlations between all dependent variables across the phases of the TICM. Upper triangle: Mean cross-correlation coefficients across participants, significance determined by permutation testing; lower triangle: Visualization of permutations tests, red line is average cross-correlation in the data, black histogram is the null distribution determined by 1000 permutations of the phase order. *Note*: $*p \leq .05$; $**p \leq .01$; $***p \leq .001$.

Individual differences

When examining the relationship between perceived dread and the dependent variables per TICM phase, only Post-encounter threat revealed a significant correlation between movement speed and dread ratings. The more participants subjectively perceived dread during Post-encounter the less they moved (Figure 7).

Figure 7. Pearson correlations between phase averages of all dependent variables and mean dread ratings per phase of the TICM.

Trait anxiety and aggression

Trait anxiety predicted differences in movement speed over the course of the TICM (Figure 8; interaction phase × trait anxiety, $F_{(5,486.71)} = 2.67$, $p = .021$). While the progression of movement speed across phases was similar regardless of trait anxiety scores, individuals scoring high in trait anxiety generally moved less than those with low trait anxiety scores. This difference was significant for the Safety and Pre-encounter phases. Otherwise, linear mixed models did not reveal a significant relationship between trait anxiety and any of the other movement measures throughout the phases of the TICM (see supplemental Table S2).

Trait aggression did not significantly relate to any of the dependent variables (supplemental Table S2) except for eye rotations (Figure 9, interaction phase × trait aggression: $F_{(5,486.95)} = 3.77$, $p = .002$). Thereby, the difference in eye rotations between participants scoring high vs. low on trait aggression only descriptively manifested in the Safety towards the Post-encounter phases, although none of the simple slopes within phases reached significance. Specifically, more aggressive participants showed a small tendency to move their eyes less during these early phases than less aggressive participants.

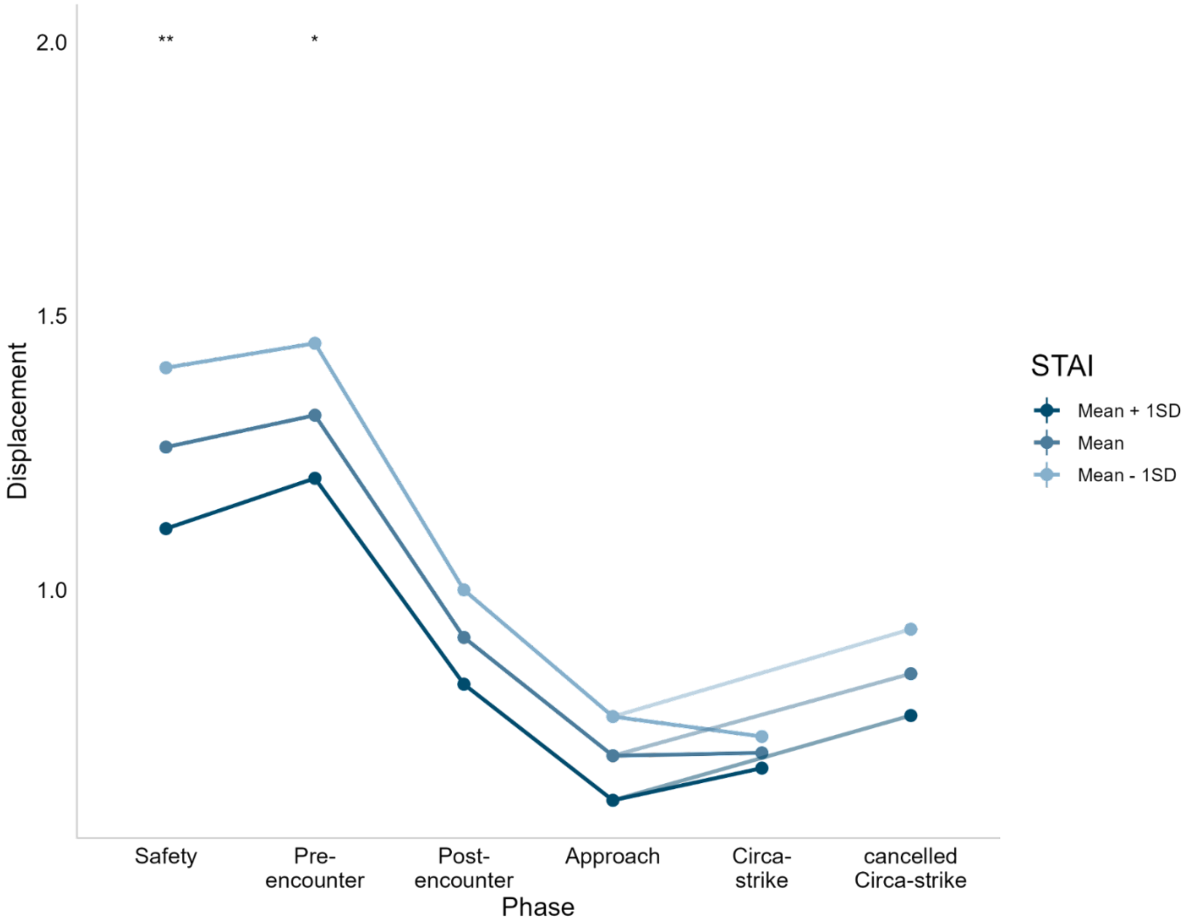

Figure 8. Relationship between trait anxiety and movement speed for all phases of the TICM.

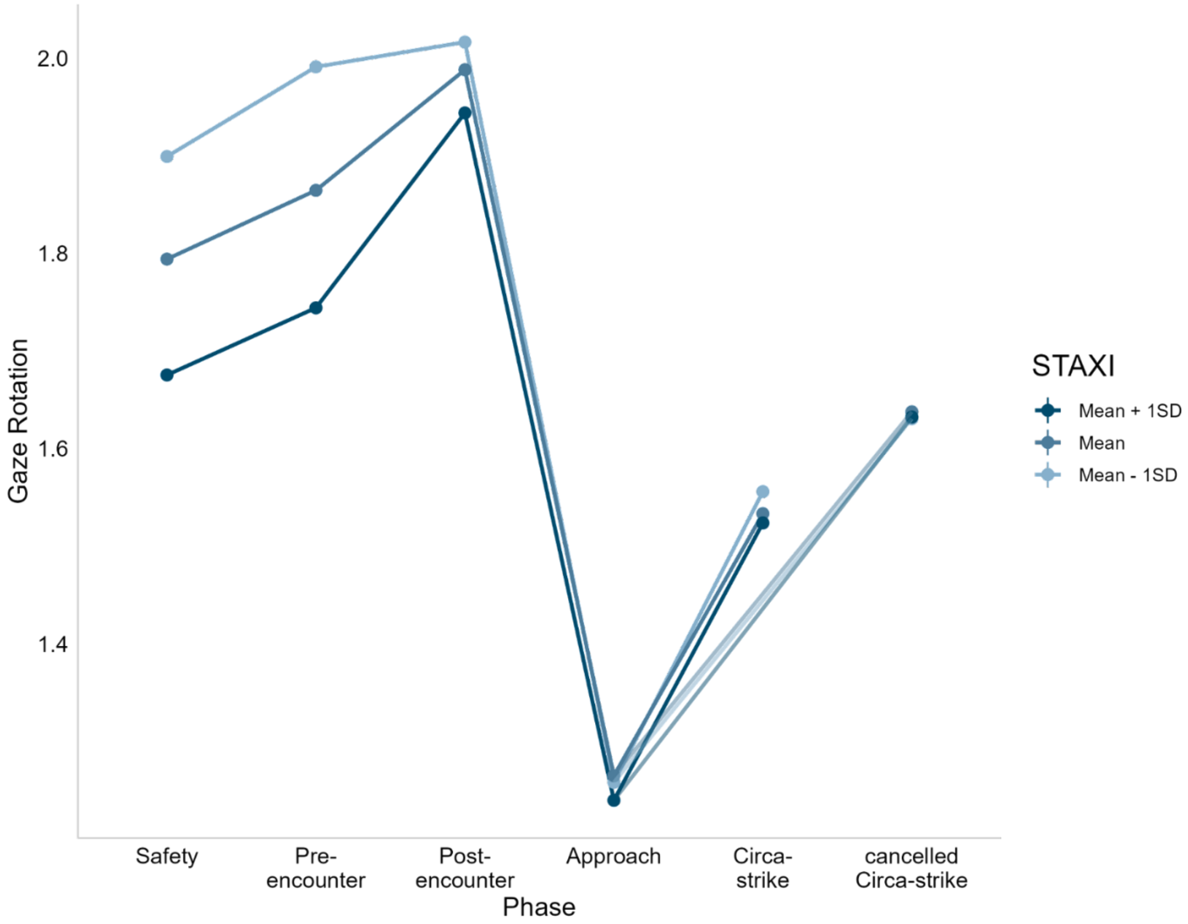

Figure 9. Relationship between trait aggression and eye rotations for all phases of the TICM.

Discussion

This study was designed to explore defensive states in humans upon increasing levels of threat based on the Threat Imminence Continuum Model by Fanselow and Lester (1988). We used a virtual reality environment to allow for both full immersion and unconstrained movement, and measured a variety of potential markers of defensive states. As intended, participants perceived more threatening phases of the TICM as more dreadful, verifying our threat manipulation. The Post-encounter and our introduced Approach phases were the crucial episodes in which we expected signs of freezing or attentive immobility, such as reduced body, head and eye movements, and heart rate decelerations. In this epoch we indeed found movement inhibitions denoted by reduced movement speed (Post-encounter and Approach) and fewer body, head, and eye rotations (Approach). Reductions in movement speed were thereby more pronounced the more dreadful the Post-encounter phase was perceived. This was accompanied by a slight but only marginally significant decrease in heart rate between the Post-encounter and Approach phases, as well as generally elevated skin conductance levels with increasing threat imminence. Accordingly, Circa-strike threat evoked the highest skin conductance response as compared to all less imminent threat contexts, but also an unexpected decrease in heart rate.

Generally, we observed cross-correlated temporal profiles within participants across the phases among all movement metrics, among the autonomic measures as well as between heart rate and body, head, and gaze rotations, respectively. Moreover, dread ratings cross-correlated negatively with all movement metrics and positively with both autonomic metrics. We only observed a few inter-individual differences. Heightened trait anxiety was related to greater reductions in movement speed. Based on descriptive analyses, more anxious participants moved slower than those low in trait anxiety throughout the entire TICM. Heightened trait aggression was associated with fewer eye rotations. This relationship descriptively manifested from the Safety until the Post-encounter phase. None of the other movement metrics were associated with trait anxiety or aggression.

Overall, our subjective, behavioral, and autonomic readouts suggest that defensive reactions change with threat imminence. The observed patterns are thereby largely in line with previous non-human and human animal work suggesting that the spatiotemporal and psychological distance of a threat elicits qualitatively distinct defensive states (Fanselow & Lester, 1988; Mobbs et al., 2007, 2020). Confirming previous findings, the Post-encounter and, even more strongly, the Approach phase evoked movement inhibitions reminding of freezing behaviors in response to threat in rodents (Hamm, 2020; Hashemi et al., 2021; Merscher et al., 2022; Roelofs, 2017; Roelofs & Dayan, 2022; Rösler & Gamer, 2019). Reductions in movements were thereby reflected in reduced movement speed, body and head rotations (Gladwin et al., 2016; Hashemi et al., 2021) as well as gaze movements (Rösler & Gamer, 2019; Merscher et al., 2022). These measures cross-correlated, which hints towards an underlying, broad, freezing-like movement inhibition. Instead of relying on a single measure to adduce complex defensive behaviors such as freezing (which is often indicated by reductions in postural sway in humans; e.g., Roelofs, 2017; Roelofs & Dayan, 2022), the current study thereby provided a more holistic proxy using multiple behavioral readouts. Given that freezing in rodents is usually assessed using video tracking or force transducers that record displacements of the entire body of the animal within a chamber (Chang et al., 2009; Trott et al., 2022), this also seems to be a more comparable translational indicator for such defensive behaviors in humans. Similar to what has been observed in animals (de Toledo & Black, 1966; Gentile et al., 1986; Roelofs & Dayan, 2022; Fanselow, 1984; Vianna & Carrive, 2005; Walker & Carrive, 2003; but see Carrive, 2000; LeDoux et al., 1984), both phases were accompanied by skin conductance increase and heart rate deceleration, suggesting a co-activation of both branches of the autonomic nervous system (Gladwin et al., 2016; Hamm, 2020).

Although the experimentally designed Post-encounter and Approach phases both represented relatively distal threat usually subsumed under Post-encounter threat alone, our findings suggest that conceptually distinct defensive responses were evoked in each phase. During Post-encounter threat (i.e., the hooded man appeared somewhere in the distant surroundings), participants stopped ongoing behaviors (i.e., moving forward to forage) and looked around rotating their bodies, heads, and eyes, presumably to orient towards the locus of danger. Similarly, when facing novel or innately aversive stimuli such as the odor of a cat, rodents have been observed to intermittently cease to move and orient towards the potentially threatening stimulus (e.g., by pointing their ears forward or even approaching it). This has been suggested to help in optimizing sensual uptake, attentional processing, and risk assessment (Blanchard & Blanchard, 1989, 1990) and may be suitably described as a form of ‘Attentive Immobility’ (Volchan et al., 2017). Regarding the spatiotemporal and psychological distance of the threat as categorized in the TICM, this state seems to occur at the intersection between Pre-encounter and Post-encounter threat: A potential threat has already been encountered but the situation is still ambiguous.

As soon as there were signs of an approaching threat (the Approach phase), all forms of movements became inhibited. Now, the location of the opponent was presumably known - and the attacker seemed to have located the participant as well - so that preparations for a potential counterattack could be started. This is in line with previous studies suggesting a decrease in movement and heart rate to facilitate fast subsequent actions (Gladwin et al., 2016; Jennings et al., 2009; Merscher et al., 2022; Obrist et al., 1970; Rösler & Gamer, 2019). While rodents usually flee when there are signs of an approaching attack, they also freeze when escape routes are blocked. This switches to vocalization, display of teeth and eventually jumping when the distance to the threat stimulus decreases (Blanchard et al., 1986, 1991). Despite the contextual differences in the current study and the mentioned rodent work (escape routes available vs. blocked), the observed motor inhibition in both species seems comparable as it suggests to aid in preparing further defensive actions when under attack. Taking up a previously introduced classification by Volchan and colleagues (2017), the observed defensive state in the Approach phase may thus be referred to as 'Immobility under Attack'. Within the TICM, it seems to mark the transition between Post-encounter threat and Circa-strike attack.

That movement inhibition manifested in both body and eye movements, which were positively cross-correlated, suggests that both movement metrics reflect freezing-like behaviors in humans as previously proposed (Rösler & Gamer, 2019; Merscher et al., 2022). Further in line with previous studies, their temporal profiles across the phases were positively related to heart rate changes (Hashemi et al., 2021). This supports the suitability of eye-tracking techniques to measure components of threat-related defense states (Merscher et al., 2022). Their versatility in a variety of different contexts such as brain imaging environments seems of particular importance to research on defensive states in humans (e.g., Hashemi et al., 2019; Wendt et al., 2017) as other measures are less applicable in that regard (i.e., body movements cannot be assessed in a brain scanner, and heart rate deceleration has been suggested to constitute a component of both defensive and appetitive states, Merscher et al., 2022). Given the demand to link neural defensive circuits to their systematic readouts, this may not only improve human but also translational research on defensive states across species in general (Signoret-Genest et al., 2022).

The expected increase in sympathetic arousal upon Circa-strike threat was only partly reflected in our findings. In line with previous findings, skin conductance levels increased with more imminent threat levels, reaching their peak during Circa-strike. Seemingly at odds with the literature, however, heart rate decreased from the Approach to the Circa-strike phase (Hamm, 2020; Löw et al., 2015; Roelofs & Dayan, 2022; Rösler & Gamer, 2019; Merscher et al., 2022; McCall et al., 2016). Given that participants strongly moved to pursue the foraging task until the Post-encounter phase, this decrease in heart rate may have also been influenced by the pause in physical exercise (Karvonen & Vuorimaa, 1988).

As previously shown, changes in both behavior and psychophysiology were related to how dreadful each phase was perceived (McCall et al., 2016). Heightened subjectively perceived dread was thereby associated with greater movement inhibitions, particularly during Post-encounter threat, as well as with higher skin conductance levels and heart rate, respectively. This corroborates that defensive responses intricately vary with both the context and individual aspects (Barrett, 2017; Blanchard, 2017; Curzon et al., 2009; Maren, 2008; Trott et al., 2022). Further underlining individual differences in threat-responding, we found heightened trait anxiety to be related to reduced movement speed. More anxious participants in our sample generally moved more slowly than less anxious individuals but this difference only became significant in the rather safe but potentially ambiguous phases (Safety and Pre-encounter - when no concrete threat was visible yet). Similar differential effects in highly anxious individuals have been demonstrated in previous studies but these predominantly manifested in enhanced freezing behaviors during Post-encounter threat (Gladwin et al., 2016; Hashemi et al., 2021). Extremely reduced body movements have hence been suggested as vulnerability markers for psychopathologies, potentially perturbing the adaptivity of defensive behaviors and the execution of other survival-related non-defensive tasks (Hashemi et al., 2021; Roelofs et al., 2010). Although differences in trait aggression have previously also been positively linked to altered defensive responding, particularly to movement inhibitions (Gladwin et al., 2016), we could only find a potential relationship between heightened trait aggression and greater inhibitions of eye movements among all movement metrics. The role of aggression in threat-responding hence remains relatively elusive. As both anxiety and aggression scores were generally low in our sample, however, more research into the relationship between individual predispositions and defensive responding in (sub)clinical samples is mandated to elucidate their role in the development and maintenance of psychopathologies (Niermann et al., 2017, 2019).

There are several limitations to the study. First, although our manipulation check suggests that participants perceived more threatening contexts as more dreadful, it seems difficult to disentangle the influence of task demands and threat imminence on the dependent variables. For instance, one could argue that ceasing to move forward and turning around to look for the opponent could be strategic behaviors within the game regardless of the threatening context. Although the threat-sensitivity and functionality of single behavioral and autonomic components of defensive states still need to be conclusively determined, previous studies have pointed toward threat-specific aspects (i.e., reduced eye movements) of defensive states and other components that seem sensitive but not exclusive to threat (e.g., heart rate deceleration, Merscher et al., 2022). Second, while virtual reality offers an immersive experience that is considered high in ecological validity as compared to other laboratory experiments, participants are still aware that the threat is not real, which limits the generalizability of our findings. Third, we merely manipulated the spatiotemporal and psychological distance of a threat as supposed in the TICM (Fanselow & Lester, 1988) but other contextual (e.g., availability of escape routes or hiding places) and individual factors (e.g., previous experience, age, gender) may be equally important to the expression of defensive states, which remains an important question for future studies (Blanchard, 2017; Curzon et al., 2009; Maren, 2008; Trott et al., 2022). Lastly, our sample mainly consisted of female students and is thus not representative of the entire population.

To conclude, the current study provided important insights into the phenomenology of defensive states in humans. Addressing concerns that simplified laboratory studies may be ineffective at triggering naturalistic defensive responses (Balban et al., 2021), we demonstrated virtual reality to be a well-suited ethological method to differentiate between distinct defensive patterns upon increasing levels of threat based on the Threat Imminence Continuum model by Fanselow and Lester (1988). We also answered the call for integrating different measures into broader defense patterns, adducing multiple subjective, behavioral, and autonomic measures and mapping their distinct patterns onto different threat contexts (Signoret-Genest et al., 2022). Specifically, we found evidence for a defense state characterized by a cessation of ongoing behaviors and orienting during Post-encounter threat, previously termed ‘Attentive immobility’. Upon approaching threat, another defense state became visible denoted by movement inhibition along with heart rate deceleration and skin conductance increase, which can be subsumed under the term ‘Immobility under attack’ (Volchan et al., 2017). Together, given methodological constraints in comparing defensive responses between rodents and humans, our study opened up a new avenue of merging insights from animal and human research that could eventually inform our understanding about the role of defensive states in mental health and disease.

6. General Discussion

The main goal of the current thesis was to elucidate defensive states in humans, with a particular focus on their (oculo)motor and autonomic dynamics. Prior human and animal work reported a decrease of motion in response to threat (i.e., freezing). This defensive behavior has been used as a readout to quantify fear, but scientists have raised objections to its investigation, conceptualization, and interpretation. For example, previous studies exposed that freezing occurs across a variety of contexts, suggesting it to reflect different overarching defensive states. I therefore adopted a conceptual distinction of three defensive states, Attentive Immobility, Immobility under Attack, and Tonic Immobility, proposed by Volchan and colleagues (2017). Critically, each state involves, but is not reduced to an inhibition of motor activity. According to this account, the exhibited defensive state is determined by the spatial, temporal, or psychological likelihood of a threat and the availability of escape routes (Volchan et al., 2017). Attentive Immobility is a defensive response to potential, uncertain, or ambiguous threats, a situation also labeled Post-encounter (Fanselow & Lester, 1988). Immobility under Attack is exhibited when an attack has been initiated and escape routes are blocked while the proximity of the threat is still (perceived as) relatively distant. Tonic Immobility is the last resort to unavoidable threat, displayed during and after Circa-strike contact.

Each of these defensive behaviors is principally adapted to protect an individual from harm but may pose a risk to their physical or mental health when disproportionately expressed (Pittig et al., 2020). However, despite the fundamental role of defensive behaviors in well-being and mental health, we are still only scratching the surface of understanding the integrated phenomenology of human threat-responding. We thus embarked on a series of experiments to explore and unravel the behavioral and autonomic dynamics of immobility-related defensive states in human adults.

We were particularly interested in a recently found oculomotor effect that was observed in anticipation of avoidable threat (Rösler & Gamer, 2019). Specifically, Rösler and Gamer (2019) found that gaze dispersion diminished when participants prepared for a fast motor response (i.e., button press) to escape from an aversive electrical stimulation (vs. no stimulation or an inevitable stimulation). Along with this oculomotor pattern, they observed bradycardia and an increase in skin conductance, presumably indicating a co-activation of both branches of the autonomic nervous system. More pronounced reductions in both gaze dispersion and heart rate were thereby related to faster motor responding, suggesting an action-preparatory mechanism. Reminded of an inhibition of motion in response to threats that prepares for fast subsequent defensive actions, this centralization of gaze has been discussed as an index for threat-related immobility in humans.

Despite the promising theoretical contribution of Rösler and Gamer (2019), certain design limitations left this conclusion ambiguous. Motivated by the potential of eye-tracking technology to provide a marker for fear-related motion in otherwise movement-restricted environments (e.g., brain scanning), we designed four studies to elucidate the robustness, threat-specificity, and validity of diminished gaze dispersion and its concomitant autonomic dynamics upon avoidable threat. We thereby adapted (Study 1-3) and extended (Study 4) Rösler and Gamer's (2019) study design.

Study 1 comprised two experiments pursuing whether the previously observed decrease of oculomotor activity would

- i) robustly occur during preparation for threat-avoidance irrespective of task demands (Experiment 1);
- ii) reflect an action-preparatory state, along with its concomitant autonomic dynamics, independent of valence or a threat-specific defensive state (Experiment 2).

Two further studies explored whether this inhibition of oculomotor activity would

- iii) interact with other notorious gaze dynamics such as attentional biases for social and threatening stimuli (Study 2);
- iv) be related with inhibitions of somatomotor activity, as both have been discussed as indicators for freezing-like behaviors in humans (Study 3).

Finally, Study 4 elucidated this gaze effect

- v) among multiple behavioral (movement speed, body, head, and eye rotations), autonomic (heart rate and skin conductance), and individual (subjectively perceived dread, trait anxiety, straight aggression) measures in response to increasing levels of threat in an immersive virtual reality environment. With that, we aimed to address limitations of previous work on defensive states in humans that mainly employed movement-restricted experimental designs with low ecological validity and assessed complex defensive behaviors with single measures.

6.1. Reduced Gaze Dispersion upon Avoidable Threat

6.1.1. Robustness and Threat-Specificity

To test whether the observed reductions in visual scanning were specific to the experimental task by Rösler and Gamer (2019), we adapted their design such that shock avoidance required more spatially distributed attention (Study 1, Experiment 1). While previously shocks could be avoided by quickly pressing the space bar that was centrally located in front of the participant, it was now necessary to react upon peripherally presented response prompts with a quick joystick movement in the correct direction to avert a shock. Besides replicating the previously shown autonomic patterns (i.e., bradycardia, skin conductance increase, pupil dilation) during the preparatory period for shock avoidance, we corroborated a reduction in gaze dispersion that positively predicted the speed of subsequent motor responses. In contrast to previous findings, however, differences in fixation durations and frequency were not replicated (Rösler & Gamer, 2019). Although it remains to be determined whether this is due to changes in experimental design, low reliability of measurements, or the genuine absence of effects on these fixation metrics, our findings suggest that reductions in gaze dispersion may best reflect oculomotor changes during the expectation of avoidable threat.

Building on this first evidence for the robustness of narrowed visual exploration during the anticipation of avoidable threat, we addressed whether the observed effects were reflective of an action-preparatory mechanism, irrespective of contextual valence, or a threat-specific defensive state. We thus transferred the paradigm employed in Experiment 1 (Study 1) into an appetitive context (Study 1, Experiment 2). Instead of avoiding shocks, participants could now achieve a financial reward (vs. no or a guaranteed reward) when quickly reacting upon a peripherally presented response prompt. We showed that preparing to achieve rewards was not accompanied by globally narrowed fixations but that autonomic responses resembled those in the threatening context. Comparing the findings of Experiment 1 and 2, we proposed that bradycardia and a concomitant activation of the sympathetic nervous system may not only be sensitive to threat but to action preparation in general. In contrast, globally reduced gaze dispersion seemed to be more threat-specific.

In both experiments of Study 1, however, the image material we presented was characterized by a homogeneous distribution of visual features to prevent picture-immanent gaze dynamics from interfering, which resulted in a relatively low ecological validity (Rösler & Gamer, 2019). To address this issue and further elucidate the robustness of reduced visual scanning upon avoidable threat across more heterogeneous stimulus contexts, Study 2 investigated whether and how this oculomotor pattern would interact with other notorious gaze behaviors. As certain social and threatening stimuli have been shown to draw overt visual attention, we replaced the previous pictures with images of various indoor and outdoor scenes containing one or more social (i.e., faces, Experiment 1) or threatening stimuli (i.e., spiders and snakes, Experiment 2). Importantly, the stimuli of interest differed in their distance from the image center. We found evidence for two sequential oculomotor effects indicating orienting and action preparation upon approaching threat. Relevant visual features such as social or threatening stimulus elements initially captured attention, but this pattern was superseded by a strong decrease of visual exploration as a function of action preparation under threat. The latter was accompanied by an increase in skin conductance levels, pupil dilation and bradycardia.

Taken together, the findings of our first two studies provide evidence for signs of a defensive state called Attentive Immobility upon approaching threat (Volchan et al., 2017). Specifically, we corroborated previously observed reductions of gaze dispersion along with a co-activation of parasympathetic (bradycardia) and sympathetic (increase in skin conductance and pupil width) branches of the autonomic nervous system in anticipation of avoidable threat (Gladwin et al., 2016; Hashemi et al., 2021; Rösler & Gamer, 2019). Supporting the notion that this state adaptively supports action preparation (Gladwin et al., 2016; Rösler & Gamer, 2019; Löw et al., 2015), we found associations between reduced gaze dispersion and heart rate, respectively, and faster motor-reactions on a trial-wise basis. Globally reduced visual scanning was thereby only apparent in a threatening context, which indicates that this oculomotor effect may constitute an action-preparatory, threat-specific, and robust component of a defense state upon avoidable threat. Bradycardia, in contrast, occurred in both defensive and appetitive contexts. Despite the widely assumed robust association between bradycardia and reduced postural sway, which has led to the use of heart rate deceleration as an indicator for freezing (Lojowska et al., 2019; Wendt et al., 2017), cardiac and motor decrease seem to constitute different components of a defense state. This is supported by previous work showing that bradycardia can be evoked as an action-preparatory response irrespective of threat (Jennings et al., 2009; Löw et al., 2008; Obrist et al., 1970). Intrigued by the potential suitability of eye-tracking technology in discriminating between defensive and appetitive states, we followed up on testing whether the observed reductions in oculomotor activity would be indicative of a broad, underlying state of movement inhibition, also visible in simultaneous reductions in body movements.

6.1.2. *Validity*

Freezing or movement inhibitions in response to threats in humans have been mainly deduced by reductions in postural sway (Roelofs, 2017; Roelofs & Dayan, 2022). Due to similar temporal dynamics and functionality, the observed decrease in oculomotor activity reminded of such freezing-like behaviors. In Study 3, we therefore tested whether inhibitions of gaze and body movements would indeed reflect the same underlying construct. Specifically, we examined their co-occurrence and relationship using the same design and measures as Rösler and Gamer (2019) plus an additional measure of postural sway. The choice of this measure was based on previous work (Roelofs, 2017; Hashemi et al., 2021; Gladwin et al., 2016), where reductions in body sway, assessed as weight shifts in the center of pressure on a stabilometric platform, were adduced as indicators for freezing behaviors in humans. We replicated a decrease in gaze, body, and heart rate activity and an increase in skin conductance during the anticipation of avoidable shocks. We failed to find a trait-like relationship between body and gaze movements across participants, but their temporal profiles were positively related within individuals, suggesting that both metrics reflect the same construct. However, given that both measures as well as most designs investigating them might lack ecological validity, these results are not fully generalizable.

To address this limitation as well as an additionally problematic reliance on single measures to assess complex defensive behaviors, we conducted Study 4. Based on the Threat Imminence Continuum Model (TICM) by Fanselow and Lester (1988), we created a virtual reality environment simulating increasing levels of threat. With that, we allowed for both full immersion and unconstrained movement, and measured a variety of behavioral (movement speed, body, head, and eye rotations), autonomic (heart rate and skin conductance), and subjective measures (trait anxiety, trait aggression, perceived dread). Accordingly, we assessed potential inhibitions of gaze activity upon threat, which were previously reflected in reduced fixation dispersion, by measuring the amount of eye rotations in this now 3-dimensional context.

Our design involved all stages of the TICM (i.e., Safety, Pre-encounter, Post-encounter, and Circa-strike) plus an additional phase (‘Approach’) to signal still distal but approaching potential threat between Post-encounter and Circa-Strike. Based on the findings of our previous studies, we expected to observe (oculo)motor inhibitions along with bradycardia and a skin conductance increase in the Post-encounter stage, presumably reflecting a state of Attentive Immobility. Indeed, movement speed significantly reduced in the Post-encounter phase (i.e, when a hooded man appeared somewhere in the distant surroundings) but body, head, and eye rotations increased compared to the Pre-encounter phase. Only during the Approach stage (i.e., when the man started to approach the participant, but it was still unclear whether he would attack), all movement metrics became inhibited. The transition from Post-encounter to Approach was further accompanied by a slight but only marginally significant heart rate decrease and elevated skin conductance levels that generally increased with threat imminence. We further observed within-participant cross-correlations between the temporal profiles of gaze and body movements, among the autonomic measures as well as between heart rate, and body, head, and gaze rotations across the simulated phases, which hints towards an underlying, broad, defensive state.

Considering that both experimental phases (i.e., Post-encounter and Approach) are reflecting still distant and only potential threat, which is usually subsumed under Post-encounter alone (Fanselow & Lester, 1988), our findings suggest that this period indeed elicits signs of the previously labeled state of Attentive Immobility in humans (i.e., reduced body and gaze activity, heart rate deceleration, and skin conductance increase). Our segmentation of this phase that seemingly evokes different defensive behaviors, however, calls for refinement of this interpretation (Fanselow & Lester, 1988).

6.2. Theoretical Implications

Based on the TICM, the rules guiding the selection of defensive responses are based on the psychological likelihood or spatiotemporal distance of contact with a life-threatening situation (Fanselow & Lester, 1988; Mobbs, 2018; Mobbs et al., 2007). Although the model describes a continuum, it has segregated the likelihood of threat into four phases that supposedly elicit qualitatively distinct defensive behaviors. Accordingly, immobility-related states have been reported as an indicator for Post-encounter threat (i.e., a threat is present but seemingly not attacking yet) while activity bursts (fight-or-flight) have been considered typical Circa-strike responses (i.e., threat contact). The findings of Study 4 challenge such clear segmentation (for further objections and extensions of the TICM, see Trott et al., 2022). Depending on the proximity of the threat, the Post-encounter phase may elicit different defensive behaviors and should thus be further differentiated. Ambiguous threats that have not been located or assessed yet may trigger a pause in ongoing behaviors, prompting individuals to redirect their attention towards the location of the threat and evaluate the level of risk. When a potentially threatening stimulus has been located and is approaching, in turn, it may require immediate attention and mobilization of the body for potential defensive actions if an actual attack is initiated.

Indeed, findings of Study 4 suggest that participants stopped to forage as soon as there were signs of potential danger (i.e., decrease of movement speed) to locate the opponent (i.e., reflected in increased head, body, and eye movements). A similar behavior has been found in rodents. When sensing a novel, threat-related, or ambiguous stimulus (e.g., the odor of a cat), they have been observed to stop ongoing behaviors and orient towards the potential locus of danger, exhibiting slow explorative movements as to evaluate the risk (Blanchard & Blanchard, 1989, 1990). Transient immobility and orienting movements may thus be reflective of a behavioral module facilitating risk assessment during early Post-encounter threat. Taking up previous definitions, Attentive Immobility may best describe this state, although the focus on immobility may be misleading and not appealing to everyone. Other researchers thus speak of an ‘Orienting’ response (e.g., Roelofs & Dayan, 2022) or ‘Risk Assessment’ (Blanchard et al., 2011). Whichever term is adopted, it should be noted that this seemingly integrated module during early Post-encounter threat may actually involve different types of behaviors.

As soon as danger was more likely (i.e., the man approached the participant), our findings show a shift in defense state characterized by a decrease of motion visible in the entire body (i.e., reduced movement speed, body, head, and eye rotations). Now, participants had probably located the approaching man, aligning their full body and attention towards him as to prepare for thwarting his (still potential) attack. As with Attentive Immobility, rodents also seem to display such behaviors. Although fleeing is usually the best and most common option for rodents to survive an approaching threat, they must choose different defensive strategies when escape routes are not available. In such situations, rats have been observed to freeze when approached by a threat in an inescapable context, which switches to more active defensive behaviors (i.e., vocalization, display of teeth, and jumping) upon even closer distances of predatory contact (Blanchard et al., 1986, 1991; Blanchard & Blanchard, 1989). Besides contextual differences that are supposed to be pivotal for the distinction of defensive states (i.e., presence of escape routes in our study vs. no escape routes in rodent work), the observed reductions in motion in both rodents and humans bear some resemblance and may reflect a similar defense state. The previous term Immobility under Attack seems to be a suitable description, but its exhibition may not necessarily be determined by the availability of escape routes as suspected. Rather, we propose, the potential necessity of a fast subsequent defensive action, particularly fight reactions, may evoke this behavior. Which circumstances favor or require what response to heighten the chance of survival or reduce harm, may thereby depend on the individual, the circumstances, and the threat cue itself (Blanchard et al., 2001). For example, while the constitution of a mouse regarding their predators requires them to prefer flight over fight, when possible, the human potential to use weapons and their slowness may favor fight over flight across a wider range of situations.

This also implies reconsideration of conclusions we drew from our first studies and previous literature (Roelofs, 2017; Hashemi et al., 2021). Based on these new observations and theoretical deliberations, instead of finding signs of Attentive Immobility upon avoidable threat, the observed cardio-behavioral state may rather reflect Immobility under Attack. Indeed, the finding that both inhibitions of oculomotor and heart rate activity predicted faster motor reactions in Study 1-3 supports the notion that this state facilitates action preparation for fast subsequent defensive actions. Along these lines, other studies involving an active option to avert a threat also reported heart rate decelerations, skin conductance increase, and decrease in body sway to increase as a function of action preparation (Löw et al., 2015; Roelofs, 2017; Gladwin et al., 2016; Hashemi et al., 2021).

If this cardio-behavioral pattern upon avoidable threat indeed reflects Immobility under Attack, paradigms not allowing for active threat-avoidance, which we had previously placed in this category, may require a new classification (e.g., Azevedo et al., 2005; Hagenaars et al., 2012; Lopes et al., 2009; Roelofs et al., 2010). It is note-worthy that studies of this type have significant limitations and produced mixed findings. Specifically, some studies reported freezing-like reductions of body sway on the medio-lateral axis (Facchinetti et al., 2006) while others found them on the anterior-posterior axis of the balance board (e.g., Niermann et al., 2015; Roelofs et al., 2010). Moreover, they are low in ecological validity and do not entail a realistic manipulation of an orienting response that would be required to assume a state such as Attentive Immobility.

A relatively more ecologically valid stimulation in a passive design was implemented by Hagenaars and colleagues (2014), however. Instead of static pictures of aversive stimuli, they presented unpleasant 1-minute film clips (vs pleasant and neutral), allowing them to assess dynamics of body sway and heart rate over time. Aligning with the orienting response we observed in Study 2 (i.e., initial fixations were predicted by the location of social or threatening stimuli), the film induced early reductions in postural sway (between 1 and 2 s after stimulus onset). Meanwhile, heart rate steadily declined and remained low during viewing of the aversive film clip. As bradycardia has been associated with heightened perceptual decision-making (de Voogd et al., 2022) and sensual intake of visually coarse information during threat of shock (Lojowska et al., 2015, 2019) these passive viewing paradigms may measure a state of Attentive Immobility or Orienting upon relatively distal, early Post-encounter threat. This also aligns with the notion that orienting responses occur at moderate to low stimulus intensity (Hagenaars, Roelofs, et al., 2014). Given that we did not find evidence for bradycardia during Post-encounter threat (only in the Approach phase, Study 4), the sensitivity of this measure for movement and action-preparatory effects irrespective of threat (Study 1; Merscher et al., 2022), as well as mixed findings in the rodent literature (Signoret-Genest et al., 2022), the autonomic dynamics of the state of Attentive Immobility remain to be further explored in future research.

The division of defensive behaviors during Post-encounter threat into two subcategories fits with the idea of two underlying anxiety and fear-related states. Accordingly, Mobbs and colleagues (2020) proposed that an early Post-encounter stage may evoke 'Anticipatory Anxiety' and late Post-encounter 'Cognitive Fear', associated with qualitatively distinct defensive behaviors. Converging our findings with Mobbs and colleagues' (2020) proposal, we suggest that 'Anticipatory Anxiety' would come along with Attentive Immobility while 'Cognitive Fear' with Immobility under Attack. Whether these states are linked to different neural circuitries needs to be examined in future studies, however.

Lastly, although Volchan and colleagues (2017) only described three different categories of immobility-related defensive states (i.e., Attentive Immobility, Immobility under Attack, Tonic Immobility), there may be more states involving a decrease of motor activity. Specifically, Trott and colleagues (2022) pointed out that the exhibition of freezing may further depend on the availability of hiding places where detection by a predator is particularly unlikely. Supporting this claim, it has been shown that, if available, rats move to places against a wall or a dark cave before they freeze as this may heighten their chances of avoiding an attack (de Oca et al., 2007; Grossen & Kelley, 1972; Sigmundi, 1997). Whether humans display similar behaviors when hiding places are available remains an important task for future studies in further deciphering different defensive states.

6.3. Clinical Implications

Persistent or otherwise disproportionate defensive behaviors are classified as key features of anxiety-related disorders (Pittig et al., 2020). Along these lines, previous studies have pointed towards associations between interindividual differences in defensive responding and presumed vulnerability markers for psychopathologies (e.g., Frank et al., 2006; Hashemi et al., 2021; Roelofs et al., 2010). Specifically, individual differences in reduced body sway in response to threat, and sometimes also heart rate decrease, have been associated with several (pre- or sub-) clinical mental health conditions such as panic disorder (Lopes et al., 2009), trait anxiety (Frank et al., 2006; Niermann et al., 2017; Roelofs et al., 2010), PTSD (Adenauer et al., 2010; Fragkaki et al., 2017; Orr & Roth, 2000), low hair cortisol concentrations (Hashemi et al., 2021), and trait aggression (Gladwin et al., 2016). However, other studies failed to find such associations (e.g., Rösler & Gamer, 2019) and rather underlined several adaptive functions such as optimized perceptual decision making (de Voogd et al., 2022), sensory processing (Lojowska et al., 2015, 2019) and action preparation (Gladwin et al., 2016; Rösler & Gamer, 2019), which is also corroborated in our findings across all studies (i.e., relationship with action preparation but zero to few indications of clinically relevant interindividual differences regarding trait anxiety, anxiety sensitivity, and trait aggression). While this may point towards a general adaptivity of the observed defensive states in a sample of healthy young adults, more research into the relationship between individual predispositions and defensive responding in more heterogeneous, especially (sub)clinical, samples is mandated to elucidate their role in mental health and disease (Niermann et al., 2017).

Previous work already hints at how and why aberrations of these defensive states may manifest. For example, it has been suggested that in those who may be predisposed for trauma or anxiety-related psychopathology, the perceived distance of threat-imminence may be altered from that in healthy individuals, resulting in discrepancies between perceived and actual proximity of a threat (Hamm, 2020; Hamm et al., 2016; McTeague, 2016; Perusini & Fanselow, 2015). For instance, those prone to clinically relevant panic attacks, which are normally ignited in response to circa-strike threat, may (fear to) exhibit these behaviors in relatively innocuous or ambiguous contexts where they are not required (McTeague, 2016). Conversely, an absence of circa-strike behaviors (i.e., fight-flight) or prematurely falling into a state of Tonic Immobility when other options are still available may also cause mental disturbances such as PTSD (e.g., Rizvi et al., 2008). Along these lines, our finding that highly anxious participants moved more slowly in the Safety and Pre-encounter phase than less anxious individuals in Study 4 may point towards premature Post-encounter defenses, potentially perturbing other survival-relevant non-defensive behaviors. Indeed, increased vigilance and caution have been shown to be particularly pronounced in animals who may be or perceive themselves as particularly vulnerable (Monclús & Rödel, 2009; Pangle & Holekamp, 2010; Quenette, 1990).

Further sensitivity for psychological disturbances seems to lie in an inability to flexibly adapt behaviors to ever-changing surroundings and individual needs (Notebaert et al., 2020). In line with this notion, it has been suggested that rigid attention bias toward threatening stimuli irrespective of their relevance in a given situation may reflect a disproportionate allocation of attention resources that may hinder the performance of other relevant behaviors (Abado, Richter, et al., 2020; Abado, Sagi, et al., 2020; Okon-Singer, 2018). For example, highly anxious individuals showed a rigid attention allocation to threatening stimuli, irrespective of whether later avoidance of an aversive outcome relied on their detection. Less anxious individuals, in contrast, flexibly adapted their attention to the given task demands. Inflexible and dysfunctional alignment of attention has hence been suggested to constitute a marker of vulnerability for psychopathology (Notebaert et al., 2020; Lazarov et al., 2019). Based on our observations of two consecutive effects on overt visual attention in Study 2, which probably indicate an initial orienting response and subsequent action preparation, it is crucial for future research to investigate how exaggerated or reduced expressions of either behavior may perturb the adaptiveness of the other. In support of this notion, increased or decreased attentional bias to threatening stimuli has been shown in psychopathology such as phobia (Gremsl et al., 2018) and psychopathy (Baskin-Sommers & Brazil, 2022).

Besides such interindividual differences, a further question remains as to how specific features of the context may cause harm to a normally functioning organism. While it seemed that in our, as well as in several previous, designs on the matter (Roelof, 2017), participants were given sufficient time to exhibit both orienting responses and action preparation to perform the required action (Study 2), it remains unclear whether and how their interaction would change in more time-critical, overextending, or potentially traumatic situations. Previous work suggests that being taken by surprise may evoke panic-like states (Mobbs et al., 2020) or Tonic Immobility, which has been proposed to play a role in the development and severity of PTSD (Volchan et al, 2011; Rizvi et al., 2008)

6.4. Limitations

There are several limitations to our studies. First, all studies involved a highly homogeneous and gender-unbalanced (except for Study 3) sample. Although we are not aware of gender differences in (oculo)motor and autonomic responding in healthy individuals upon avoidable threat, our findings are mainly representative of young female students. In rodents, in contrast, the majority of studies has been conducted on males (Bangasser, 2015; Beery & Zucker, 2011) but more recent work examined both sexes, demonstrating sex differences in freezing behaviors in fear conditioning paradigms (e.g., females tend to freeze less than males, Russo & Parsons, 2021; Shanazz et al., 2022). In light of these findings in rodents and the notion that anxious psychopathology is more prevalent in women than men (Christiansen, 2015; Kessler et al., 2012), both rodent and human research should aim at investigating more heterogeneous samples to increase their generalizability and impact to a greater population.

Second, although Study 1 suggests that a centralization of gaze may be a threat-specific component of a defensive state, it is difficult to draw an ultimate conclusion in this regard due to comparability constraints between Experiment 1 (threat context) and 2 (reward context). Precisely, we cannot rule out that the intensity of unpleasantness in Experiment 1 was different from the intensity of pleasantness in Experiment 2. There have been efforts to account for differences in pain avoidance and reward preference in previous experiments (Andreatta & Pauli, 2015) but creating exact comparability between absolute valence of incentives for shocks and rewards is notoriously difficult to accomplish. As 1) our autonomic data suggests a comparable activation of the sympathetic and parasympathetic branches of the autonomic nervous system in avoidable shock and achievable reward trial types in both experiments and 2) behavioral responses were very similar between avoidable shock and achievable reward trials for accuracy and response times, we still assumed that there were no gross differences in the motivation to escape a shock and obtain a reward in this study. However, it remains to be tested whether the inhibitions of oculomotor activity upon avoidable threat are indeed specifically sensitive to threat or reflecting an attentional shift as part of an anticipatory state.

Consequently, this affects the interpretation of all our findings. In Study 4, for example, we cannot clearly disentangle whether the behavioral and autonomic effects are due to threat imminence or task demands. That participants perceived increasingly threatening contexts as more dreadful hints at defensive aspects of these dynamics but we cannot guarantee these would exclusively occur upon threat, particularly in light of the notion that defensive and appetitive states bear fundamental resemblance (Lang et al., 2013; Löw et al., 2008).

Third, none of the studies contained a real threat and, due to ethical constraints, shock intensities were relatively low. Although shocks are considered effective in triggering fear-like responses, their intensity has been shown to be a main determinant of different defensive behavior in rodents (Mobbs et al., 2019; Moyer & Korn, 1964). Furthermore, participants in our studies were already familiar with the aversive stimulation due to shock calibration procedures prior to the experiments, which also differs from corresponding rodent work (e.g., Signoret-Genest et al., 2022). Whether human studies implementing relatively weak and familiar shocks are thus tapping into the same neural systems as respective rodent work where shocks are not known beforehand and usually delivered at high intensities to trigger antipredator defense modes, remains an outstanding question.

Fourth, when inferring from rodent studies about human defensive responding, it is worth noting that besides striking homologies there are significant differences between these species, ranging from basal biological features (e.g., certain proteins, Oberheim et al., 2009; or epigenetic marks, Gräff et al., 2012) to highly evolved cognitive processes in humans, which may play a crucial role in the development and maintenance of psychopathology (e.g., meta-cognitions, Wells, 2011; socialization, Schwarz, 2017). While rodent research has contributed to great advances in understanding the human brain, body, and mental health, there may be species-specific factors causing limitations and challenges to translational work from bench to bedside (Blanchard, 2017; Dragunow, 2020).

As previously suggested, theoretical models on fear- and anxiety-related states should thus account for and further investigate potentially relevant factors of the context, the affected organism, and the threat cue itself (Blanchard et al., 2011). This may also help reconcile seemingly contradictory theories about fear and anxiety where some propose that these concepts have hard-wired neuroanatomical profiles while others state that fear and anxiety may be dynamic, ad-hoc constructs, inferred based on an individual's biotope and experiences (Mobbs et al., 2019). Our findings support resembling defensive responses in humans and rodents and thus point towards certain underlying states concerting similar defensive responses across species, but the displayed behaviors may differ depending on an individual's subjective experiences and context (Barrett, 2017; Bateson & Mead, 1942).

6.5. Conclusion

This thesis set out to elucidate and unravel (oculo)motor and autonomic components of defensive states in humans. We embarked on a series of experiments testing the robustness, threat-specificity, and validity of previously shown defensive states. Specifically, we showed an inhibition of (oculo)motor activity (i.e., denoted by reduced eye movements), heart rate decrease, and a concomitant increase in sympathetic arousal (i.e., skin conductance and pupil width increase) upon avoidable threat. Importantly, reductions in oculomotor activity seemed to not only robustly but also specifically occur upon avoidable threat, constituting an adaptive action-preparatory mechanism to avert an incoming threat. Action preparation was further supported by bradycardia, which seemed to occur irrespective of contextual valence.

Importantly, our last study provided evidence that helps disentangle two previously proposed defensive states involving a decrease of (oculo)motor activity. Specifically, we observed a state consisting of a cessation of ongoing behaviors and orienting upon relatively distal, ambiguous threat (Attentive Immobility). Upon approaching potential threat, in turn, an entire immobilization and presumed allocation of attention to the threat stimulus became apparent (Immobility under Attack). With that, we corroborated previous theories (e.g., the Threat-Imminence-Continuum model) stating that the spatiotemporal or psychological distance of threat elicits qualitatively distinct defensive states (Fanselow & Lester, 1988) and shed light onto different immobility-related states that were previously confounded (Volchan et al., 2017). The latter seems particularly pertinent as conceptual imprecision has hindered fruitful inner- and interdisciplinary exchange on fear- and anxiety-related states.

We further proved the usability and potential of virtual reality to portray a variety of behavioral and autonomic defense patterns in humans in an ecologically valid setting. We thereby provided an approach to overcome critical constraints and limitations of previous studies that assessed complex defensive behaviors based on single measures in highly simplified, artificial designs.

Lastly, significant cross-correlations between the temporal profiles of the (oculo)motor and autonomic measures upon increasing levels of threat point towards the existence of broader underlying defensive states. Whether the different defensive response patterns are linked to distinct neural circuits, remains to be tested in future studies. Eye-tracking techniques may thereby help elucidate the neuroanatomical profiles of defensive states in humans as they may provide seemingly robust and threat-specific markers of defensive motion and attention in otherwise movement-restricted environments.

Together, our findings gather evidence for specific oculomotor and autonomic dynamics upon increasing levels of threat that may inspire future translational work in rodents and humans on shared mechanisms of threat processing, ultimately supporting the development of novel therapeutic approaches.

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Appendix

Supplemental Material for Study 1

Centralized gaze as an adaptive component of defensive states in humans

Alma-Sophia Merscher, Philip Tovote, Paul Pauli, and Matthias Gamer

Details on the experimental set-up, procedure, and shock calibration

Experiment 1. After providing written informed consent, participants read instructions about which color would signal each of the three different outcomes. They were told that the naturalistic pictures appearing after the color cues are irrelevant for the task but could be freely explored. Participants were additionally instructed to fixate the center of the screen when a fixation cross was visible. Subsequently, electrodermal and electrocardiographic electrodes were attached. The electrode for shock delivery was attached to the left calf. Participants were then seated in a dimly lit sound-proof cabin with their head stabilized by a chin rest and forehead bar to prevent confounding movements. With their dominant hand, they reached for the grip of the joystick, which was fixed on the table with lashing straps at a distance where participants could easily move it towards the left and right side. On the horizontal axis (left/right), the placement of the joystick was set in line with the center of the screen. The experimenter sat right outside the cabin during the experiment to monitor its progression. After participants were seated, they underwent a shock calibration procedure. In line with our previous work (Rösler & Gamer, 2019), they were asked to rate the shock intensity on a visual analogue scale from 0 to 10 (VAS; 0 = "Not painful at all" to10 = "unbearably painful") with 4 marking the point where the perceived intensity turns from very unpleasant to slightly painful. Shock intensity was incremented from 0 mA in steps of 0.1 mA until reaching a rating of 4. This procedure was repeated three times. The amperages of every repetition that were rated at a VAS level of 4 were then averaged and the result amplified by 50%to counteract habituation effects during the experiment.

Experiment 2. The procedure was identical to Experiment 1 but since no shocks were delivered, the shock electrode was not attached and no shock calibration procedure was necessary.

Details on the prediction of response times

Similar to our previous study (Rösler & Gamer, 2019), we examined whether physiological and oculomotormeasures during the anticipation phase could be used to predict the speed of behavioral responses in the avoidable threat (Experiment 1) and achievable reward (Experiment 2) conditions. Overall, participants were very accurate (Experiment 1: *M* = 91.7%, *SD* = 9.7%; Experiment 2:*M* = 89.0%, *SD* = 9.3%) and fast (Experiment 1: *M* = 489.8 ms, *SD* = 61.2 ms; Experiment 2:*M* = 472.7 ms, *SD* = 56.4 ms) to respond to the respective cues and we neither observed significantdifferences between experiments for accuracy, $t_{(97.82)} = 1.44$, $p = .154$, nor for response times, $t_{(97.36)} = 1.46$, $p = .148$.

For each experiment, we computed a generalized linear mixed model (GLMM) using both heart rate and centralization of gaze separately as predictors for reaction times in the avoidable threat and achievable reward trials, respectively. Subject ID was added as a random intercept into the GLMM to account for individual differences in reaction times between participants. Based on the results of our previous study (Rösler & Gamer, 2019), we considered only the second half of the picture viewing period in these analyses since differences between trial types were most pronounced in this phase. Accordingly, we averaged four bins (second 5 to 8 of picture viewing) for heart rate and centralization of gaze on a trial-wise basis. Trials with erroneous responses were excluded. To perform the GLMM in R, we used the lmer4 package (version 1.1-15; Bates et al., 2015). Model estimates were chosen to optimize the restricted maximum likelihood criterion and *p*-values for each predictor were calculated using Satterthwaite’s approximation of degrees of freedom with the lmerTest package (version 2.0-25; Kuznetsova et al., 2017).

Table S1: Results of the 3 (trial types) × 8 (bins in seconds) repeated-measures ANOVAs on individual oculomotor responses measured in Experiment 1 and 2.

Study	**Dependent Variable**	**Predictor**	df_{Num}	df_{Den}	**ε**	***F***	***p***	η^2_g
Experiment 1 (Threat context)	Global Center Bias	(intercept)	1	49		996.01	.000	.93
		bin	7	343	.41	42.36	.000	.10
		cue	2	98	.82	30.23	.000	.08
		bin × cue	14	686	.31	13.64	.000	.03
	Horizontal Center Bias	(intercept)	1	49		852.45	.000	.90
		bin	7	343	.44	29.85	.000	.09
		cue	2	98	.83	11.79	.002	.04
		bin × cue	14	686	.32	6.71	.000	.02
	Vertical Center Bias	(intercept)	1	49		681.95	.000	.90
		bin	7	343	.45	46.56	.000	.09
		cue	2	98	.79	58.33	.000	.13
		bin × cue	14	686	.48	19.57	.000	.04
	Fixation Numbers	(intercept)	1	49		2062.42	.000	.97
		bin	7	343	.41	112.45	.000	.19
		cue	2	98	.78	0.96	.367	.00
		bin × cue	14	686	.42	2.30	.036	.01
	Fixation Durations	(intercept)	1	49		1170.64	.000	.92
		bin	7	343	.41	51.78	.000	.13
		cue	2	98	.83	1.96	.155	.01
		bin × cue	14	686	.43	2.81	.011	.01
Experiment 2 (Reward context)	Global Center Bias	(intercept)	1	49		504.21	.000	.85
		bin	7	343	.36	18.17	.000	.03
		cue	2	98	.59	0.84	.381	.00
		bin × cue	14	686	.19	.89	.438	.00
	Horizontal Center Bias	(intercept)	1	49		396.59	.000	.79
		bin	7	343	.35	14.64	.000	.02
		cue	2	98	.56	.95	.342	.01
		bin × cue	14	686	.19	2.23	.097	.01
	Vertical Center Bias	(intercept)	1	49		433.98	.000	.86
		bin	7	343	.41	23.38	.000	.04
		cue	2	98	.84	80.91	.000	.17
		bin × cue	14	686	.51	6.35	.000	.01
	Fixation Numbers	(intercept)	1	49		1237.89	.000	.95
		bin	7	343	.29	88.34	.000	.15
		cue	2	98	.71	0.19	.749	.00
		bin × cue	14	686	.49	3.17	.003	.00
	Fixation Durations	(intercept)	1	49		571.81	.000	.87
		bin	7	343	.30	42.54	.000	.12
		cue	2	98	.69	0.71	.446	.00
		bin × cue	14	686	.29	3.55	.007	.01

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S2: Results of the 3 (trial types) × 10 (bins in seconds) repeated-measures ANOVAs on individual autonomic responses measured in Experiment 1 and 2.

Study	**Dependent Variable**	**Predictor**	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
Experiment 1 (Threat context)	Heart Rate	(intercept)	1	49		13.19	.001	.08
		bin	9	441	.26	378.72	.000	.16
		cue	2	98	.91	32.72	.077	.02
		bin × cue	18	882	.27	13.07	.000	.04
	Skin Conductance	(intercept)	1	49		59.41	.000	.24
		bin	9	441	.18	32.17	.000	.07
		cue	2	98	.87	1319	.000	.10
		bin × cue	18	882	.16	12.50	.000	.04
	Pupil Dilation	(intercept)	1	49		146.73	.000	.65
		bin	9	441	.10	178.87	.000	.45
		cue	2	98	.97	45.45	.000	.09
		bin × cue	18	882	.15	24.47	.000	.03
Experiment 2 (Reward context)	Heart Rate	(intercept)	1	49		30.52	.000	.11
		bin	9	441	.26	327.23	.000	.18
		cue	2	98	.82	6.42	.005	.04
		bin × cue	18	882	.28	23.38	.000	.09
	Skin Conductance	(intercept)	1	49		1.39	.024	.00
		bin	9	441	.26	3.38	.029	.01
		cue	2	98	.56	52.90	.000	.33
		bin × cue	18	882	.09	39.68	.000	.27
	Pupil Dilation	(intercept)	1	49		114.47	.000	.51
		bin	9	441	.12	173.15	.000	.49
		cue	2	98	.79	72.70	.000	.22
		bin × cue	18	882	.08	35.63	.000	.06

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

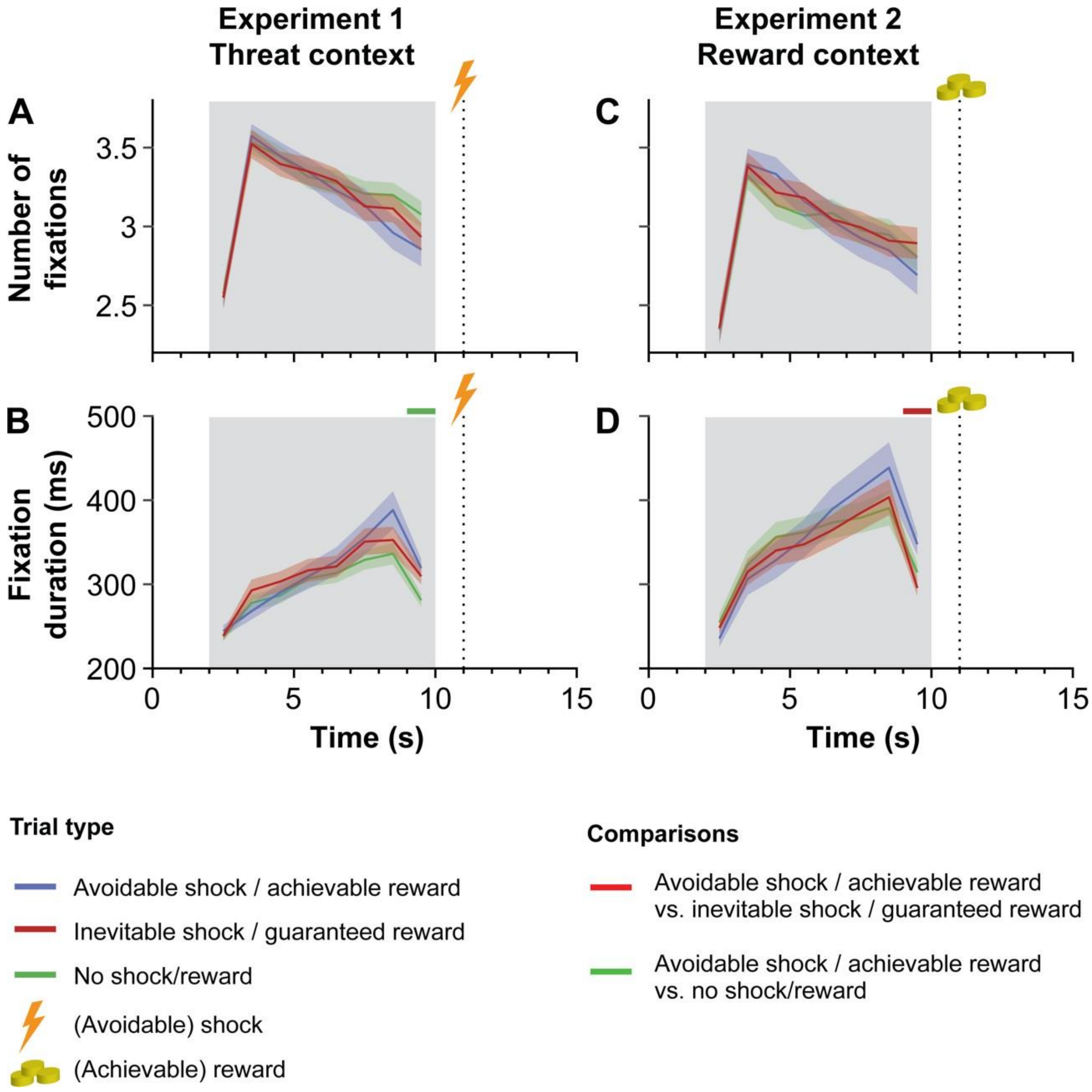


Figure S1: Fixation numbers and durations during the anticipation of an inevitable, no, or an avoidable shock in Experiment 1 (fixation numbers: A, fixation durations: B) or a guaranteed, no or achievable reward in Experiment 2 (fixation numbers: C, fixation durations: D. Shaded ribbons denote standard errors of the mean. Horizontal lines at the top of each figure indicate significant differences between avoidable shock (A and B) or achievable reward (C and D) trials and the other trial types (after false discovery rate correction). Shading in grey denotes the phase between onset and offset of picture presentation with the offset prompting quick responses in the avoidable shock and achievable reward trials, respectively.

Table S3: Comparisons between the time course of global center bias in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		1406.82	.000	.89
experiment	1	98		6.96	.010	.04
bin	7	686	.40	58.55	.000	.05
cue	2	196	.65	10.95	.000	.02
experiment × bin	7	686	.40	3.58	.000	.00
experiment × cue	2	196	.65	4.75	.010	.01
bin × cue	14	1372	.25	4.08	.000	.00
experiment × bin × cue	14	1372	.25	8.91	.000	.01

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S4: Comparisons between the time course of horizontal center bias in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		1139.12	.000	.85
experiment	1	98		6.54	.012	.03
bin	7	686	.40	43.00	.000	.05
cue	2	196	.61	0.75	.412	.00
experiment × bin	7	686	.40	2.78	.045	.02
experiment × cue	2	196	.61	5.56	.014	.01
bin × cue	14	1372	.25	1.40	.240	.00
experiment × bin × cue	14	1372	.25	6.80	.000	.01

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S5: Comparisons between the time course of vertical center bias in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		967.59	.000	.84
experiment	1	98		3.71	.006	.02
bin	7	686	.38	44.22	.000	.04
cue	2	196	.58	2.91	.085	.01
experiment × bin	7	686	.38	3.38	.023	.00
experiment × cue	2	196	.58	10.87	.000	.03
bin × cue	14	1372	.24	1.63	.177	.00
experiment × bin × cue	14	1372	.24	10.82	.000	.01

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S6: Comparisons between the time course of heart rate in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 10 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		38.75	.000	.09
experiment	1	98		0.00	.947	.00
bin	9	882	.26	69.91	.000	.16
cue	2	196	.86	9.48	.000	.03
experiment × bin	9	882	.26	0.83	.585	.00
experiment × cue	2	196	.86	0.24	.784	.00
bin × cue	18	1764	.30	31.53	.000	.05
experiment × bin × cue	18	1764	.30	3.45	.000	.00

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S7: Comparisons between the time course of skin conductance in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 10 (bins in seconds) ANOVA.

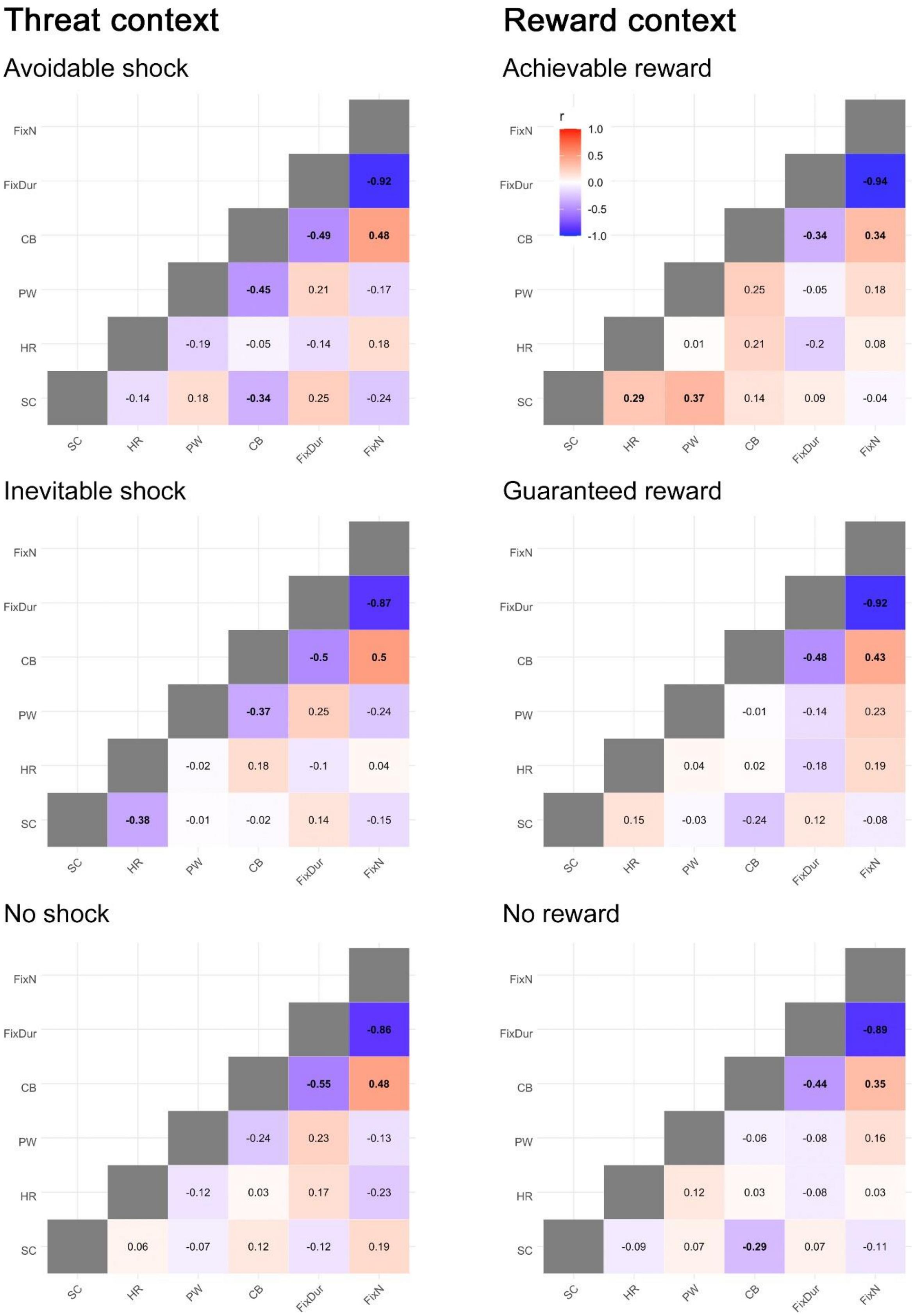
Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		87.22	.000	.24
experiment	1	98		35.76	.000	.12
bin	9	882	.19	27..23	.000	.03
cue	2	196	.93	42.24	.000	.12
experiment × bin	9	882	.19	23.64	.000	.03
experiment × cue	2	196	.93	16.63	.000	.05
bin × cue	18	1764	.14	41.40	.000	.08
experiment × bin × cue	18	1764	.14	17.59	.000	.03

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S8: Comparisons between the time course of pupil width in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 20 (bins in 500 ms) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		252.34	.000	.59
experiment	1	98		24.17	.000	.12
bin	19	1862	.11	333.93	.000	.45
cue	2	196	.91	103.95	.000	.12
experiment × bin	19	1862	.11	19.46	.000	.05
experiment × cue	2	196	.91	12.48	.000	.02
bin × cue	38	3724	.13	56.28	.000	.04
experiment × bin × cue	38	3724	.13	2.39	.039	.00

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.



Threat context

Reward context

Avoidable shock

Achievable reward

Inevitable shock

Guaranteed reward

No shock

No reward

Figure S2. Pearson correlations between fixation numbers (FixN), fixation durations (FixDur), global center bias (CB), pupil width (PW), heart rate (HR) and skin conductance (SC). Values were averaged across the second half of the anticipation phase in Experiment 1 (Threat context) and Experiment 2 (Reward context).

Table S9. Internal consistency for all dependent variables across all trials per trial type.

	Experiment 1: Threat context			Experiment 2: Reward context		
	Avoidable shock	Inevitable shock	No shock	Achievable reward	Guaranteed reward	No reward
Global Center Bias	.94	.87	.88	.95	.94	.90
Fixation Numbers	.96	.91	.89	.97	.94	.92
Fixation Durations	.95	.86	.77	.97	.91	.89
Skin Conductance	.68	.81	.80	.87	.71	.83
Heart Rate	.61	.62	.65	.73	.15	.41
Pupil width	.92	.92	.93	.95	.93	.94

Note. All dependent variables are averaged across the second half of the anticipation phase.

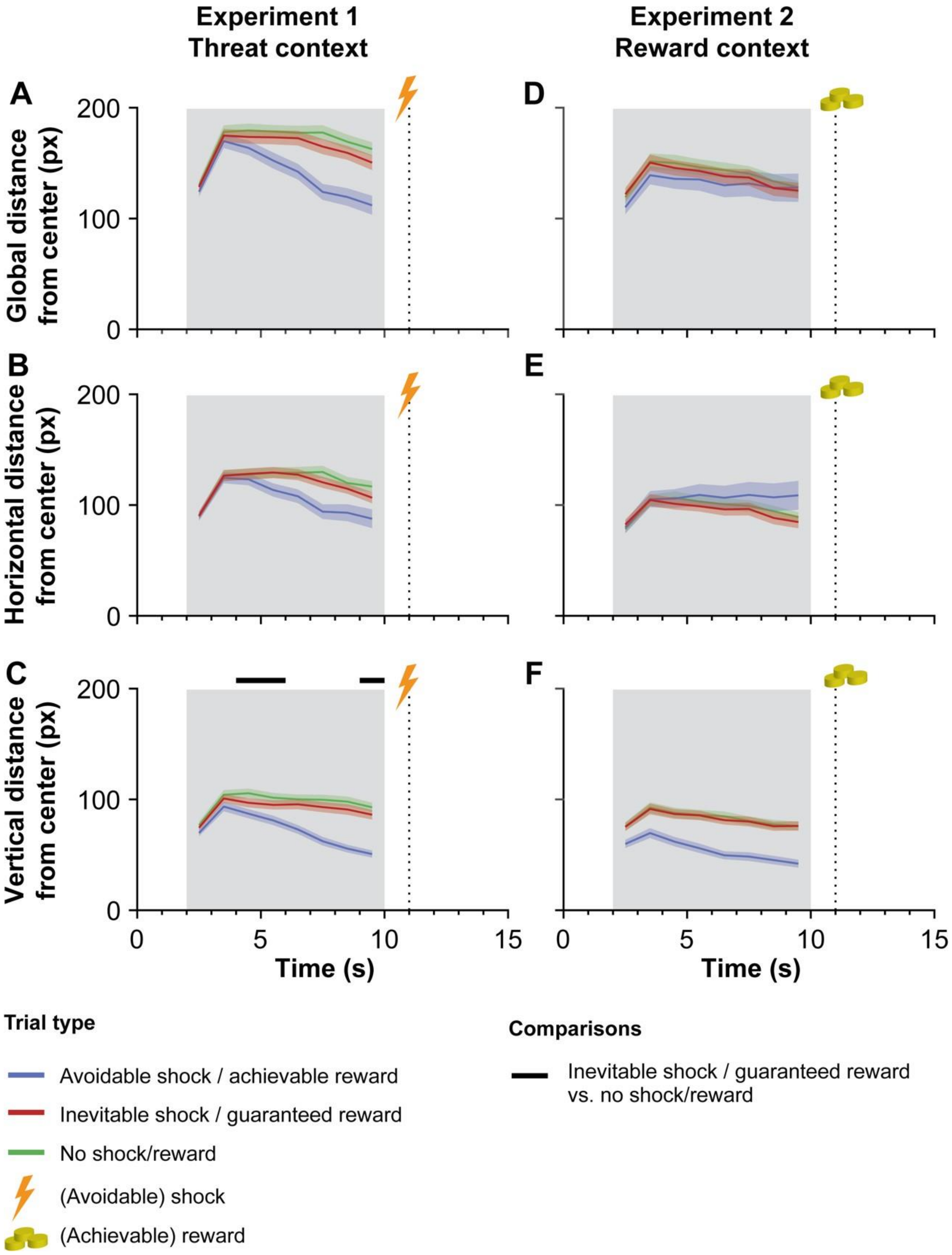


Figure S3. Changes in center bias during the anticipation of an inevitable, no or avoidable shock in Experiment 1 (global center bias: A, horizontal center bias: B, vertical center bias: C) or reward in Experiment 2 (global center bias: D, horizontal center bias: E, vertical center bias: F). Shaded ribbons denote standard errors of the mean. The black horizontal line at the top of each figure indicates significant differences between inevitable shock (A, B, and C) or guaranteed reward (D, E and F) trials and no shock/no reward trials (after false discovery rate correction). Vertical black lines depict onset and offset of picture presentation with the offset prompting quick responses in the avoidable shock and reward trials, respectively.

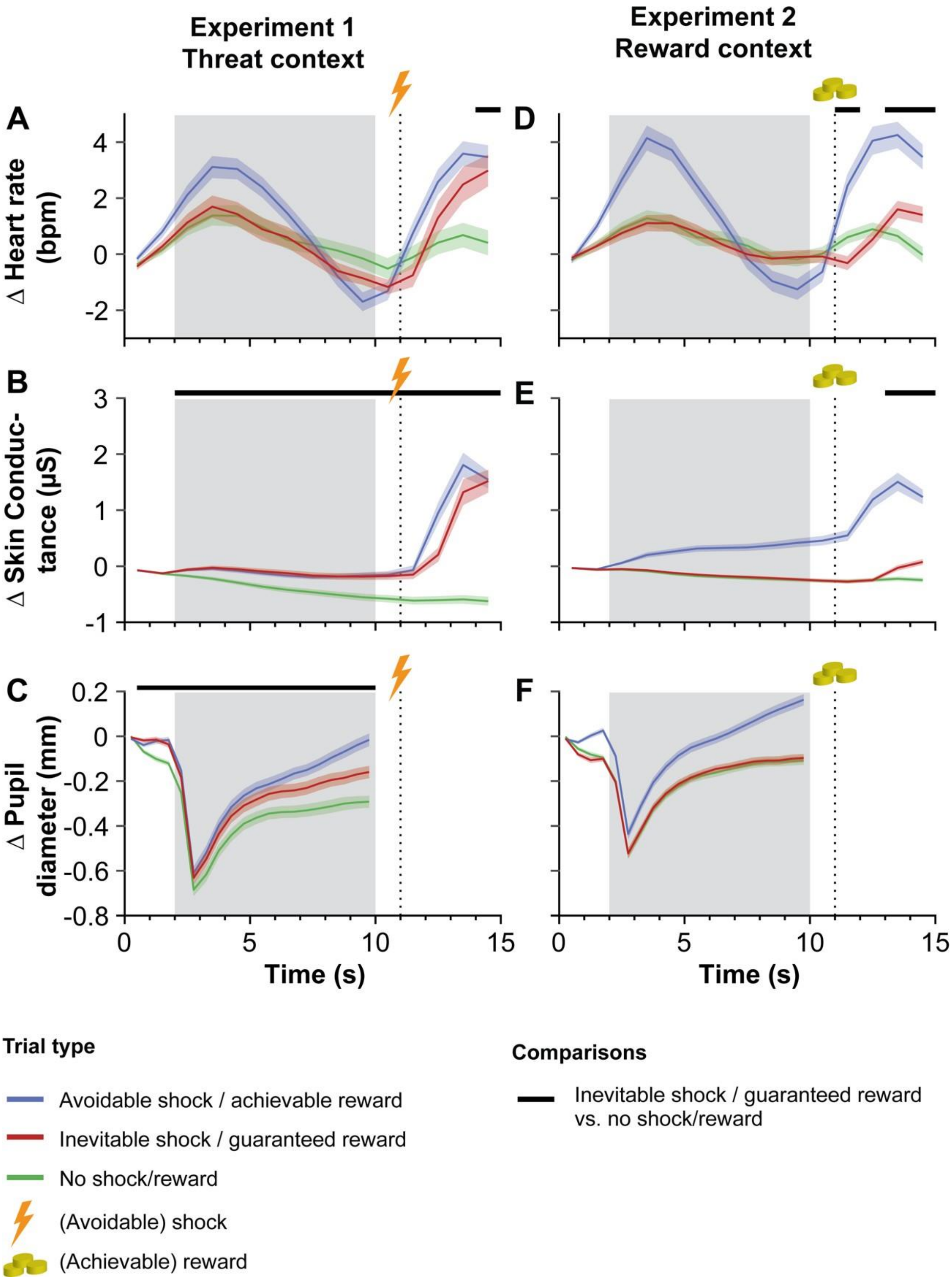


Figure S4. Autonomic responses during the anticipation of an inevitable, no or avoidable shock in Experiment 1 (heart rate: A, skin conductance: B, pupil width: C) or reward in Experiment 2 (heart rate: D, skin conductance: E, pupil width: F). Shaded ribbons denote standard errors of the mean. The black horizontal line at the top of each figure indicates significant differences between inevitable shock (A, B, and C) or guaranteed reward (D, E and F) trials and no shock/no reward trials (after false discovery rate correction). Vertical black lines depict onset and offset of picture presentation with the offset prompting quick responses in the avoidable shock and reward trials, respectively.

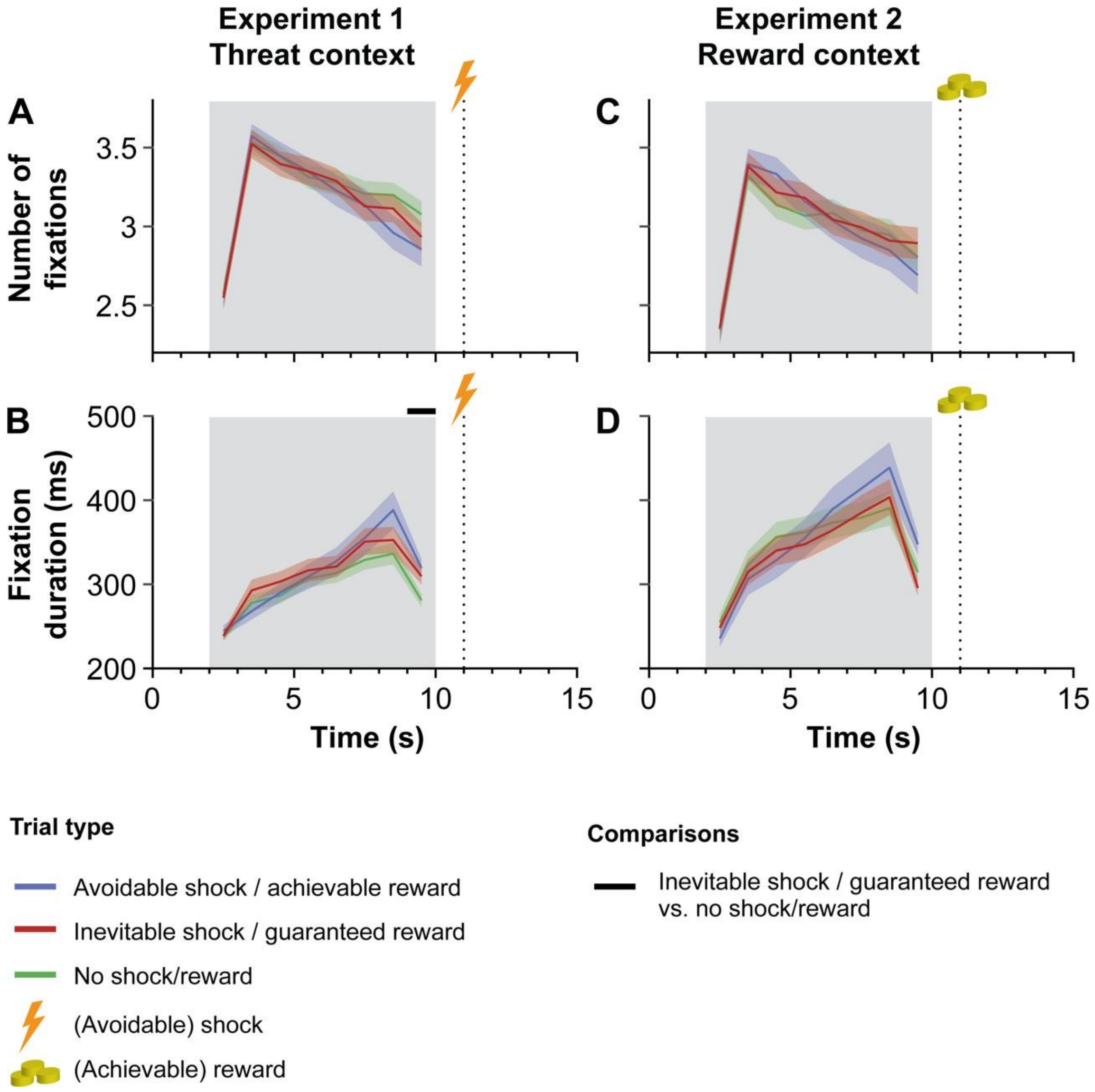


Figure S5. Changes in fixation numbers and durations during the anticipation of an inevitable, no or avoidable shock in Experiment 1 (A and B) or reward in Experiment 2 (C and D). Shaded ribbons denote standard errors of the mean. The black horizontal line at the top of each figure indicates significant differences between inevitable shock (A, B, and C) or guaranteed reward (D, E, and F) trials and no shock/no reward trials (after false discovery rate correction). Vertical black lines depict onset and offset of picture presentation with the offset prompting quick responses in the avoidable shock and reward trials, respectively.

Supplemental Material for Study 2

Fear lies in the eyes of the beholder – robust evidence for reduced gaze dispersion upon avoidable threat

Alma-Sophia Merscher and Matthias Gamer

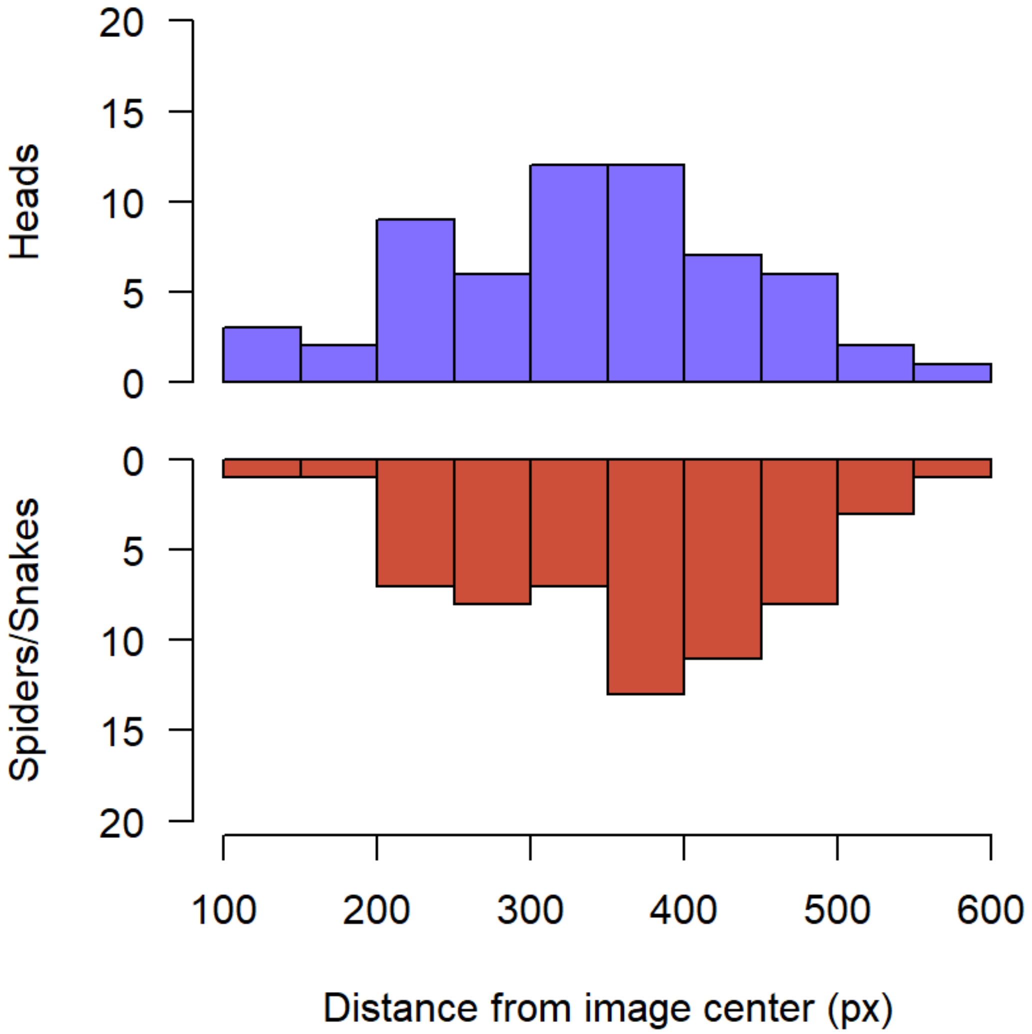


Figure S1. Distribution of heads (Experiment 1) and spiders/snakes (Experiment 2) across all pictures from the image center in pixels.

Table S1: Results of the 3 (trial types) × 8 (bins in seconds) repeated-measures ANOVAs on individual oculomotor responses measured in Experiment 1 and 2.

Study	**Dependent Variable**	**Predictor**	df_{Num}	df_{Den}	ε	F	p	η^2_g
Experiment 1 (Social Stimuli)	Center bias	(intercept)	1	49		6909.18	.000	.98
		bin	7	343	.41	37.94	.000	.12
		trial type	2	98	.66	55.64	.000	.26
		trial type × bin	14	686	.43	32.49	.000	.13
	Fixation numbers	(intercept)	1	49		2462.42	.000	.97
		bin	7	343	.39	51.85	.000	.11
		trial type	2	98	.63	36.52	.000	.10
		trial type × bin	14	686	.37	39.57	.000	.09
	Fixation durations	(intercept)	1	49		211.62	.000	.60
		bin	7	343	.27	39.45	.000	.10
		trial type	2	98	.55	20.18	.000	.12
		trial type × bin	14	686	.22	24.84	.000	.08
	Fixation durations on social stimuli	(intercept)	1	49		521.59	.000	.82
		bin	7	343	.50	187.45	.000	.45
		trial type	2	98	.86	1.20	.303	.00
		trial type × bin	14	686	.61	1.02	.420	.00
Experiment 2 (Threatening Stimuli)	Center bias	(intercept)	1	49		3097.76	.000	.97
		bin	7	343	.43	37.65	.000	.09
		trial type	2	98	.72	32.96	.000	.14
		trial type × bin	14	686	.37	20.56	.000	.06
	Fixation numbers	(intercept)	1	49		3665.37	.000	.98
		bin	7	343	.47	89.18	.000	.18
		trial type	2	98	.71	3.05	.071	.01
		trial type × bin	14	686	.49	7.72	.000	.02
	Fixation durations	(intercept)	1	49		133.16	.000	.58
		bin	7	343	.21	38.60	.000	.08
		trial type	2	98	.63	1.67	.202	.01
		trial type × bin	14	686	.15	5.43	.005	.01
	Fixation durations on threatening stimuli	(intercept)	1	49		968.49	.000	.91
		bin	7	343	.59	39.89	.000	.11
		trial type	2	98	.90	4.13	.023	.01
		trial type × bin	14	686	.61	1.17	.314	.00

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Fixation durations and numbers

The temporal profile of fixation durations (interaction trial type × second in Experiment 1: $F_{(14,686)} = 24.84$, $\varepsilon = .13$, $p < .001$, $\eta^2_g = .08$, Experiment 2: $F_{(14,686)} = 5.43$, $\varepsilon = .15$, $p = .005$, $\eta^2_g = .01$) and numbers (interaction trial type × second in Experiment 1: $F_{(14,686)} = 39.52$, $\varepsilon = .37$, $p < .001$, $\eta^2_g = .09$, Experiment 2: $F_{(14,686)} = 10.79$, $\varepsilon = .40$, $p < .001$, $\eta^2_g = .03$) differed between the trial types, with fewer and longer fixations in flight trials towards the end of the anticipation phase as compared to shock and safety trials (Fig. S2).

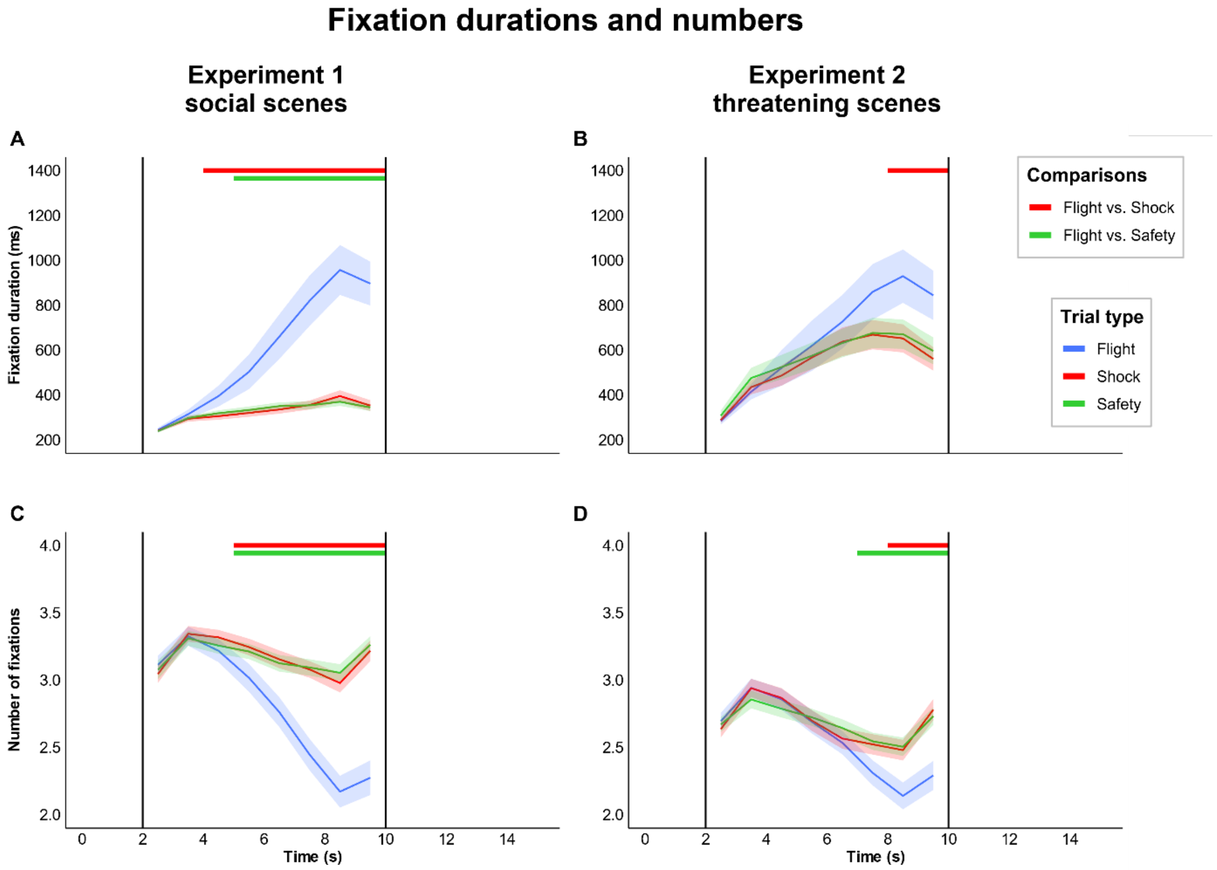


Figure S2. Temporal profile of fixation durations (Experiment 1: A, Experiment 2: B) and numbers (Experiment 1: C, Experiment 2: D) as a function of trial type. Vertical lines denote the onset and offset of the anticipation (i.e., picture viewing) phase. Horizontal lines on top of the graphs illustrate significant pairwise comparisons between avoidable shock trials and the other two trial types after false discovery rate correction. Note that average fixation durations might exceed bin size when fixations were spanning multiple bins.

Table S2: Results of the 3 (trial types) × 10 (bins in seconds) repeated-measures ANOVAs on individual autonomic responses measured in Experiment 1 and 2.

Study	**Dependent Variable**	**Predictor**	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
Experiment 1 (Social Stimuli)	Heart rate	(intercept)	1	49		0.13	.720	.00
		bin	9	441	.30	44.21	.000	.18
		trial type	2	98	.95	1.41	.250	.01
		trial type × bin	18	882	.29	10.50	.000	.04
	Skin conductance	(intercept)	1	49		56.00	.000	.34
		bin	9	441	.24	22.28	.000	.07
		trial type	2	98	.99	14.80	.000	.08
		trial type × bin	18	882	.19	18.37	.000	.05
	Pupil dilation	(intercept)	1	49		3410.17	.000	.98
		bin	19	441	.10	75.58	.000	.06
		trial type	2	98	.98	30.38	.000	.01
		trial type × bin	38	882	.17	24.06	.000	.00
Experiment 2 (Threatening Stimuli)	Heart rate	(intercept)	1	49		6.04	.018	.04
		bin	9	441	.30	28.66	.000	.13
		trial type	2	98	.98	1.66	.197	.01
		trial type × bin	18	882	.30	14.57	.000	.06
	Skin conductance	(intercept)	1	49		46.47	.000	.23
		bin	9	441	.24	18.53	.000	.05
		trial type	2	98	.83	17.75	.000	.11
		trial type × bin	18	882	.14	13.04	.000	.05
	Pupil dilation	(intercept)	1	49		3734.44	.000	.99
		bin	19	441	.12	118.85	.000	.11
		trial type	2	98	.94	18.47	.000	.01
		trial type × bin	38	882	.14	10.70	.000	.00

Note. ε calculated according to Greenhouse-Geisser; η^2_g refers to the generalized η^2.

Table S3: Comparisons between the time course of global center bias in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		8575.37	.000	.97
experiment	1	98		0.08	.775	.00
bin	7	686	0.38	74.44	.000	.10
trial type	2	196	0.69	87.02	.000	.19
experiment × bin	7	686	0.38	1.14	.330	.00
experiment × trial type	2	196	0.69	1.67	.198	.00
trial type × bin	14	1372	0.43	52.47	.000	.09
experiment × trial type × bin	14	1372	0.43	1.44	.196	.00

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S4: Comparisons between the time course of fixation durations in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		296.51	.000	.58
experiment	1	98		8.10	.005	.04
bin	7	686	0.21	74.67	.000	.08
trial type	2	196	0.59	13.92	.000	.04
experiment × bin	7	686	0.21	3.13	.061	.00
experiment × trial type	2	196	0.59	2.89	.085	.01
trial type × bin	14	1372	0.14	25.30	.000	.03
experiment × trial type × bin	14	1372	0.14	2.72	.068	.00

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S5: Comparisons between the time course of fixation numbers in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		4555.57	.000	.97
experiment	1	98		22.93	.000	.12
bin	7	686	0.41	98.53	.000	.10
trial type	2	196	0.69	31.02	.000	.05
experiment × bin	7	686	0.41	1.32	.267	.00
experiment × trial type	2	196	0.69	7.80	.002	.01
trial type × bin	14	1372	0.40	45.10	.000	.06
experiment × trial type × bin	14	1372	0.40	5.71	.000	.01

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S6: Comparisons between the time course of fixation durations on rois in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 8 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	***F***	***p***	η^2_g
(Intercept)	1	98		1489.98	.000	.88
experiment	1	98		127.16	.000	.38
bin	7	686	0.57	196.51	.000	.26
trial type	2	196	0.89	1.91	.156	.00
experiment × bin	7	686	0.57	27.16	.000	.05
experiment × trial type	2	196	0.89	4.60	.014	.01
trial type × bin	14	1372	0.67	0.73	.688	.00
experiment × trial type × bin	14	1372	0.67	1.48	.147	.00

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S7. Generalized linear mixed model for each second of the anticipation period with distance of social or threatening stimuli, respectively, and trial types (flight vs. shock vs. safety) as predictors. Subject ID was inserted as random intercept.

		Experiment 1		Experiment 2	
	bin	β	*p*	β	*p*
Intercept	1	113.68	**<.001**	84.24	**<.001**
	2	172.05	**<.001**	99.05	**<.001**
	3	220.92	**<.001**	136.08	**<.001**
	4	223.59	**<.001**	130.18	**<.001**
	5	211.26	**<.001**	119.35	**<.001**
	6	194.91	**<.001**	99.75	**<.001**
	7	181.29	**<.001**	121.09	**<.001**
	8	183.42	**<.001**	102.98	**<.001**
Distance of stimuli of interest (Experiment 1: social stimuli, Experiment 2: threatening stimuli)	1	0.42	**<.001**	0.49	**<.001**
	2	0.37	**<.001**	0.56	**<.001**
	3	0.20	**<.001**	0.42	**<.001**
	4	0.15	**<.001**	0.41	**<.001**
	5	0.13	**<.001**	0.39	**<.001**
	6	0.10	**.008**	0.38	**<.001**
	7	0.09	**.019**	0.28	**<.001**
	8	0.01	.733	0.29	**<.001**
Shock vs. flight	1	-20.32	.112	-29.98	**.041**
	2	3.22	.839	3.71	.829
	3	2.06	.908	26.61	.169
	4	7.40	.695	7.79	.697
	5	19.30	.313	39.97	.054
	6	33.79	.080	48.18	**.024**
	7	59.42	**.003**	34.30	.114
	8	38.87	.055	52.23	**.015**
Safety vs. flight	1	-7.45	.555	-2.97	.844
	2	1.31	.933	-24.94	.158
	3	-30.85	.080	4.67	.815
	4	-7.60	.684	-12.06	.558
	5	10.50	.579	23.30	.275
	6	27.88	.143	48.81	**.027**
	7	59.43	**.002**	22.16	.322
	8	54.21	**.007**	37.45	.089

Table S7 continued

Distance of stimuli of interest × shock vs. flight	1	0.09	**.013**	0.10	**.011**
	2	0.04	.933	0.03	.510
	3	0.08	.116	-0.01	.833
	4	0.11	**.040**	0.06	.234
	5	0.14	**.010**	0.02	.709
	6	0.15	**.005**	0.05	.399
	7	0.12	**.033**	0.11	.066
	8	0.24	**<.001**	0.09	.101
Distance of stimuli of interest × safety vs. flight	1	0.05	.142	0.02	.554
	2	0.05	.297	0.11	**.020**
	3	0.17	**.001**	0.06	.284
	4	0.14	**.011**	0.11	**.054**
	5	0.16	**.004**	0.07	.189
	6	0.19	**.001**	0.05	.390
	7	0.12	**.030**	0.16	**.007**
	8	0.20	**<.001**	0.13	**.022**

Note. Significant effects are indicated in bold.

Table S8: Comparisons between the time course of heart rate in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 10 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		1.94	.167	.01
experiment	1	98		3.71	.057	.01
bin	9	882	0.31	69.35	.000	.15
trial type	2	196	0.97	1.14	.320	.00
experiment × bin	9	882	0.31	3.17	.028	.01
experiment × trial type	2	196	0.97	1.92	.150	.00
trial type × bin	18	1764	0.31	24.44	.000	.05
experiment × trial type × bin	18	1764	0.31	0.99	.431	.00

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S9: Comparisons between the time course of skin conductance in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 10 (bins in seconds) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		102.43	.000	.29
experiment	1	98		1.29	.259	.00
bin	9	882	0.25	39.30	.000	.06
trial type	2	196	0.95	29.89	.000	.09
experiment × bin	9	882	0.25	1.51	.220	.00
experiment × trial type	2	196	0.95	3.13	.049	.01
trial type × bin	18	1764	0.18	27.70	.000	.04
experiment × trial type × bin	18	1764	0.18	2.73	.040	.00

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S10: Comparisons between the time course of pupil width in Experiment 1 and Experiment 2, using a 2 (experiments) × 3 (trial types) × 20 (bins in 500 ms) ANOVA.

Predictor	df_{Num}	df_{Den}	**ε**	F	p	η^2_g
(Intercept)	1	98		7144.58	.000	.99
experiment	1	98		13.52	.000	.11
bin	19	1862	0.12	189.62	.000	.09
trial type	2	196	0.98	45.12	.000	.01
experiment × bin	19	1862	0.12	10.69	.000	.01
experiment × trial type	2	196	0.98	0.06	.943	.00
trial type × bin	38	3724	0.16	29.25	.000	.00
experiment × trial type × bin	38	3724	0.16	1.21	.299	.00

Note. ε calculated according to Greenhouse-Geisser; η^2g refers to the generalized η^2.

Table S11. Correlations between the self-report anxiety measures and the main dependent variables across participants in flight trials in both Experiments.

		STAI-T	ASI	SNAQ	FSQ
Experiment 1	Center bias	.03	.13	-	-
	Fixation duration	-.14	.09	-	-
	Fixation number	.19	.13	-	-
	Fixation duration on ROI (1st half of picture presentation)	-.10	.08	-	-
	Fixation duration on ROI (2nd half of picture presentation)	-.08	.08		
	Skin conductance	-.10	-.15	-	-
	Heart rate	-.12	-.02	-	-
	Pupil dilation	.14	.04	-	-
Experiment 2	Center bias	.17	.01	**-.33**	.21
	Fixation duration	.03	-.07	**.32**	-.16
	Fixation number	.12	.19	-.22	.25
	Fixation duration on ROI (1st half of picture presentation)	-.10	-.14	-.15	.06
	Fixation duration on ROI (2nd half of picture presentation)	.01	.01	-.25	.18
	Skin conductance	.03	.17	-.07	.10
	Heart rate	-.04	-.13	-.13	.26
	Pupil dilation	**.30**	.26	.01	.20

Note. STAI-T = State-Trait Anxiety Inventory, trait version (Laux et al., 1981), ASI = Anxiety Sensitivity Index (Kemper et al., 2009), SNAQ = Snake Questionnaire (Klorman et al., 1974), FSQ = Fear of Spiders Questionnaire (Rinck et al., 2002); ROI = Region of interest. Please note that SNAQ and FSQ were only assessed in Experiment 2. Autonomic and oculomotor measures were averaged across all time bins during the second half of the stimulus presentation (bin 5 to 8), except for Fixation durations on ROIs, which was averaged for both the first (bin 1 to 4) and second half of the stimulus presentation (bin 5 to 8). Only one of the observed correlations was statistically significant. Bold values denote significant correlations ($p < .05$).

Supplemental Material for Study 3

Can I see it in the eyes? – An investigation of freezing-like motion patterns in response to avoidable threat

Alma-Sophia Merscher and Matthias Gamer

Mediolateral and omnidirectional body sway

To provide a more complete understanding of changes in postural sway during anticipation of avoidable threat, beyond those reported in the manuscript in the anterior-posterior direction, we additionally explored changes in body sway on the mediolateral (Figure S1A) and both axes (Figure S1B) using 3 (trial type) × 10 (bins in seconds) repeated measures ANOVAs, respectively. Note that we considered 15 bins for data illustration. There were no significant differences in mediolateral sway between trials types (main effect trial type: $F_{2,98}= 1.70$, $\varepsilon = 0.78$, $p = .195$, $\eta^2_g = 0.02$), over time (main effect bin: $F_{9,441}= 1.98$, $\varepsilon = 0.31$, $p = .123$, $\eta^2_g = 0.01$) or between the temporal profiles of the trial types (interaction effect trial type × bin: $F_{18,882}= 1.48$, $\varepsilon = 0.38$, $p = .176$, $\eta^2_g = 0.01$, see Figure S1A). When considering an integrated assessment of movements on both axes, body sway did not generally differ between trial types (main effect trial type: $F_{2,98}= 2.78$, $\varepsilon = 0.80$, $p = .080$, $\eta^2_g = .03$) but over time (main effect bin: $F_{9,441}= 7.41$, $\varepsilon = 0.32$, $p < .001$, $\eta^2_g = 0.02$). Specifically, when awaiting an avoidable shock, participants swayed increasingly less towards the end of the anticipation phase as compared to the other two trial types (interaction effect trial type × bin: $F_{18,882}= 2.13$, $\varepsilon = 0.38$, $p = .041$, $\eta^2_g = 0.01$; see Figure S1B).

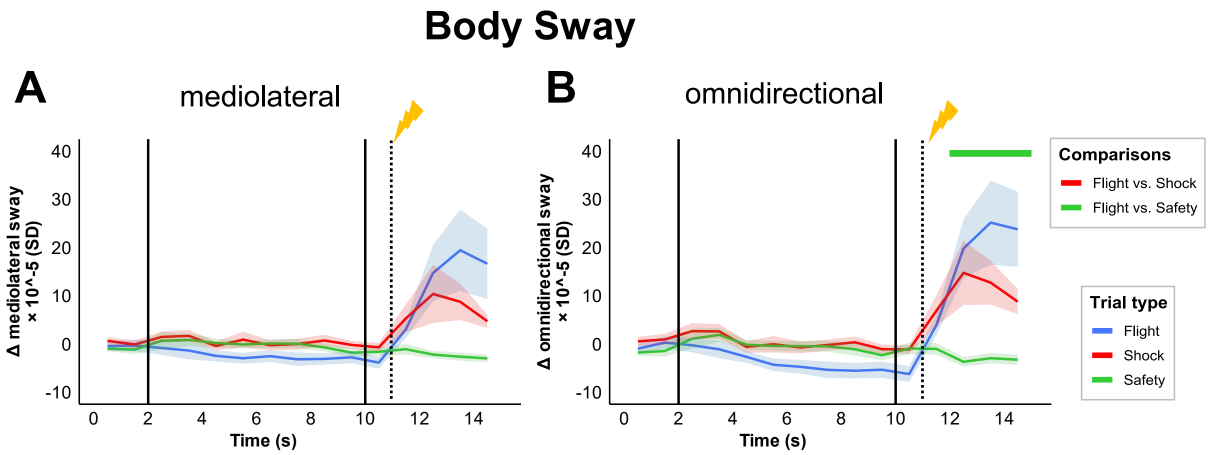


Figure S1. Temporal changes in body sway on the mediolateral axis (A) and both axes (B, Euclidian distance from the center of pressure) as a function of trial type starting at cue onset. The black vertical lines denote picture on- and offsets. The green and red horizontal bars on top of each plot indicate false-discovery rate corrected significant pair-wise comparisons between flight and shock (red) or safety trials (green), respectively.

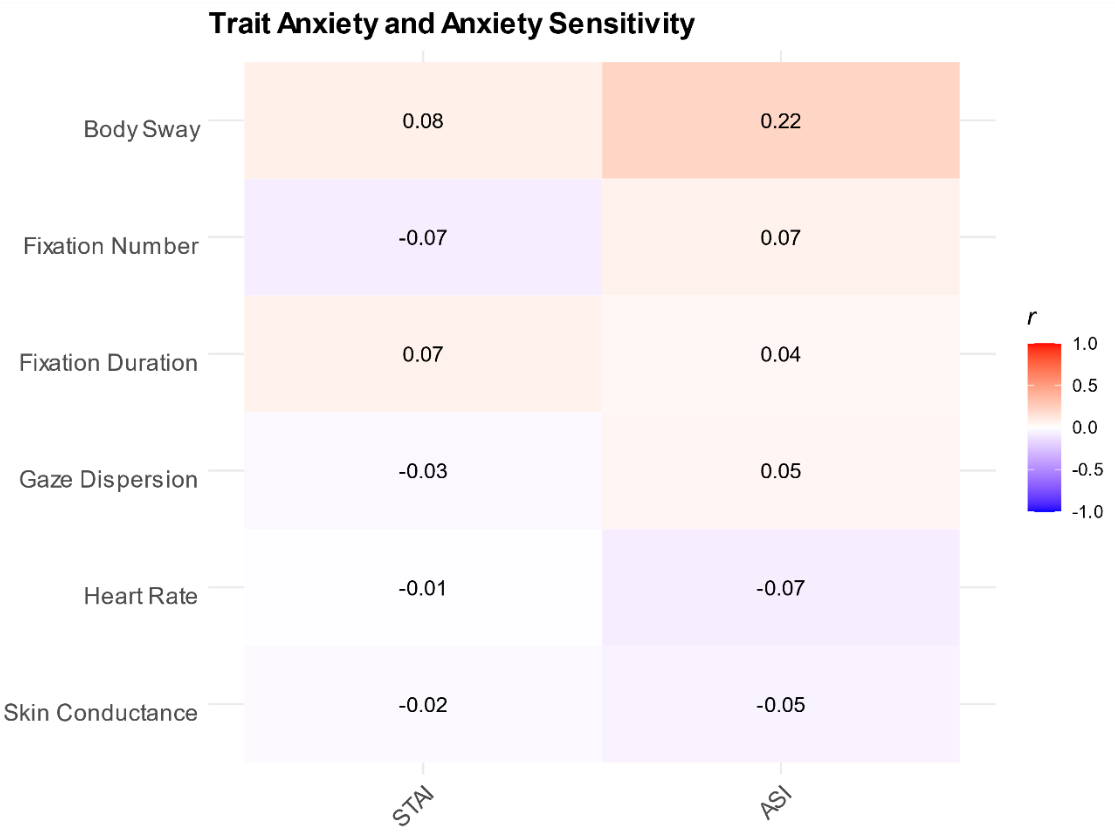


Figure S2. Correlations between the self-report anxiety measures and the main dependent variables across participants in flight trials. STAI = State-Trait Anxiety Inventory, trait version (Laux et al., 1981), ASI = Anxiety Sensitivity Index (Kemper et al., 2009). Autonomic and (oculo)motor measures were averaged across all time bins during the second half of the stimulus presentation (bin 5 to 8). None of the observed correlations was statistically significant.

Supplemental Material for Study 4

Unraveling the (oculo)motor and autonomic patterns of defensive states in humans in an unconstrained, immersive virtual world

Lea Hildebrandt, Alma-Sophia Merscher, and Matthias Gamer

Post-experimental questionnaire

Based on a questionnaire developed by Mobbs and colleagues (2008), subjects completed a post-experimental questionnaire which asked them to indicate on a 10-point analog Likert scale how much they dreaded each phase. To support their memory for each phase, we showed them exemplary screenshots of each phase in the virtual world (see Figure S1). An example of a question is: ‘How anxious were you when you in the situation depicted in the picture?’

Figure S1. Selection of exemplary pictures per phase that were part of the post-experimental questionnaire. Based on these pictures, participants were asked to rate how much they dreaded each of the phases as experienced in the experiment on a 10-point Likert scale.

Table S1. Distribution of shots fired.

Average no. shots	Shots	No shots	Correct hits	Forced misses	Real misses	Errors
7.73	83.78 %	16.23 %	61.23 %	16.77 %	12.66 %	9.35 %

Note. Average no. shots: Mean number of shots fired per participant across the experiment; Shots: Shots fired during the Circa-strike trials (regardless of whether correct hit or not); No shots: percentage of Circa-strike trials where no shot was fired; Correct hits: Participant shot and hit attacker during the Circa-strike phase; Forced misses: Participant correctly shot attacker during Circa-strike phase but shot was counted as miss (~ in 20% of the cases); Real misses: Participant shot but missed the attacker during Circa-strike phase; Errors: Participant shot the attacker too early (i.e. when the attacker had not drawn the baseball bat and was not attacking yet). A Pearson's product-moment correlation showed that trait aggression was not significantly related to shooting errors as previously shown ($r_{(100)}$= -.04, p = .698; Gladwin et al, 2016).

Table S2. Effect of trait anxiety and trait aggression on the dependent variables.

Interindividual factors	**Dependent Variable**	**Predictor**	df_{Num}	df_{Den}	***F***	***p***	η^2_g
Trait Anxiety	Perceived dread	Phase	5	501.93	14.54	<.001	0.13
		STAI-T	1	99.31	0.46	.497	0.05
		Phase × STAI-T	5	501.93	2.71	.020	0.03
	Movement Speed	Phase	5	486.76	12.49	<.001	0.11
		STAI-T	1	97.62	3.30	.072	0.03
		Phase × STAI-T	5	486.71	2.67	.021	0.03
	Body Rotation	Phase	5	486.28	0.96	.442	<0.01
		STAI-T	1	96.93	0.90	.345	<0.01
		Phase × STAI-T	5	486.16	0.15	.981	<0.01
	Head Rotations	Phase	5	486.92	1.41	.218	0.01
		STAI-T	1	97.78	2.77	.099	0.03
		Phase × STAI-T	5	486.87	0.75	.585	<0.01
	Eye Rotations	Phase	5	486.97	6.85	<.001	0.07
		STAI-T	1	97.94	0.80	.375	<0.01
		Phase × STAI-T	5	486.97	2.16	.057	0.02
	Heart rate	Phase	5	435	1.18	.317	0.01
		STAI-T	1	87	1.31	.256	0.01
		Phase × STAI-T	5	435	0.51	.766	<0.01
	Skin Conductance	Phase	5	440	0.28	.924	<0.01
		STAI-T	1	88	0.12	.726	<0.01
		Phase × STAI-T	5	440	0.15	.980	<0.01
Trait Aggression	Perceived dread	Phase	5	501.92	5.57	<.001	0.05
		STAXI	1	101.69	2.53	.115	0.02
		Phase × STAXI	5	501.92	0.75	.588	<0.01
	Movement Speed	Phase	5	486.83	11.47	<.001	0.11
		STAXI	1	97.70	0.04	.835	<0.01
		Phase × STAXI	5	486.79	1.85	.102	0.02
	Body Rotation	Phase	5	486.42	0.89	.486	<0.01
		STAXI	1	97.08	0.56	.456	<0.01
		Phase × STAXI	5	486.32	1.12	.346	0.01
	Head Rotations	Phase	5	486.79	3.10	.009	0.03
		STAXI	1	97.66	1.61	.207	0.02
		Phase × STAXI	5	486.75	0.66	.653	<0.01

Table S2 continued

	Eye Rotations	Phase	5	486.97	13.84	<.001	0.12
		STAXI	1	97.92	0.40	.529	<0.01
		Phase × STAXI	5	486.95	3.77	.002	0.04
	Heart rate	Phase	5	435	0.67	.669	<0.01
		STAXI	1	87	1.46	.231	0.02
		Phase × STAXI	5	435	0.24	.943	<0.01
	Skin Conductance	Phase	5	440	0.74	.597	<0.01
		STAXI	1	88	2.53	115	0.03
		Phase × STAXI	5	440	0.66	.653	<0.01

Note. Degrees of freedom were adjusted using Satterthwaite’s approximation.

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Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design Methods Development	MG	AM			
Data Collection	AM				
Data Analysis and Interpretation	AM,MG	PT			
Manuscript Writing					
Writing of Introduction	AM	MG	PT	PP	
Writing of Materials & Methods	AM,MG	PT			
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Data Collection	AM				
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Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design Methods Development	LH	AM	MG		
Data Collection	LH	AM			
Data Analysis and Interpretation	LH	AM, MG			
Manuscript Writing					
Writing of Introduction	AM, LH	MG			
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3	LH	AM,MG			
4	LH	AM,MG			
5	LH	AM,MG			
6	LH	AM,MG			
7	LH	AM,MG			
8	LH	AM,MG			
9	LH	AM,MG			
Table	**Author Initials,** Responsibility decreasing from left to right				
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2	AM,LH	MG			

I also confirm my primary supervisor’s acceptance.

Doctoral Researcher’s Name Date Place Signature

Publication list

Peer-reviewed journal article

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Peer-reviewed short-paper for a conference

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Contributions to international conferences

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Merscher, A. S., & Gamer, M. Autonomic and oculomotor indices of action preparation in response to threat and reward. Abstract for poster presentation, 2021, Jahrestagung Psychologie und Gehirn, virtual congress, June 2021.

Merscher, A. S., & Gamer, M. Centralized Gaze as a threat-specific component of defensive states in humans. Abstract for poster presentation, 2021, World Association for Stress Related and Anxiety Disorders (WASAD), 3rd International Congress of the World Association for Stress Related and Anxiety Disorders, Vienna, October 2021.

Merscher, A. S., & Gamer, M. Fear lies in the eyes of the beholder – reduced gaze dispersion upon avoidable threat. Abstract for poster presentation, 2022, Jahrestagung Psychologie und Gehirn, virtual congress, June 2022.

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I hereby confirm that my thesis entitled *To Fear or not to Fear: Unraveling the (Oculo)motor and Autonomic Components of Defensive States in Humans* is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

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Hiermit erkläre ich an Eides statt, die Dissertation *Vor Furcht Erstarren: Charakterisierung der (Okulo)motorischen und Autonomen Komponenten Menschlicher Defensivreaktionen* eigenständig, d.h. insbesondere selbstständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

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