nature reviews neuroscience

https://doi.org/10.1038/s41583-023-00736-3

Review article

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Defensive responses: behaviour, the brain and the body

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Abstract

Most animals live under constant threat from predators, and predation has been a major selective force in shaping animal behaviour. Nevertheless, defence responses against predatory threats need to be balanced against other adaptive behaviours such as foraging, mating and recovering from infection. This behavioural balance in ethologically relevant contexts requires adequate integration of internal and external signals in a complex interplay between the brain and the body. Despite this complexity, research has often considered defensive behaviour as entirely mediated by the brain processing threat-related information obtained via perception of the external environment. However, accumulating evidence suggests that the endocrine, immune, gastrointestinal and reproductive systems have important roles in modulating behavioural responses to threat. In this Review, we focus on how predatory threat defence responses are shaped by threat imminence and review the circuitry between subcortical brain regions involved in mediating defensive behaviours. Then, we discuss the intersection of peripheral systems involved in internal states related to infection, hunger and mating with the neurocircuits that underlie defence responses against predatory threat. Through this process, we aim to elucidate the interconnections between the brain and body as an integrated network that facilitates appropriate defensive responses to threat and to discuss the implications for future behavioural research.

Sections

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Introduction

Most animals live under constant threat of predation, and most predators are themselves prey to other, larger predators. Consequently, natural selection endowed humans and other animals with various innate and automatically activated defensive behaviours[1], which provide protection from being harmed or killed in dangerous situations. In a landmark paper[2] in 1970, in stark contrast to the prevailing theories at the time[3,4], Bolles argued that predatory threats induced specific innate defence responses rather than arbitrary actions whose usefulness to the animal in threat avoidance required reinforcement learning (provided that the animal survived long enough). This was later elaborated in the models developed by Bolles and Fanselow[5] and Fanselow and Lester[6], the latter of which described defensive response selection along a predatory imminence continuum. The perceived spatiotemporal proximity or imminence of the predator by an animal determines which defensive responses might be effective and how much time is available to select any of them.

Defensive responses vary with threat imminence in most animals studied, including humans, rodents and non-human primates[7–9]. Defensive behaviours such as risk assessment, avoidance, freezing, escape, defensive attack or death feigning in response to threatening stimuli along the predatory imminence continuum have been characterized in a variety of species, ranging from crayfish to humans[10,11]. Moreover, Fanselow also proposed that certain kinds of stress can distort the predatory imminence continuum such that defensive responses "intrude into times when organisms should be engaging in other adaptive behaviors"[11]. Many neuropsychiatric disorders, particularly anxiety-related or fear-related disorders, such as generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD), are characterized by an inappropriate intrusion of defensive responses into daily life situations[11,12]. Thus, the pathology of these disorders might be better understood by studying defensive responses within this threat imminence framework.

Box 1

A selection of brain-control theories of defensive behaviour

Many comprehensive reviews that consider defensive behaviour to be entirely mediated by brain processes have been published. For example, Gross and Canteras emphasize data that support the existence of multiple distinct fear pathways in the brain and describe how each pathway is specialized to deal with different types of innate and learned environmental threat[95]. Silva et al. summarize current knowledge on the common organization of brain circuits processing innate fear responses to various threats including predators, aggressive conspecifics and painful stimuli[205]. Evans et al. review evidence supporting escape as a flexible behaviour that relies on the intersection between cortical and subcortical circuits and highlight the power of using escape behaviour as a model for systems neuroscience[124]. Branco and Redgrave discuss the computational challenges of generating escape behaviour and provide an overview of the neural mechanisms that implement different stages of escape[206].

Past research has often considered defensive behaviours to be entirely mediated by brain processes (Box 1). Although behaviour is the readout of the inner workings of an organism, the functions of peripheral processes are often neglected or viewed as contingent outputs of brain processes. However, increasing evidence suggests that the endocrine, immune, reproductive and digestive (including gut microbiota) systems, as well as the autonomic nervous system, influence behavioural responses to threats[13–27]. For example, the endocrine, immune and gastrointestinal systems become altered under various conditions, such as infection or starvation[15,28–33]. In turn, alterations in these systems influence defensive behaviour by shifting defence response activation thresholds and/or modifying the vigour of their execution[14–18,20–22,32–35]. In addition, sex hormones influence the behavioural responses of an organism to perceived threats[24–27].

Moreover, the enduring selection pressure of predatory threats probably shaped not only the neurocircuits underlying defensive responses in the brain but also the structure of the peripheral nervous system and organs, including the neuroendocrine and neuroimmunological interplay between them. Defence against predators needs to be balanced against other adaptive behaviours that are essential for survival and reproduction, such as foraging, mating and recovery from illness, and this balance requires adequate integration of internal and external signals in a complex interplay between the brain and the body[36]. In humans, this interplay can be unfavourably affected by several factors in modern environments, such as psychosocial stress or inadequate nutrition. For example, gut dysbiosis, inflammation and metabolic syndrome are associated with an increased risk for several neuropsychiatric diseases, such as anxiety disorders or PTSD[37–40]. Thus, a comprehensive perspective should acknowledge that the behavioural responses of an organism to threat are realized by the mutual regulation of processes in both the brain and body[41].

In this Review, we aim to discuss the neurocircuits underlying defence behaviour while considering two crucial aspects: their function within the threat imminence framework and their interplay with peripheral systems in different ethologically relevant contexts. First, we discuss the different phases within the threat imminence framework and the mechanisms that regulate the selection of defence responses in rodents. We then summarize the neural circuits recruited during the defence responses found within the threat imminence framework, with an emphasis on the amygdala and other subcortical structures[42]. Second, we discuss how various peripheral signals related to infection and nutritional and reproductive state shape defensive behaviours, highlighting the important roles of the subcortical structures in the neural circuits underlying defence behaviour for integrating these peripheral responses. Last, we briefly discuss the implications of brain–body interactions and resulting distortions in the threat imminence continuum for future behavioural and neuropsychiatric research.

Threat imminence and defensive behaviour

Over the past century, empirical naturalistic observation and laboratory research, as well as theoretical studies, have established that a defensive behaviour framework in response to threat is exhibited across species[43]. Within the threat imminence framework, animals choose from a distinct set of defensive behaviours that reduce the likelihood of predation or physical damage resulting from threats. Pre-encounter, post-encounter and circa-strike phases, characterized by distinct defensive responses from animals, can be defined by the imminence (or proximity) of the threat – ranging from low (potential) threat, through detection of a predator in the environment, to detection

by the predator and predator contact[2,5,6,44] (Fig. 1a). The pre-encounter phase is the period during which a risk of threat is present but there is no immediate evidence of danger. In this phase, risk assessment and avoidance behaviours predominate[45]. When a predator has been detected and the threat imminence increases, the post-encounter phase commences, during which animals exhibit the freezing state as a defensive response[46]. Lastly, the circa-strike phase begins when the predator starts to pursue the prey, increasing the proximity of the threat to an animal. The behavioural responses in this phase depend on the perceived spatiotemporal proximity of the predator and can be further subdivided into low-proximity (escape and persistent freezing) or high-proximity (defensive attack and tonic immobility) defensive responses. In humans, the pre-encounter, post-encounter and circa-strike phases within the threat imminence framework have been proposed to map to the behaviours and neural circuits underpinning the emotional states of anxiety, fear and panic, respectively[44,47].

Animals use risk orienting and risk assessment processes, such as sniffing or visual and auditory screening of the environment, to evaluate the perceived threat level and its proximity, and the threat imminence detected determines the utilized distinct defensive responses, which often proceed sequentially following the order of the pre-encounter, post-encounter and circa-strike phases[1,48–50]. In rodents, the pre-encounter phase is characterized by a combination of risk assessment through active exploration of the environment and risk avoidance, typically reflected in a preference for dark and protected places over open areas[45]. The freezing state involves not only an immediate stilling of all movement, which decreases the likelihood of detection by the predator in the post-encounter phase, but also increased muscle tone and behavioural vigilance to the threat, which are needed for successful escape or defensive attack — the 'fight or flight' response — in the circa-strike phase[46,51]. However, freezing is not necessarily constrained to the post-encounter phase and it can be the only defensive response observed in the circa-strike phase under certain circumstances, rather than only preceding escape or defensive attack responses[52–54]. Indeed, a freezing state sustained for long time periods — persistent freezing — can occur during the circa-strike phase[52,53,55]. High-imminence threats can elicit an escape response in the circa-strike phase only if an escape route is available. Otherwise, persistent freezing or launching a defensive attack are the defensive responses used by rodents during an inescapable attack, depending on the proximity of the threat[56]. Tonic immobility — death feigning — can be viewed as the last-resort defensive response to a predator attack when either escape or defensive attack are too dangerous[1,57–59]. This can typically be observed when animals are physically restrained, and it can last from seconds to hours, even after release from the constraint[59]. Besides reduced breathing and heart rates, tonic immobility also involves tongue protrusion and wide open eyes. The latter two are features also seen in dead animals across several mammalian species, but not observed during freezing[60]. Thus, in this Review, tonic immobility has been incorporated as the last defensive response animals use during the circa-strike phase (Fig. 1a).

Although the triggers for and the execution of an innate threat response are species specific[61,62] — human defensive behaviours are typically more complex than those of rodents described above[63] — a continuum of escalating defensive responses to threat including freezing, fight or flight responses and tonic immobility has been evolutionarily conserved in humans[1]. High-imminence proximal threats provoke fast, reflexive defensive behaviours (such as the fight–flight–freeze response) in humans, whereas more distal threats of low imminence permit risk assessment and exploration (such as weighing the threat value and searching for safety)[64]. Thus, these defensive responses probably reflect conserved threat coping mechanisms that were present in the most recent common ancestor of primates and rodents[65]. Next, we discuss the neural circuits subserving defensive behaviours across the threat imminence continuum.

Neural circuits underlying defensive behaviour

It is difficult to obtain evidence of causal mechanisms underlying specific defensive behaviours and to identify the neural circuits and specific neuronal populations involved in humans. An alternative approach for investigating causality in the neural circuits underlying defensive behaviour is to use rodent models, which are more accessible and manipulable. Modern tools, such as optogenetics, allow investigation of whether specific neural circuits have a causal role in various types of defensive behaviour.

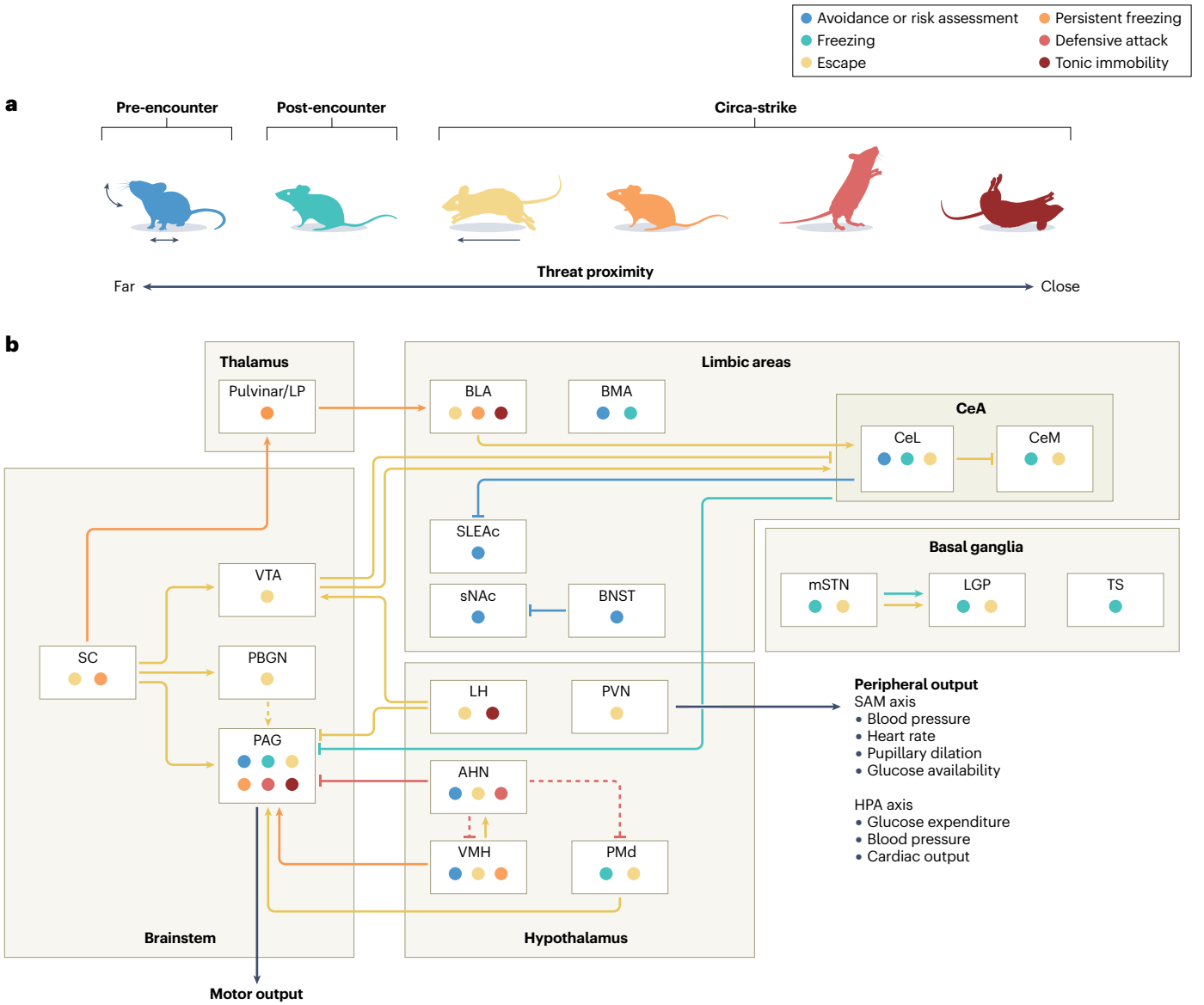
The visual system provides one of the major sensory inputs for detecting imminent threats across animal species[66–69]. Experimentally, the visual looming assay provides rapidly expanding dark overhead spots as visual cues of an imminent 'looming' threat that can trigger circa-strike phase defensive behaviours, such as escape and persistent freezing, in non-human primates and rodents[52–54,69,70]. Imminent threat detected by the auditory system can also influence emotion and visual information processing in humans and non-human primates[71–74]. The auditory looming assay, which presents sudden increasing sounds induced by broadband white noise as an imminent looming threat, can trigger sequential freezing and escape responses in rodents[51]. During the auditory looming assay, freezing can be considered a post-encounter stage response that prepares the animal for a successful fight or flight response in the circa-strike phase.

The systematic modulation of defensive behaviour can be achieved experimentally by taking advantage of the accurate temporal control available for visual and auditory looming stimuli and the ability to vary multiple parameters such as luminance, contrast, speed and sound intensity[51,69,75,76]. In 'Circuits involved in high-imminence threats', we review studies investigating neural circuits in subcortical brain regions that mediate defensive behaviour, with most of them using either the visual or auditory looming assay, the threat of an actual predator or optogenetic or pharmacologic activation of specific brain areas to trigger defensive responses in animals. In some contexts, foot-shock or physical restraint that can mimic more proximal threat are also discussed. The studies predominantly investigate defensive behaviour in mice because they are highly suitable for experimental investigation of neural circuits, but when appropriate, related findings in other species, including humans, are noted explicitly.

Circuits involved in high-imminence threats

The dominant forms of defensive response to visual and auditory looming stimuli are associated with the post-encounter phase (freezing) and with the more distal predator interactions in the circa-strike phase when the threat proximity is lower (persistent freezing and escape). When the threat proximity is higher in the circa-strike phase, the more proximal predator interactions are commonly associated with mechanosensory stimulation, and the dominant forms of defensive response are defensive attack or tonic immobility (Fig. 1a). Various subcortical neural circuits have been revealed as responsible for mediating defensive behaviours in response to high-imminence threats.

The superior colliculus (SC) evokes behavioural responses, such as persistent freezing or escape, to visual and auditory looming



stimuli (Fig. 1b). In the rodent brain, visual threat information can reach the SC directly via inputs from retinal ganglion cells[77,78]. Visual pathways extending from the retina to the SC have also been described in non-human primate brains[79]. Furthermore, the activity of excitatory neurons in the deep layers of the medial SC represents the threat imminence of visual looming stimuli and is predictive of escape in rodents[54]. The SC also responds to somatosensory and auditory inputs in both rodents and non-human primates[51,80,81]. In rodents, corticofugal inputs from the auditory cortex to the SC are required for escape but not post-encounter freezing induced by auditory looming stimuli[51].

The SC can coordinate the behavioural response selection between persistent freezing and escape in the circa-strike phase through its projections to the lateral posterior thalamic nucleus (LP) and the parabigeminal nucleus (PBGN) (Fig. 1b). Excitatory inputs from the SC to the LP can induce persistent freezing, which is mediated through excitatory projections from the LP to the basolateral amygdala (BLA)[52,53]. This SC–thalamus–amygdala pathway is also activated in humans during behavioural responses when presented with threatening images[82]. In contrast to the persistent freezing induced by the SC–LP pathway, activation of glutamatergic SC projections to the PBGN induces escape[53,83]. Furthermore, bilateral inactivation of the LP results in escape responses to visual looming stimuli, whereas persistent freezing to the same stimuli occurs following bilateral inactivation of the PBGN in mice[53].

The SC also promotes escape behaviour through another pathway. After receiving imminent visual threat information from the SC, GABAergic neurons in the ventral tegmental area (VTA) can also induce escape responses via inhibitory projections to the central nucleus of the amygdala (CeA)[84]. In rodents, if escape is feasible, excitatory projections from the BLA activate the lateral CeA (CeL), which in turn inhibits the medial CeA, thereby facilitating the shift in defensive response from persistent freezing to escape[85]. Despite the considerable differences in structural organization and complexity of rodent and primate brains, the subcortical defensive pathways originating from the SC and eventually delivering threat-relevant information to

Fig. 1 | Defensive responses across the three phases of the threat imminence continuum and the underlying brain circuits. a, The defensive responses exhibited across the pre-encounter, post-encounter and circa-strike phases of the threat imminence continuum (modified from a model developed by Fanselow and Lester[6]) depend on the threat imminence and its proximity to the rodent. When the threat imminence is low, pre-encounter defensive responses of avoidance and risk assessment are based on the innate tendency of rodents to avoid open and bright areas, where there is increased potential to encounter a predatory threat. Threat imminence increases from low to high in the post-encounter phase after a rodent detects a predatory threat, which evokes freezing as a defensive response that enables assessment of the nature of the threat if the situation is still ambiguous. In the circa-strike phase, when the threat has become imminent, escape responses are triggered or a state of persistent freezing can be maintained if no escape routes are available. Close proximity of a predator involving physical contact elicits the last-resort circa-strike defence responses, expressed as defensive attack or tonic immobility. **b**, The brain circuits that underlie pre-encounter, post-encounter and circa-strike defensive responses. The thalamus and limbic areas are involved in relatively lower-imminence defence responses compared to the hypothalamus and brainstem. The periaqueductal grey (PAG) drives defensive behaviours through projections delivering outputs to the brainstem motor control centre. In addition to motor output, information regarding low-imminence and high-imminence threats is processed in limbic areas by the amygdala and the bed nucleus of the stria terminalis (BNST) and is then relayed to the hypothalamus to stimulate the paraventricular nucleus (PVN), which induces sympathetic–adrenomedullary (SAM) and hypothalamic–pituitary–adrenal (HPA) axis activation. SAM activation generally increases heart rate and force of contraction, peripheral vasoconstriction and energy mobilization. The HPA axis initiates the production and release of glucocorticoid hormones (for example, corticosterone in rodents and cortisol in humans). Circulating glucocorticoids then trigger the mobilization of stored energy and potentiate several sympathetically coordinated effects, such as peripheral vasoconstriction. Thus, the SAM and HPA axes allow coordination between brain and body functions that are geared towards coping with threat. Lines of different colours represent excitatory (arrowheads) or inhibitory neuron projections in circuits underpinning distinct defensive responses. Dashed lines represent the proposed neural pathway by which the parabigeminal nucleus (PBGN) and the anterior hypothalamic nucleus (AHN) control escape and defensive attack, respectively[204]. Black lines indicate behavioural and physiological outputs. BLA, basolateral amygdala; BMA, basomedial amygdala; CeA, central nucleus of the amygdala; CeL, lateral CeA; CeM, medial CeA; LGP, lateral globus pallidus; LP, lateral posterior thalamic nucleus; LH, lateral hypothalamus; mSTN, medial subthalamic nucleus; PMd, dorsal premammillary nucleus; SC, superior colliculus; SLEAc, central sublenticular extended amygdala; sNAc, nucleus accumbens shell; TS, tail of the striatum; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area.

the amygdala appear to be evolutionarily conserved between these mammalian orders[85,86].

The periaqueductal grey (PAG), which interfaces with the peripheral motor systems that execute defensive behaviour, receives direct inputs from both the SC and the CeA[54,87,88] (Fig. 1b). Earlier studies in cats and rats established the PAG as a critical node for post-encounter freezing as well as escape and persistent freezing responses elicited by a lower-imminence threat in the circa-strike phase. For example, pharmacological activation of the dorsal PAG (dPAG) induces escape responses such as running and jumping in cats[89,90]. Indeed, dPAG neurons encode the choice to escape and govern escape vigour after receiving visual looming threat information from the SC, and inhibition of dPAG neurons abolishes the escape response elicited by SC activation in mice[54]. Projections from the PBGN to the PAG have been discovered[91,92], which possibly transmit threat-relevant visual information to the motor systems that drive escape responses. Furthermore, an earlier study used the contextual cues in an inescapable situation (a conditioned fear paradigm) to evoke post-encounter freezing responses in rodents, and electrolytic lesions of the ventrolateral PAG (vlPAG) reduced these responses[93]. In mice, optogenetic activation of vlPAG excitatory neurons promotes post-encounter freezing behaviour[88] and the inhibitory inputs from the CeA promote post-encounter freezing by disinhibiting these vlPAG excitatory outputs to the motor system[88].

In an avoidance paradigm in which a virtual predator — endowed with the ability to chase, capture and inflict pain — pursued human volunteers through a maze, increased proximity of the virtual predator shifted brain activity from the ventromedial prefrontal cortex to the PAG, as measured by functional MRI[94]. This shift in brain activity became more prominent when human volunteers anticipated a high degree of pain, suggesting that more proximal (higher-imminence) predatory threats are conveyed to the PAG. In mice, such higher-imminence threatening stimuli are first processed via medial hypothalamic–PAG circuits consisting of the anterior hypothalamic nucleus (AHN), the dorsomedial part of the ventromedial hypothalamic nucleus (VMHdm) and the dorsal premammillary nucleus (PMd)[95] (Fig. 1b).

The peak responses of excitatory VMHdm neurons vary with the intensity of threat stimuli to encode higher-imminence threats. For instance, a virtual predatory rat produces stronger long-lasting peak responses in VMHdm neurons in mice, whereas conspecific mice (social threat) produce moderate responses and an overhead visual looming disk produces weaker, short-lasting peak responses[96]. In addition, inhibition of excitatory VMHdm neurons reduces avoidance in the open field test (OFT) (a pre-encounter defence response) after exposure to a virtual predatory rat[96].

Interestingly, activation of excitatory neurons in the VMHdm can induce defensive behaviours suitable for the environment. For instance, their activation elicits escape to a hiding box if present in an open field (probably mediated through their projections to the AHN) and persistent freezing in the absence of a hiding box (through their projections to the PAG)[97] (Fig. 1b). Consistent with an evolutionarily conserved role for the VMHdm in defensive responses, deep brain stimulation of the VMH in awake human volunteers evokes anxiety and panic attacks[98,99]. These findings in humans support the idea that the VMH mediates defensive responses to threat ranging from the pre-encounter (anxiety) to circa-strike (panic) phases.

The PMd also sends excitatory projections to the PAG[100] (Fig. 1b). PMd neurons in mice respond strongly during exposure to an actual predator (a rat)[100]. Other threat-indicating stimuli, such as aversive lights, noises and social defeat, also activate the rodent PMd[101]. Lesions of the PMd reduce post-encounter freezing while increasing risk assessment activities of rats during exposure to a distal predatory threat (a cat)[102]. Furthermore, in mice, escape responses from a predatory rat require excitatory projections from the PMd to the dPAG, and inhibition of the excitatory PMd projection neurons lowers escape speeds[103]. Together, these results suggest that the VMHdm and PMd encode increased threat imminence and that reduction in the functions of either brain area can increase the threshold for activating the execution of defensive responses.

Using a behavioural paradigm that mimics the circa-strike phase with proximal threat in laboratory mice, a recent study showed that

an AHN–PAG pathway is critical for defensive attack[104]. Activation of GABAergic neurons in the AHN prioritized defensive attack over other forms of pre-encounter and post-encounter defensive behaviour such as risk assessment, avoidance and freezing[104]. The prioritization of defence attack is probably mediated by concurrent GABAergic inhibition of the VMHdm and PMd by the AHN[104] (Fig. 1b).

Other hypothalamic nuclei also control defensive behaviour during the post-encounter and circa-strike phases. GABAergic neurons in the lateral hypothalamus (LH) projecting to the PAG inhibit escape behaviour in mice during initial exposure to unfamiliar prey that may bring risk during predation, such as cockroaches[105]. In addition, excitatory neuronal populations in the VTA receive inputs from the LH that evoke escape responses to visual looming and olfactory predatory cues[106]. Interestingly, optogenetic activation of excitatory VTA neurons projecting to the CeA also promotes fleeing, expressed as increased total distance travelled and mean velocity in the OFT[107]. These excitatory neurons probably work together with GABAergic VTA neurons by activating and inhibiting the CeA neurons responsible for mediating escape and persistent freezing responses, respectively[84,85] (Fig. 1b).

Corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) also encode increased threat imminence and facilitate escape responses to looming stimuli[108]. Other extrahypothalamic CRH neurons are involved in post-encounter and circa-strike responses to threats received through multiple sensory modalities. For example, a study has shown that CRH neurons in the medial subthalamic nucleus (mSTN) projecting to the lateral globus pallidus in the basal ganglia mediate freezing in the post-encounter stage in response to predator odour or escape in response to visual looming stimuli[109]. Interestingly, these mSTN–CRH neurons also promote an increase in rapid eye movement (REM) sleep in mice under sustained exposure to predatory threat (a rat). The same study showed that awakening from REM sleep after presentation of predator odour is more rapid than that from non-REM sleep, suggesting an adaptive function of this REM sleep increase[109]. Together, these results indicate that mSTN–CRH neurons can coordinate different survival-related behaviours (defence against predators and sleep–wake state regulation). Another brain area in the basal ganglia, the tail of the striatum (TS), can also control freezing in the post-encounter stage[51]. For example, optogenetic inhibition of the TS reduces the freezing response that often precedes escape responses to auditory looming cues[51].

The circuits that underlie tonic immobility are relatively less studied because guinea pigs are the only rodents in which a long-lasting tonic immobility defensive response is easily induced[60]. In guinea pigs, the vlPAG controls the duration of tonic immobility through a complex interaction of cholinergic, opioidergic and GABAergic mechanisms[110]. Neuromodulation in the LH and BLA is also known to mediate tonic immobility in guinea pigs[111,112]. Further dissection of the circuits involved will require the development of adequate behavioural paradigms to reliably induce tonic immobility in laboratory mice.

Circuits involved in low-imminence threats

Rodent defence behaviour in the pre-encounter phase is dominated by risk assessment and avoidance of brightly lit and open areas, presumably because there is potential to engage with threats such as predators that could appear at any time[9,45]. Thus, avoidance of central areas of the OFT and open arms of the elevated plus maze (EPM) are frequently reported as measures of rodent pre-encounter defensive behaviour (often interpreted as anxiety-like behaviours)[45]. The amygdala not only is involved in the discussed defensive responses to high-imminence threats, but also is largely engaged in the response to low-imminence threats in the pre-encounter phase (Fig. 1b).

The complex control of low-imminence defence responses is orchestrated by a variety of cell types in the CeA. For example, the CeL contains two major GABAergic populations of neurons that are marked by protein kinase C-δ (PKC-δ) and somatostatin (SOM)[113,114]. Activation of PKC-δ neurons in the CeL promotes increased exploration of the open arms in the EPM[113]. By contrast, activation of SOM neurons in the CeL projecting to the sublenticular extended amygdala triggers increased avoidance responses during the EPM (movement into the closed arms) and OFT (thigmotaxis)[114]. Furthermore, basomedial amygdala (BMA) neurons encode anxiety-related contextual features (movement into the closed arms during the EPM), and optogenetic activation of BMA neurons decreases threat-anticipatory-related post-encounter freezing in a conditioned fear paradigm and decreases avoidance responses (increased open arm exploration)[115]. Thus, BMA neurons allow the animal to differentiate safe and aversive environments during risk assessment processes.

Optogenetic activation of GABAergic neurons projecting from the bed nucleus of the stria terminalis (BNST) to the nucleus accumbens shell promotes avoidance of the open arms on the EPM[116]. Although the amygdala and BNST are both involved in mediating pre-encounter stage defence responses, they seem to have very different roles. When the threat imminence is low, the ecological demands faced by an animal are to outwit predators while balancing homoeostatic threats, such as depletion of food[64]. Thus, animals in the pre-encounter phase need to perform a noncontinuous risk assessment to weigh the threat value and to search for safety, while simultaneously maintaining sustained hypervigilance to cope with unpredictable threat[64,117]. Rodent studies have clarified that the amygdala mediates short-term, phasic responses for rapid switching among safe and aversive environments, suggesting that it has a role in noncontinuous risk assessment[115]. By contrast, the BNST mediates the sustained hypervigilant response in the pre-encounter phase[118]. In humans, activity in the amygdala increases in a threatening pre-encounter phase context involving anticipation of painful electric shocks[119]. Individuals with GAD display increased activity in the amygdala during the onset of threat anticipation, whereas they display sustained elevated BNST activity[120]. These findings point to a conserved role of the amygdala and the BNST in the control of pre-encounter phase defensive responses (Fig. 1b).

Furthermore, lesions of the dPAG in mice attenuate pre-encounter phase risk assessment during the entry to the post-encounter phase by exposure to a predator threat (rat), and excitatory dPAG neuron activation leads to increased thigmotaxis in the OFT, indicative of enhanced risk assessment[121,122]. Single-unit in vivo electrophysiology in specific subsets of dPAG neurons showed that they encode risk assessment in mice during the predatory threat exposure and that this subset of dPAG neurons are largely distinct from those encoding post-encounter phase freezing and circa-strike phase escape responses[122]. These results reveal that several distinct PAG circuits also coordinate pre-encounter defence responses to low-imminence threats.

Modulation of defensive behaviours

The survival and reproductive potential of an animal depend on fundamental processes for the maintenance of physiology within an optimal homoeostatic range and for the appropriate allocation of time and resources to conflicting needs. These fundamental processes both rely on the accurate integration of peripheral signals into brain circuits to determine the most appropriate behavioural responses to

be executed[123]. For example, the risk of predation must be weighed against the benefits of fulfilling sleep needs, recovering from infection or pursuing tasks such as foraging or mating. Instinctive defensive behaviours displayed across all stages of the threat imminence continuum have evolved as flexible action patterns that can be adjusted or inhibited according to external circumstances, internal signals and previous experience[124].

Thus, attention is warranted not only to the advances in understanding the subcortical circuitry of threat responses, as discussed above, but also to brain periphery communication and the modulation of defence circuitry by internal signals and physiological processes. As pointed out by Flavell et al., internal states not only consist of changes in brain function but also involve changes in other parts of the body[36]. The perceived imminence of a threat and the resulting defence behaviours depend not only on the threat's actual spatiotemporal distance but also on the internal state of the animal. To a large extent, modulation of defence vigour or phase shifts in the threat imminence continuum are caused by several factors, such as the presence or absence of infection or mating opportunities and nutritional status, which are reflected in the interactions between the periphery and subcortical circuitry summarized above. In 'Infection and inflammation', 'Hunger and nutritional state' and 'Sex hormones and reproductive state', we discuss how peripheral systems tailor defensive behaviours in different ethologically relevant contexts in an adaptive manner, which may help to understand the distortions in the threat imminence continuum that underlie maladaptive behaviour patterns in psychiatric disorders, as discussed in the 'Conclusions and perspective' section.

Infection and inflammation

Besides predators, infectious pathogens have been among the strongest selective forces in animal evolution. As a result, a constellation of immunological and behavioural responses to infection exists that conserves energy for fighting pathogens, while simultaneously maintaining vigilance to detect changes in threat level during risk assessment and higher-imminence defence behaviours[13–16]. In rodents, peripheral inflammatory cytokines predominantly orchestrate infection-related behavioural modifications, including reduced exploration and increased avoidance, which can be considered as increased defence vigour during the pre-encounter phase[15,16]. In rodents, intraperitoneal administration of lipopolysaccharide (LPS) mimics bacterial infection by triggering the release of inflammatory cytokines in various cell types and causing an acute inflammatory response, which induces a pre-encounter phase defensive response during the EPM (anxiety-like behaviour)[17]. In humans, two pro-inflammatory cytokines, tumour necrosis factor (TNF) and interleukin-1β (IL-1β), are elevated in individuals with anxiety-related and fear-related disorders, including GAD, PTSD, panic disorder and phobias[40,125]. These results hint at the important role of cytokines in modulating defence responses across all stages of the threat imminence framework.

Cytokines can influence defensive behaviours by several mechanisms. They can cross the blood–brain barrier[126], so cytokines derived from local or peripheral sources can both modulate brain activity and behavioural responses[127] (Fig. 2). For example, LPS endotoxin — which does not cross the blood–brain barrier — induces peripheral immune activation and leads to de novo IL-1β, IL-6 and TNF synthesis in the amygdala that correlates with increased neuronal activity and increased anxiety-like behaviour in rats[128]. Local IL-1β administration into the medial hypothalamus or the dorsal PAG in cats potently facilitates defensive rage, a circa-strike phase defensive response that protects against attacks perceived as inescapable from predators or conspecifics[129,130]. Cytokine signals can be detected by peripheral neurons expressing cytokine receptors and transmitted via sensory afferent fibres of the vagus nerve to the brain to modulate the activity of subcortical structures[131,132]. For example, peripheral injections of LPS endotoxin increase c-fos expression in PVN neurons and activate the hypothalamic–pituitary–adrenal (HPA) axis, and subdiaphragmatic vagotomy blocks these effects[19,133]. Peripheral IL-1β can increase action potential firing in vagal afferents and activate the nucleus tractus solitarii (NTS), the central projection area of vagal afferent nerve fibres in the medullary brainstem[134–136]. Ascending efferent projections from the NTS form a wide-ranging network spanning multiple subcortical brain regions involved in mediating defensive responses, including the PAG, LC, PVN, CeA and BNST[137,138]. Thus, visceral information collected by the vagus nerve might ultimately influence defensive behaviour by modulating these brain areas involved in processing defence responses to low-imminence and high-imminence threats (Figs. 1b and 2). Indeed, the NTS has been shown to mediate defensive behaviours in the pre-encounter stage. For example, bilateral glucocorticoid receptor antagonism in the NTS reduces exploratory behaviour in the EPM[139], whereas chemogenetic activation of NTS neurons is sufficient to elicit moderate increases in anxiety-like behaviours in the OFT[140]. Consistent with these results in rodents, vaccination-induced inflammation in healthy humans results in deteriorated mood (including anxiety) compared with controls. Furthermore, the mood deterioration correlated with greater reactivity in the dorsal pons, the PAG and the amygdala during an implicit emotional face perception task, in which randomly displayed sad, happy, angry and neutral faces triggered implicit rather than explicit emotion processing[141].

The immune system can influence defensive responses by another pathway involving the passage of circulating molecules secreted from immune cells into the brain (Fig. 2). For example, in the pre-encounter phase, *Rag2* knockout mice (which do not produce mature lymphocytes) exhibit reduced anxiety-like behaviour and increased locomotor activity (indicative of a reduced avoidance response) during the OFT[142]. The modulation of pre-encounter defence responses by lymphocytes is partially mediated by xanthine secreted by peripheral $CD4^+$ T cells, which can enter the brain and trigger the proliferation of oligodendrocytes, thereby resulting in hyperactivation of amygdala neurons[143]. In sum, these mechanisms indicate that peripheral inflammatory responses have important roles in shaping defensive behaviours across the threat imminence continuum, including the pre-encounter and the circa-strike stages.

Peripherally generated pro-inflammatory cytokines such as IL-1, IL-6, TNF and IFN-γ could activate PVN–CRH neurons to promote counter-regulation of the above-mentioned peripheral inflammation through the potent anti-inflammatory effects of glucocorticoids[144–146]. This interplay of peripheral inflammation and the HPA axis has an important role in adjusting defensive behaviours during infection. During low-imminence and high-imminence threat exposure, PVN–CRH neurons secrete CRH into peripheral circulation and CRH activates peripheral neuroendocrine responses via the HPA axis, which further induces the secretion from the adrenal gland of glucocorticoids — cortisol in primates — into the bloodstream[147–152] (Fig. 2). Circulating glucocorticoids reach every organ and allow coordination between the brain and the body of functions geared towards behavioural responses to current and subsequent threats[151–153]. Open arm exposure during the EPM increases plasma corticosterone, which is positively correlated with risk assessment behaviour in rodents[154]. In primates, HPA axis activity is also

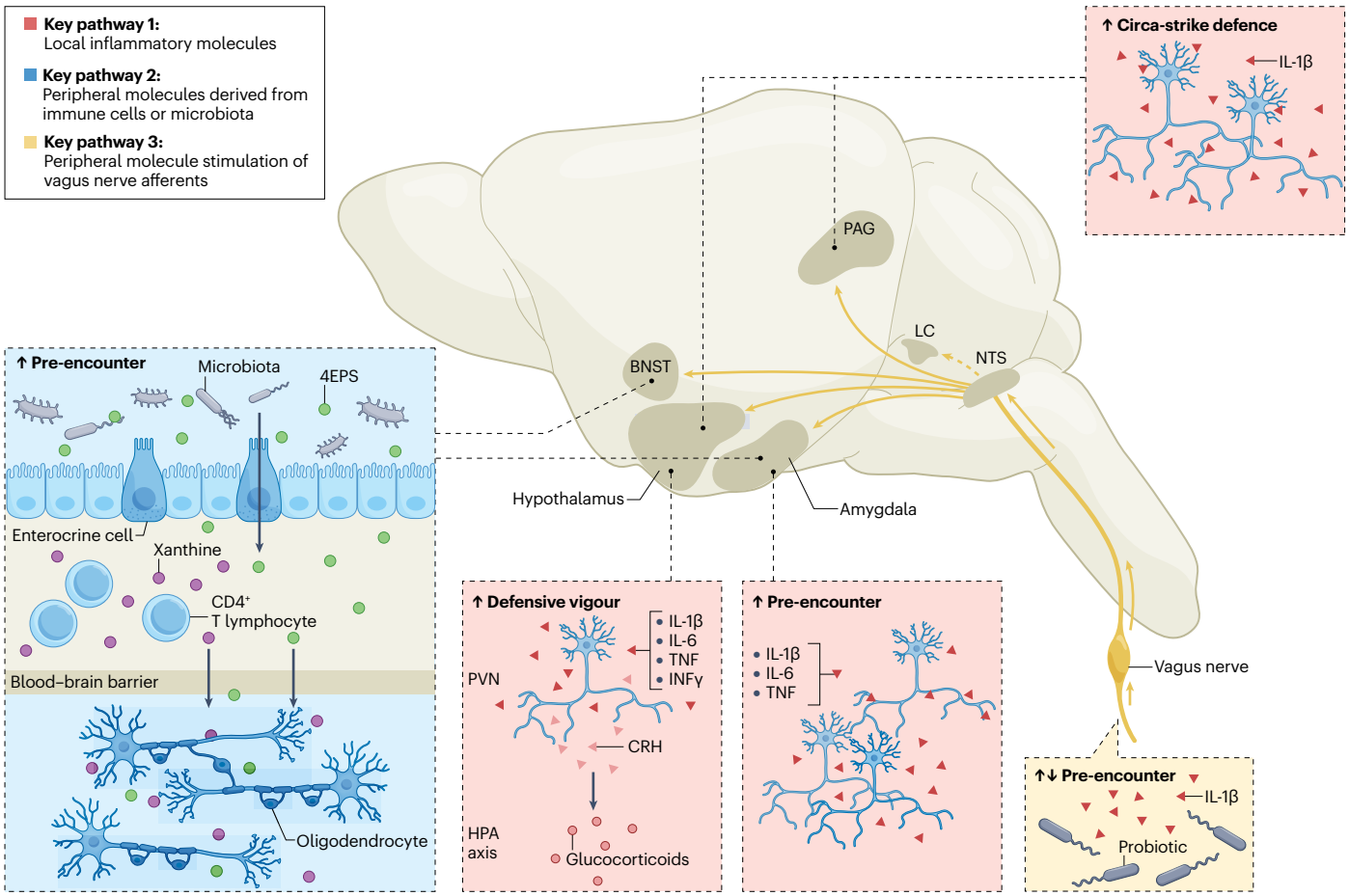


Fig. 2 | Transmission routes of infection signals to the brain. In the context of infection, cytokines released by activated immune cells and gut microbes stimulate sensory afferent nerves of the vagus, which transmit that information to the nucleus tractus solitarii (NTS). The NTS projects to multiple subcortical structures including the amygdala, hypothalamus and the bed nucleus of the stria terminalis (BNST), a pathway by which infection signals received by the vagus nerve may ultimately influence defensive behaviour. Cytokines derived from local or peripheral sources can both function in the brain. Interleukin-1β (IL-1β) in the periaqueductal grey (PAG) and the hypothalamus can increase circa-strike defence responses, and in the amygdala can promote pre-encounter defence. IL-6 and tumour necrosis factor (TNF) increase anxiety-like behaviour by modulating amygdala activity and activate the hypothalamic–pituitary–adrenal (HPA) axis via release of corticotrophin-releasing hormone (CRH) from paraventricular nucleus (PVN) neurons. Metabolites, such as xanthine and 4-ethylphenyl sulfate (4EPS), derived from activated immune cells and microbiota, can access the brain through humoral routes. They alter oligodendrocyte function in the brain and change region-specific activity in the amygdala and BNST, which further increases anxiety in mice. Solid yellow lines indicate direct neural connections between the NTS and downstream targets, and dashed lines indicate putative indirect connections from the NTS to the locus coeruleus (LC) through which the vagus nerve modulates defensive behaviour.

an important regulator of defensive vigour in the face of high-imminence threat. For example, infant rhesus monkeys with high cortisol levels engage in longer persistent freezing in the circa-strike stage (induced by exposure to a room containing a human intruder standing in proximity to a caged monkey with no eye contact)[152]. Human children with comparatively high basal and reactive cortisol levels display longer persistent freezing episodes in the circa-strike stage (induced by a male stranger entering the room, approaching the child, kneeling down and looking at the child without speaking)[153]. Thus, it can be speculated that HPA axis activation could lead to infection-related changes in defensive behaviour, but this has not yet been causally determined.

Evidence supports the idea that the gut–immune–brain axis also modulates pre-encounter defensive behaviour during infection and inflammation. In mice, gut infections increase patterns of behaviour in the OFT that are thought to represent pre-encounter phase defensive responses[20,155], and simultaneously occurring dysbiotic microbiota exacerbate intestinal inflammation[28,156]. Furthermore, gut infection causes vagal nerve ganglia activation, and vagotomy alleviates the associated anxiety-like behaviour[20,155]. Administration of probiotic bacteria reduces anxiety-like behaviour after gut infection, chemically induced colitis, or during exposures to low-imminence threat, and vagotomy revealed that a vagal route mediates these reductions in anxiety-like behaviour[20–23]. Further supporting evidence exists in rodents that received a probiotic intervention after experiencing threat. For example, chronic oral administration with the probiotic bacterium *Lactobacillus rhamnosus*

ameliorates EPM-induced anxiety-like behaviour, which possibly results from vagus nerve-mediated modulation of activity in the amygdala and locus coeruleus[21]. Probiotic consumption by rats decreases pre-encounter defence responses following a single foot-shock, and by humans, it decreases anger (hostility) and anxiety level measured by self-report questionnaires[157]. In addition, germfree mice display disproportionally elevated glucocorticoid levels in response to physical threat, and recolonizing germfree mice with specific microorganism strains reverses the exaggerated HPA activity[15]. Furthermore, the microbial metabolite 4-ethylphenyl sulfate (4EPS) — which can cross the blood–brain barrier — induces pre-encounter defence responses in mice[158]. This behavioural modulation may result from impaired oligodendrocyte function and myelination and increased activity in the amygdala and BNST that are causally affected by the entry of 4EPS into the brain[158] (Fig. 2). Thus, circulating molecules derived from the gut microbiome can also deliver infection signals to the brain and modulate defence responses.

These results illustrate the various routes by which the gut–immune–brain axis modulates defensive behaviour during infection and inflammation. In the humoral route, metabolites and cytokines signalling infection directly target the amygdala, BNST and PAG and, thereby, influence the defensive behaviours in the pre-encounter phase and circa-strike phase. Furthermore, cytokines also promote activation of the HPA axis by triggering PVN–CRH release, which may have an important role in increasing defensive vigour against both low-imminence and high-imminence threat. In addition, the vagus nerve also conveys peripheral signals from the gut and other organs to the brain, including the NTS and its efferent subcortical network, which modulate anxiety-like behaviours in the pre-encounter phase (Fig. 2).

Hunger and nutritional state

Hunger can change the dynamics of the energy–predation risk trade-off between the benefit of acquiring food and the risk of encountering a predator. A hungry animal often decides to search for food and risk potentially encountering predators and other environmental threats rather than remaining in a safe shelter without a source of energy. By contrast, if energy and nutrition demands are met, the desire for safety could predominate and the need to risk exploring novel surroundings that are potentially rewarding and threatening might be reduced[159]. Thus, it has been proposed that hunger generally increases risk-taking in environments with acute dangers and supresses threat-anticipatory behaviours such as freezing[32], whereas satiety inhibits exploration and increases avoidance behaviour[31].

The nutritional state of an animal is represented by the interactions of peripheral molecules signalling hunger or satiety with the brain (Fig. 3). Ghrelin, a hunger signal that induces food-seeking behaviour, is released from enteroendocrine cells of the stomach during fasting, whereas feeding suppresses ghrelin release[29,30]. By contrast, leptin (primarily produced by adipose cells), cholecystokinin, glucagon-like peptide-1 and peptide YY (originating from the gastrointestinal system), and insulin (excreted by the pancreas) predominantly act as satiety signals and suppress consumption and potentially food seeking[31,160,161]. Agouti-related peptide (AgRP)- and neuropeptide Y-containing neurons in the arcuate nucleus of the hypothalamus (ARC) are activated by peripheral hunger signals and inhibited by satiety signals, and their activation promotes food-seeking behaviour[31,162] (Fig. 3). Activation of AgRP neurons in the ARC in mice suppresses predator odour-evoked freezing and avoidance defensive responses in the post-encounter and pre-encounter stages, respectively[159]. Activation of ARC–AgRP projections to the medial amygdala in fed mice promotes high-risk exploration in a chamber associated with foot-shock compared with fasted mice[163]. In addition, both the CeA and BNST receive inputs from the NTS[31], which is a relay hub of visceral metabolic information promoting hypoglycaemia-induced feeding[164], and thereby might integrate nutritional-state-related information with threat-related information in the pre-encounter and circa-strike phases.

Some effects of fasting-induced hormones, such as ghrelin and fibroblast growth factor 21 (FGF21), on subcortical brain circuits suggest that more complex interactions exist (Fig. 3). For example, fasted mice show increased plasma ghrelin levels and accelerated fear extinction[32,33]. But ghrelin administration and overexpression of the ghrelin receptor in the amygdala induce anxiety-like behaviour[34], and exogenous ghrelin directly modifies amygdala activity and enhances defensive behaviours in the pre-encounter phase, represented as increased avoidance of the chamber in which the animals experienced foot-shock[35]. Interestingly, exposure to physical and psychological threats such as foot-shock, social defeat and forced immobilization also increases gastric ghrelin, but after, it appears to have an anxiolytic effect[165]. The hormone FGF21 is synthesized in several organs including the liver, pancreas, muscle and brain,[166,167] and diverse environmental or nutritional challenges, including physical exercise, food deprivation and overfeeding, can increase FGF21 expression in peripheral organs and/or in the brain[168,169]. Hepatic synthesis of FGF21 increases CRH expression in the BLA and induces anxiety-like behaviour in both the OFT and EPM[170].

Leptin is primarily produced by white adipose tissue, and leptin levels are elevated in fed animals and are reduced upon fasting[171,172]. As a satiety agent, leptin in many contexts seems to have the opposite effect on defensive behaviours to that expected from the energy–predation risk trade-off outlined above (Fig. 3). For example, leptin-deficient mice display increased anxiety-like behaviour, suggesting a role of leptin in supressing avoidance in the pre-encounter phase[173]. In addition, mice with a selective knockout of the leptin receptor in dopamine neurons (predominately but not exclusively located in the VTA) exhibit anxiety-like behaviour during an EPM test, which is probably caused by their increased dopaminergic input to the amygdala[174]. Leptin receptors are also expressed in the amygdala, and intra-amygdala leptin injections reduces threat-anticipatory post-encounter freezing responses to auditory cues in a conditioned fear paradigm[175,176].

Furthermore, the endocrine system related to nutrient availability affects the HPA axis and may further modulate the complex execution of defensive behaviours[177,178]. For example, peripherally administered ghrelin induces not only anxiety-like behaviour during the EPM but also hypothalamic *Crh* mRNA expression, which may lead to activation of the HPA axis[179]. In contrast to non-stressed conditions, ghrelin reduces avoidance in the pre-encounter phase after exposure to an acute high-imminence threat (restraint)[180]. This reduction in defensive behaviours may partially result from increased feedback inhibition of the amygdala by partially stimulating the HPA axis at the level of the anterior pituitary[180] (Fig. 3). Leptin may reduce avoidance in the pre-encounter phase by exerting different effects on the HPA axis than ghrelin. Systematic administration of leptin supresses the activity of the HPA axis by inhibiting hypothalamic CRH release after acute restraint stress[181]. Leptin also suppresses restraint-induced corticosterone release, partially mediated by recruitment of a subpopulation of leptin-sensitive LH GABAergic neurons, and activation of these neurons normalizes hypercorticosteronaemia in

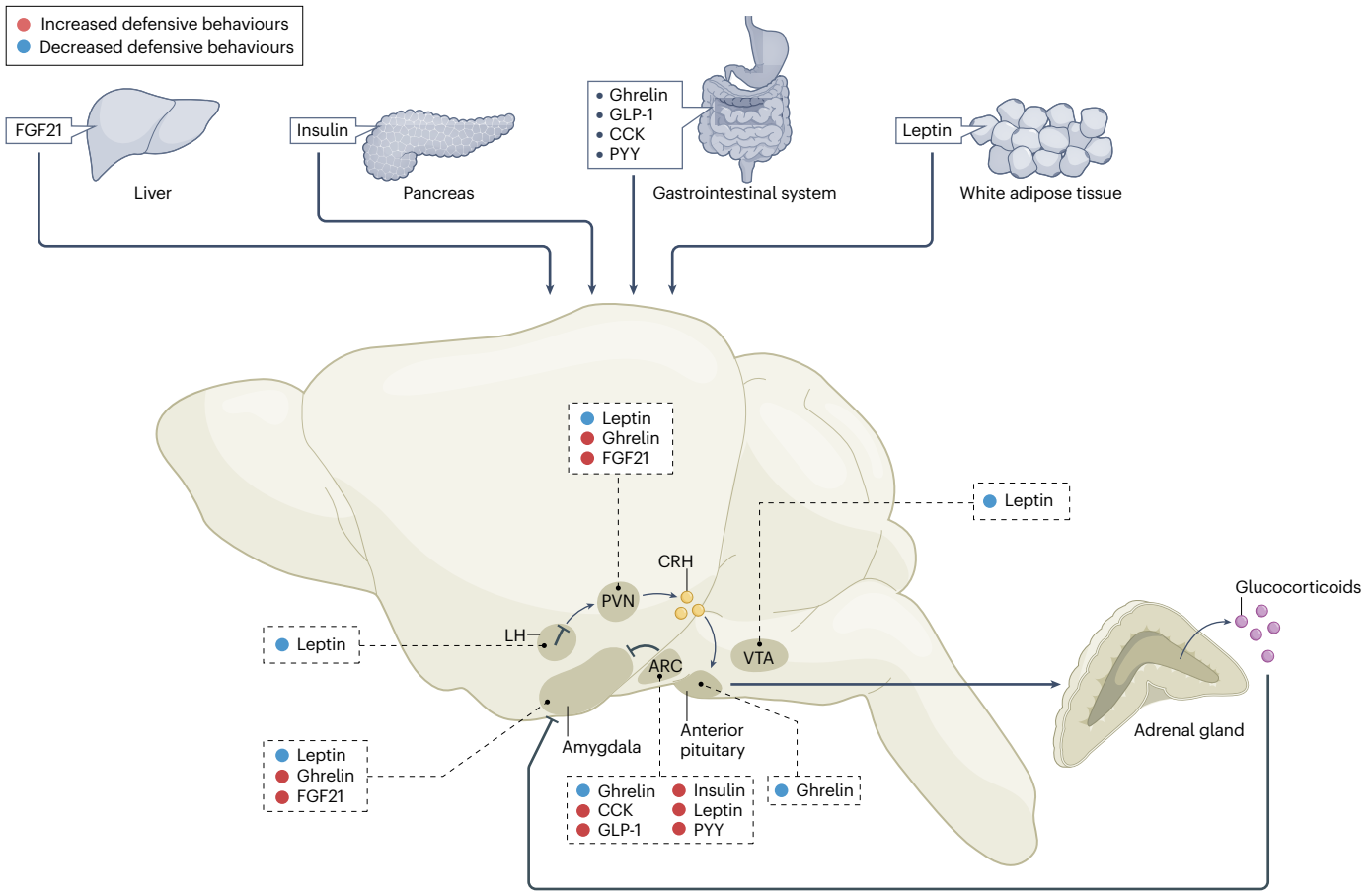


Fig. 3 | Crosstalk between the digestive system and the brain. Several hormones are produced from the gastrointestinal system, adipose tissue, pancreas and liver to control food intake and metabolism. Ghrelin is best known as a hunger signal, whereas leptin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and insulin predominantly act as satiety signals. Agouti-related peptide (AgRP) and neuropeptide Y (NPY) neurons in the arcuate nucleus of the hypothalamus (ARC) are the hub-integrating signals from these peripheral hormones; $AgRP^+$ or NPY^+ ARC neuron activity is increased by peripheral hunger signals and decreased by satiety signals. Furthermore, activity of $AgRP^+$ or NPY^+ ARC neurons suppresses defensive behaviours in the pre-encounter and post-encounter stages and activation of inhibitory $AgRP^+$ or NPY^+ ARC neuron projections to the amygdala promotes high-risk exploration. Hormones such as leptin, ghrelin or fibroblast growth factor 21 (FGF21) can further modulate defensive behaviour during the pre-encounter phase and post-encounter freezing (leptin) by exerting direct effects on the ventral tegmental area (VTA) and amygdala. Leptin, ghrelin and FGF21 can also modulate the activity of the hypothalamus–pituitary–adrenal (HPA) axis via signalling in the paraventricular nucleus (PVN) and/or anterior pituitary. First, ghrelin enhances pre-encounter defensive behaviours of naive animals by stimulating corticotrophin-releasing hormone (CRH) release from the PVN but can also directly activate the anterior pituitary, which in turn stimulates the production of glucocorticoids from the adrenal glands to exert HPA feedback inhibition on the amygdala to reduce such defensive behaviour. Second, leptin reaching the lateral hypothalamus (LH) after previous circa-strike threat exposure (such as restraint) can activate leptin-sensitive GABA neurons that can reduce glucocorticoid release by inhibiting LH–orexin excitatory projection neurons to the PVN, thereby suppressing CRH release. Lastly, prolonged fasting-induced FGF21 release can also stimulate CRH release from the PVN and glucocorticoid release. However, the modulatory effect on defensive behaviours by HPA changes mediated by leptin-sensitive LH neurons and fasting-induced FGF21 remains to be examined.

leptin-deficient mice[182]. After prolonged fasting, hepatic production of FGF21 — which is able to cross the blood–brain barrier — can stimulate CRH release in the PVN, leading to increased corticosterone release[183,184] (Fig. 3). However, the modulatory effect on defensive behaviours of HPA changes in different nutritional states remains to be examined.

In summary, neural activity in the amygdala, which is involved in both low-imminence and high-imminence threat processing, is directly regulated by endocrine signals in the satiety and hunger states. Amygdala neural activity can also be inhibited by feedback from the HPA axis and its activation by endocrine signals (Fig. 3). Of note, ghrelin and leptin have a dual role in modulating defensive behaviours during the pre-encounter stage, suggesting more complex coordination among the brain, peripheral organs and the HPA axis. This complexity may serve to optimize the energy-predation risk trade-off by integrating safety or threat signals and food availability information from the external environment with the motivational state related to feeding from satiety and hunger endocrine signals. For example, whereas food foraging behaviour requires a certain degree of risk-taking and, thus, a reduction in avoidance behaviour, adequate alertness for risk

assessment needs to be maintained, so risk-taking behaviour cannot remove the ability to execute active defence responses. This is especially the case if limited nutrient consumption has already led to a decrease in muscle mass or energy.

Sex hormones and reproductive state

In addition to the immune system and gut, sex hormones released from the gonads (testosterone, oestrogen and progesterone) are important signals of internal state. Gonadotropin-releasing hormone secreted by the hypothalamus stimulates the pituitary to synthesize and release follicle-stimulating hormone (FSH) and luteinizing hormone (LH)[185]. In male mammals, LH promotes the synthesis and secretion of testosterone in the testes[185]. Circulating testosterone can enter the brain and can be aromatized to oestrogen (predominantly in hypothalamic and limbic brain areas and during early developmental stages)[186], or it can be reduced to dihydrotestosterone[187]. In female mammals, FSH and LH stimulate oestrogen production and follicle growth in the ovaries during the first phase of the reproductive cycle (called oestrous cycle in non-primate mammals and menstrual cycle in primates). Orchestrated by positive and negative feedback loops between the female reproductive system and the hypothalamus, the cycle proceeds to ovulation, and progesterone levels start increasing at the onset of this second phase[188]. At the end of the reproductive cycle, when the potential for successful fertilization is at a minimum (around menstruation in primates), both progesterone and oestrogen levels are at their lowest[189]. Thus, sex hormones signal reproductive status in females, whereas in males of various mammalian species, sex hormones signal mating opportunity, because circulating testosterone levels increase in males when they encounter receptive females or engage in reproductive behaviour[25,190]. In human males and those of other mammalian or avian species with social monogamy and paternal care, a reduction in circulating testosterone has been observed when the female partner has successfully given birth, indicating a shift from mating efforts to parenting[191]. Short-term forms of 'sexual satiety' after (repeated) intercourse also exist in mammals but are largely independent of circulating testosterone[192,193]. Comparable to the search for food, seeking mating partners and engaging in courtship and sexual behaviour carry the risk of encountering predators or conspecific competitors. Thus, a similar risk–benefit trade-off and modulation of defence behaviours by sex hormones can be expected. Indeed, sex hormones have been found to influence the activity of subcortical neurocircuits that mediate threat responses during the pre-encounter and post-encounter stages.

Testosterone reduces defensive behaviours in the pre-encounter and post-encounter stages in rodents. For example, male rats and male mice both have decreased pre-encounter defensive responses (spend more time in the open arms of the EPM) after testosterone administration[24,26,194]. Similarly, exogenous testosterone administration leads to reduced avoidance of cat odour by male rodents (fresh cat urine signals the presence of a predator in close proximity so typically induces post-encounter defence behaviours)[25,195]. Both systemic administration (for 14 days) and local MeA administration (for 12 days) of testosterone lead to hypomethylation of the MeA arginine vasopressin (AVP) promoter and reduced post-encounter defence behaviour[25,195]. Thus, the effect of testosterone on defensive responses could be mediated by increased AVP expression and the resulting activation of AVP neurons in the MeA[195,196]. Testosterone also exerts an inhibitory effect on the HPA axis, predominantly at the hypothalamic level[197]. In summary, these observations suggest that testosterone directly or indirectly (via one of its brain metabolites) increases the threshold for defensive behaviour and, thereby, facilitates engagement in oftentimes risky mate seeking and sexual behaviours[198].

Similarly, female rats exposed to cat odour exhibit reduced anxiety-like behaviour (indicated by a shift from post-encounter freezing behaviour towards pre-encounter risk assessment) during higher-fertility oestrous cycle stages that involve elevated oestrogen and progesterone levels[27]. In humans, the symptoms of women with

Glossary

Adaptive behaviour
Behaviour is adaptive in an evolutionary sense if it increases genetic fitness, usually by promoting the survival and reproduction of an organism or that of close kin, which can coincide with negative emotional states such as anxiety or fear.

Auditory looming assay
Behavioural test in which an animal is exposed to sudden increasing sounds induced by broadband white noise, triggering circa-strike phase defensive behaviours.

Conditioned fear
A learned defensive response elicited by a previously neutral stimulus (conditioned stimulus) that has been paired with an aversive event (unconditioned stimulus).

Defence vigour
Physical strength of the execution of defensive behaviours. For example, an increase in speed during escape and increased durations of avoidance or persistent freezing can be considered increased defence vigour.

Dysbiotic microbiota
An imbalance or disruption in bacterial composition, metabolic activities or distribution within the intestinal tract, often leading to negative health consequences such as inflammation and disease.

Elevated plus maze
Behavioural test in which an animal explores elevated open and enclosed arms and in which reduced entries into open arms indicate enhanced defensive behaviour in the pre-encounter phase and an anxiety-like state.

Maladaptive behaviour
Behaviour patterns that are adaptive in some contexts can become maladaptive in an evolutionary sense when they are displayed in the wrong context or with excessive vigour and, thereby, prevent the execution of adequate adaptive behaviours, ultimately reducing the survival chances and reproductive success of the organism.

Open field test
Behavioural test in which an animal explores an unstructured open arena and the increased avoidance of central areas indicates enhanced defensive behaviours in the pre-encounter phase (often referred to as anxiety-related behaviour).

Optogenetics
An approach involving the expression of light-sensitive ion channels or pumps in specific cells, allowing cellular or organ activity to be manipulated by light with high spatial and temporal precision.

Phase shifts in the threat imminence continuum
Phase shifts in the threat imminence continuum refer to the phenomenon when behaviour patterns typical for one phase of the continuum are activated earlier (at lower imminence) or later (at higher imminence) than typically observed. They may be adaptive in some contexts or maladaptive in others.

Visual looming assay
Behavioural test in which an animal is exposed to rapidly expanding dark overhead spots that serve as visual cues simulating approaching threats, triggering circa-strike phase defensive behaviours.

anxiety disorders frequently worsen during the peri-menstrual phase of the menstrual cycle (characterized by low oestrogen and progesterone levels), and several studies also suggest the same relationship between menstrual cycle and anxiety state in women without anxiety disorders[199–201]. Furthermore, oestradiol and progesterone stimulate or inhibit the HPA axis depending on menstrual cycle phase, pregnancy status or phase of life (prepubertal, premenopausal or postmenopausal)[202], suggesting more complex modulation of defensive behaviours by sex hormones in human females. Thus, a cross-species trend of reduced fear-related or anxiety-like behaviours when fertility and receptiveness to mating are increased exists, but more research is needed to clarify the mechanisms by which defensive responses are influenced by sex hormones.

In summary, accumulating evidence supports the view that sex hormones, together with other peripheral signals such as cytokines or satiety/hunger hormones, have an important role in influencing defensive behaviours. Most studies discussed here investigate peripheral modulation of pre-encounter behaviours or responses to low-imminence threat. Similarly, most of the circuits affected by peripheral signals in the reviewed studies involve the amygdala, the hypothalamus and/or the PAG, indicating a need for future research on peripheral signals modulating the responses to high-imminence

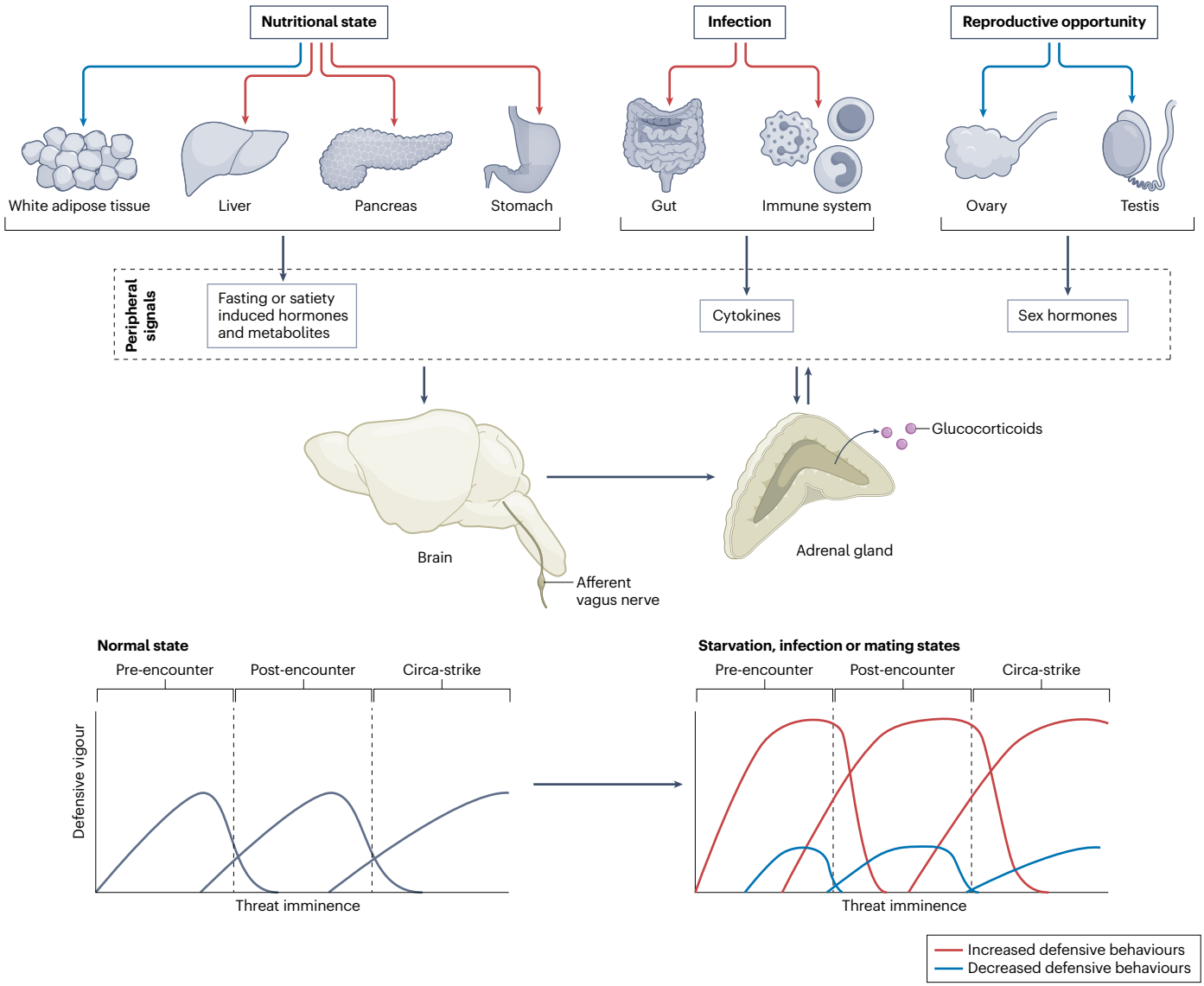


Fig. 4 | Brain–body interactions modulating defensive behaviours. Evolutionary pressure to protect from predation while balancing other needs, such as foraging, mating or recovering from infections, has shaped not only the brain but also peripheral systems and their interplay. During infection or starvation, the immune and gastrointestinal systems convey peripheral signals to the brain and influence defensive behaviour by shifting the threshold for defensive response activation and/or changing the defensive vigour during their execution (in the literature reviewed, both appear to be coupled). Reproductive states indicating mating opportunities determine whether sex hormones can exert effects on subcortical structures and/or glucocorticoid release from the activity of the hypothalamic–pituitary–adrenal axis. A resulting increase in the threshold for defensive behaviour could, thereby, facilitate engagement in often-risky mate seeking and sexual behaviours.

threat and/or the activity of other subcortical structures involved in defence behaviours.

Conclusions and perspective

The past decade has seen tremendous progress in deciphering the neural circuits underlying defensive behaviour, but negotiating threats needs to be balanced with other tasks that are important for survival and reproduction. In this Review, emphasis has been put not only on the coordination of pre-encounter, post-encounter and circa-strike defensive responses by subcortical networks but also on how defence behaviours are modulated by peripheral systems that are responsible for inflammatory, nutritional and reproductive states. Understanding this interplay can more appropriately reveal the function of defensive behaviour in ethologically relevant contexts. As many current studies focus on the effects of peripheral signals on pre-encounter defence responses, it is often not clear whether these signals equally affect the post-encounter and circa-strike defence response thresholds. Although it could be argued that high-imminence threat leaves little room for peripheral modulation, testosterone levels, infection or energy status can be expected to also affect choices such as persistent freezing versus escape, fight versus flight or defensive attack versus death feigning. Thus, future studies should investigate whether and how infection, hunger and reproduction signals influence post-encounter and circa-strike defensive behaviour, given that these peripheral signals modulate activity in the amygdala and hypothalamus, which are key structures in circuits mediating freezing, escape, defensive attack or tonic immobility responses.

Future studies should also investigate in detail the neural circuits that receive peripheral signals and execute defensive responses across the threat-imminence continuum, and the potential peripheral effects on important subcortical structures other than the amygdala, hypothalamus and PAG. For example, does the SC integrate peripheral infection and nutritional signals with external threat information and change behaviour selection across the threat-imminence continuum? Do the mSTN and its connections in the subcortical network coordinate behaviours to fulfil homoeostatic needs while coping with predatory threat (sleep demand versus escape from a predator) during infection?

Our understanding of neuropsychiatric diseases could probably benefit from insights gained through a systemic and embodied approach to research. Specifically, individuals with anxiety-related and fear-related disorders have dysregulation of threat responses across the phases of the threat imminence continuum[11]. This Review has focused on peripheral modulation of defence responses in an ethologically relevant context, which tend to be adaptive (for example, when an animal is infected and pro-inflammatory cytokines cause enhanced defence responses, signalling the increased need for recovery and risk avoidance). Understanding the nature of these modulations (increased defence vigour and/or lowered thresholds for the activation of higher-imminence defensive behaviours) and the way signals from peripheral organs can contribute to these changes is crucial also for understanding the maladaptive changes in the context of a modern environment[203], leading to distortions in the threat imminence continuum (for example, when pro-inflammatory cytokines increase owing to lifestyle factors such as diet and contribute to the development of mood disorders). Changing one component of the system that regulates defensive responses to threat, such as a pro-inflammatory cytokine of the immune system, will affect other interdependent components of the whole individual and tends to shift the thresholds for defence responses across the threat imminence framework (Fig. 4). Thus, diet and lifestyle may influence the risk for several neuropsychiatric disorders through their effects on the gut microbiome, inflammation, metabolism and hormone levels. Furthermore, any perturbation in the neural and systemic controls of defensive responses to threat may increase the susceptibility of an individual to stress-related illness.

Advanced tools that enable accurate inference of defensive behaviours at the organismal level, extracted from measurements of complex

Box 2

Interdisciplinary approaches enabling interrogation of the complex interplay between the brain and body that drives defensive behaviours

A particular challenge in studying defensive behaviours at the organismal level arises from the complication that animals can be under the influence of multiple factors at once. Past experiences, environmental factors, internal homoeostasis and hormonal influences can strongly shape how individuals react in threatening circumstances. Addressing this complexity will rely on advanced methodologies and engineering systems that enable interrogation of neural pathways that span the entire body, accurately record physiological parameters and extract meaningful information from complex high-dimensional behavioural data (see Supplementary Table 1 for a brief summary of existing and emerging techniques and their application scenarios).

Emerging single-cell sequencing technology is providing a comprehensive landscape of the cell type diversity of the brain and the body[207]. The development of advanced, implantable neurotechnologies, such as electrical, optical and microfluidic interfaces, for various parts of the nervous system has created various options for manipulating and monitoring neural activity in awake, behaving rodents[208]. Platforms involving the combination of these neurotechnologies with emerging methods in genetic neurobiology and animal physiology have provided a wide variety of research tools that enable exploration of the causal impact of neural activity and genes in the brain and the body during threat exposure[104,209–211]. The application of machine learning (ML) to animal behaviour studies is a particularly promising approach. From the kinematic features extracted from video recordings, ML algorithms provide an unbiased and high-throughput interrogation of fine behavioural variation shaped by bodily processes[36,212]. ML-based algorithms can also identify behavioural pattern modifications, such as changes in frequency, duration and temporal sequence of behavioural modules, following exposure to distinct threat types or drug applications and in disease models[36,212–216]. Discoveries using these interdisciplinary approaches will contribute to understanding the fundamental mechanisms underlying the maintenance of organismic health.

high-dimensional behavioural data and physiological parameters in a laboratory setting, will allow the dissection of the complex interplay between the brain and body (Box 2). A systems-level understanding of the defensive response to threat and its regulation provides a new lens through which one can understand the maintenance of organismic health. Successful outcomes from this research paradigm should also pave the way for the exploration of strategies to develop treatment methodologies for neuropsychiatric diseases.

Published online: 20 September 2023

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Acknowledgements
The authors thank all scientists whose studies were reviewed in this paper and apologize to those whose work was not cited owing to space limitations. The authors thank B. H. Zhao for assistance with illustrations. This work was supported by grants from the National Natural Science Foundation of China (32230042 and 31930047 to L.W., 32200826 to Y.-T.T. and 32222036 to P.W.), the Shenzhen Science and Technology Program (KCXFZ20211020163549011 to B.S. and JCYJ20220530154412028 to Y.-T.T.) and the Financial Support for Outstanding Talents Training Fund in Shenzhen (L.W.).

Author contributions
The authors contributed equally to all aspects of this Review.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41583-023-00736-3.

Peer review information *Nature Reviews Neuroscience* thanks Moriel Zelikowsky and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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