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Serotonin — A link between disgust and immunity?

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Summary Immune systems maintain the integrity of organisms by recognising and attacking foreign substances and/or pathogens. However, immune defences can only take place following direct contact with threats. Disgust can prevent infection before contact with potential pathogens: we propose that disgust is an evolved nervous response to a signal reliably co-occurring with infectious environmental disease threats, which motivates behaviour leading to the avoidance of infection. We hypothesize that disgust and immunity form a defensive continuum with overlaps: disgust acts prior to contact with the infectious agent and prevents it from getting into the body; emesis (vomiting) gets it out once inside the gastrointestinal tract, before penetration of the body boundaries; and immunity expels or kills infectious threats following penetration of the body proper. We further propose that serotonin (5-hydroxytryptamine, 5-HT) might be the link between disgust and immunity. 5-HT plays a central role in the induction of the emetic reflex and is possibly involved in the development of learned aversion; it is also a signal used by immune cells and modulates both innate and acquired immunity. We therefore propose 5-HT might mediate the interaction between these two defensive mechanisms.

Introduction

All animals are able to differentiate self from non-self, a prerequisite for life. Furthermore, despite the myriad pathogens and parasites present in the environment, an organism's ability to recognise its individuality enables it to preserve its integrity through immunity. To achieve similar defence functions, different phyla in the animal kingdom display various types of immunity; some mechanisms are convergent, while others are homologous because they share a phylogenetic history. Although not all details have been elucidated, immune defence mechanisms have been described in all animals, starting with the Porifera. For instance, these simple animals are able to reject allografts through at least two mechanisms: barrier formation and cytotoxicity [1]. More advanced animals show increasingly complex immune mechanisms. Considering the survival of an individual is at stake, it is not surprising that immunity is not based on a single mechanism. Immunity, however, is limited to responses that follow contact with a potential threat. A system that prevented contact with infectious threats would also be advantageous; it has been suggested that disgust is such a system [2,3]. We propose a biological and functional definition of disgust different from the lay

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understanding of the term as a purely human emotion. Disgust is defined as a system based in nervous tissue that evolved to detect reliable signals co-occurring with disease-causing infectious agents, which stimulates avoidance responses and/or other behaviours that tend to decrease the risk of disease [4]. In this paper we propose that there is a functional link between disgust and immunity, as both are part of a defensive continuum: disgust acts prior to or immediately following contact with infectious threats, while immunity deals with threats that persist despite disgust reactions.

The role of serotonin in disgust and aversive learning

Disgust can be thought of as having two stages: First, if detection of an infectious environmental threat is early enough (i.e., prior to contact with the pathogen and/or its getting inside the body), then the organism can take evasive action (avoidance). This can be considered *a priori* disgust. Examples include defensive behaviours such as avoiding faeces and blood, keeping away from sites perceived to contain pathogens, shunning infected conspecifics, etc. Second is *a posteriori* disgust: If detection of the reliable signal of an infectious threat only comes after contact with it or shortly after penetration of the body envelope, disgust reactions include behaviours that reduce the likelihood of developing infectious disease — for example, by actively expelling pathogens and thus reducing the infective dose within the body (e.g., nausea, vomiting, diarrhoea). We propose that *a posteriori* disgust and immunity overlap functionally and to an extent mechanically, as illustrated with the reflexive behaviour most usually associated to disgust: vomiting.

Emesis, or vomiting, is a reflex often associated with the rejection of orally-acquired pathogens [5]; for instance, dolphins and whales vomit when infected by *Helicobacter* [6], and emesis is the most common sign of infection in dogs harbouring the nematodes *Physaloptera* sp. [7] and *Spirocerca lupi* [8]. Although nematodes — and possibly other infectious agents — may be ejected from the intestine via diarrhoea [9], vomiting is a protective response which acts before diarrhoea and can eliminate pathogens present in the stomach and upper digestive organs; this is the case for *S. lupi*, where emesis can expel infective larvae before they penetrate the gastric mucosa [8]. However, emesis can be elicited by a broader range of stimuli. Emetic responses can be placed in at least six functional categories: (1) toxin rejection; (2) indigestible food residue rejection; (3) regurgitation as defence against predators or kleptoparasites; (4) response to postural instability and sensory conflict; (5) immune-mediated vomiting; and (6) ''cognitively-mediated'' (psychogenic) disgust. Thus, emesis has evolved for reasons other than disease avoidance, including defence against predators and protection against internal mechanical damage. However, types 2, 3, 4 and 6 are not considered further here, because they are not related to the particular hypothesis considered here: emesis as defence against infectious disease.

Although nausea and vomiting can be induced by a broad spectrum of stimuli, these are considered to act via three main pathways in mammals [10]: abdominal vagal afferents, the area postrema and the vestibular system. These inputs converge on the brainstem, whose structures are intimately involved in the initiation and coordination of the vomiting reflex [11—13]. Serotonin (5-hydroxytryptamine, 5-HT) plays a central role in the induction of vomiting; and we propose that it mediates both disgust- and immune-related emesis. Most (ca. 90%) 5-HT in the mammalian body occurs in the gastrointestinal (GI) tract and is produced by enterochromaffin (EC) cells and released upon stimulation of enteric nerves [12,13]. In the gut, 5-HT has a variety of motor and sensory functions through submucosal and myoenteric neurons possessing a variety of receptors and initiates an array of responses as diverse as nausea, vomiting, intestinal secretion and peristalsis, all of which can be considered to be part of the *a posteriori* disgust reaction. When the gut is stimulated (e.g., by increased intraluminal pressure or free radicals), EC cells release 5-HT which act as signalling molecules by binding to intrinsic primary afferent neurons via 5-HT_4 receptors, stimulating intrinsic (enteric) and vagal afferent nerve fibres [13]: the first initiates the peristaltic reflex; the latter mediates vomiting. Vagal nerve depolarisation induced by 5-HT through binding to 5-HT_3 receptors on enteric afferent nerves is the main pathway involved in the detection of emetic stimuli: electrical or chemical stimulation of vagal nerves results in an increased concentration of 5-HT in the brainstem and leads to emesis and, in contrast, vagotomy or the use of 5-HT_3 receptor antagonists suppress chemo- and radiotherapy-induced vomiting [11—13]. Despite the importance of 5-HT as an emetic, it must be emphasized that not all insults induce vomiting through this pathway; e.g., 5-HT_3 antagonists have no antiemetic effect against vomiting induced by morphine, apomorphine or motion sickness [12]. Nevertheless,

emesis induced by the presence of an infectious agent in the GI tract could conceivably be initiated by 5-HT, and result in increased 5-HT concentration in the brain.

Toxin intake or injection induces vomiting in many animals, ranging from copepods to humans [14,15]. Emesis results in a reduction of the toxic dose and increases survival. The classical example [16] is that of Monarch butterflies that accumulate the cardiac glycosides produced by the common milkweed, *Asclepias*, on which they feed. Glycosides affect the heart of the animal that eats them, and also the nerve centre in the brain that controls vomiting. If a bird eats milkweed toxin-containing butterflies, it will be violently ill and will never try to eat them again. By our definition, this emetic response is a reflex, but not disgust, because the toxin is a non-infectious agent directly inducing vomiting. Nonetheless, this response contains elements which are a prerequisite for disgust: nervous tissue sensing and reacting to a threat, the induction of a protective response and the association between a signal and disease. It is important to distinguish between merely unpalatable substances and those chemicals that cause GI illness. Unpalatable toxins are usually considered primary repellents, which are avoided reflexively (spat out) because they irritate the peripheral chemical senses. Chemicals that cause GI illness and elicit vomiting are known as secondary repellents, which induce learned avoidance of ancillary sensory cues that are paired with the illness. Toxin-induced emesis leading to learned aversion (or conditioned taste aversion (CTA)) is purported to be universal among animals, considering it has been documented in, among others, molluscs [17], fish [18], birds [16,19], and humans [20]. Not all emetic responses to toxins belong to the category of infectious disease avoidance because some toxins do not co-occur with infectious threats. However, in some cases, like GI bacterial infections, toxins are often the best sign of a pathogen's presence in the body, because they are chemical cues that pathogens cannot avoid secreting since they are essential for self-recognition, communication or virulence [5]. We suggest that when particular toxins do reliably co-occur with infectious agents, the mechanisms responsible for detecting toxins were exapted [21] to induce emetic reactions, thus ejecting both the toxin and the toxin-producing pathogen. A possible consequence of this would be the development of CTA against pathogen-containing foodstuffs. Evidence that such exaptation is plausible is provided by substances other than toxins associated to pathogens, which can induce learned aversion; for instance, rats avoid food flavours that have previously been administered with nematode (*Nippostrongylus brasiliensis*) infective larvae [22].

Apart from its central role in the induction of the emetic reflex, 5-HT might also be involved in the development of learned aversion (CTA). CTA follows vomiting and/or irritation of the GI tract, both of which involve 5-HT as a signalling molecule [12]. Learned aversion is primarily due to medullary, vegetative processes rather than to cerebral, cognitive processes [17,23], and might be mediated by the enteric nervous system [24], which could thus be involved in two distinct processes: sensing of toxins and emesis elicitation; and the development of learned aversion. 5-HT is an important mediator between the enteric and the central nervous systems [13], and could provide the link between emesis and learned aversion, which is possibly associated with some conscious aspects of human disgust. Disgust-related emesis would have arisen when CTA was exapted to specifically recognise signals (toxins or other compounds) and associate these to infectious agents, leading to learned aversion (≈avoidance). This might be an evolutionarily conserved mechanism, considering that the nematode *Caenorhabditis elegans* is able to distinguish between innocuous and pathogenic strains of *Bacillus thuringiensis*, and actively keep away from the latter [25]. This avoidance must be based on the use of a secondary signal which reliably identifies the one strain as dangerous when consumed, because the bacterium produces a deadly toxin only once it is in the worm's digestive tract. Similarly, *C. elegans* can learn to avoid pathogenic variants of the bacteria *Pseudomonas aeruginosa* and *Serratia marcescens*, by being able to associate chemosensory stimuli with illness and avoiding these stimuli in a choice test [26]. Exposure to pathogenic bacteria resulted in upregulated expression of 5-HT in ADF chemosensory neurons; and aversive learning required 5-HT from ADF neurons and the MOD-1 serotonin receptor, a serotonin-gated ion channel. Interestingly, 5-HT also signals GI malaise in mammals, specifically following chemo- and radiotherapy, and it does so through the 5-HT_3 receptor, also a serotonin-gated ion channel [27]. The similarity of the signalling pathways in nematodes and mammals may hint to the ancient role of 5-HT in visceral-brain communication.

The immune connection

Emesis in part overlaps functionally with an organism's innate immunity: both systems are

activated shortly after certain insults breach the body envelope. Thus, some cues will simultaneously elicit both emesis and innate immune responses; we postulate this is the case for 5-HT. Recently, it was shown that both T cells ($CD3^+$) and B cells ($CD20^+$) sit proximal to EC cells in the gut of rhesus macaques [28], and it was suggested that 5-HT released by EC cells could affect nearby lymphocytes and modulate immune responses. 5-HT is usually considered a neurotransmitter, which regulates appetite, mood and pain [13]. However, large quantities of this compound exist in the epithelial tissue of the GI tract, airways and skin [12], and this localization might indicate the role of 5-HT in protective or defensive reactions of the body to external insults. Moreover, 5-HT is actively transported into different immune cell types possessing the serotonin transporter (SERT), with platelets being an important reservoir of this compound in humans, and mast cells in rodents [29]. Stored 5-HT can be released quickly upon activation of these immune cells, which is accomplished by a variety of signals, such as platelet-activating factor, thrombin, complement fragments C3a and C5a, and immunoglobulin E (IgE)-immune complexes [30]. 5-HT affects both innate and acquired immune responses: e.g., it is a potent pro-inflammatory signal and upregulates phagocytosis in peritoneal macrophages [30]; and there is evidence of increased mitogenic proliferation of lymphocytes (both T and B) in response to serotonin, acting via a $5\text{-}HT_{1A}$/NF-κB-dependent amplification loop [31]. 5-HT might also be used as a ''neurotransmitter'' by the immune system, as suggested by the recent finding of dendritic cells delivering this compound to T cells across the immunological synapse in a manner similar to that which occurs between neurons [32,33]. Thus, 5-HT is recognised as a key player in neuroimmunoendocrine interactions in humans and rodents.

A further example of the functional overlap of disgust and immunity is provided by IgE-mediated emesis. Perhaps the best example of immune-mediated vomiting is the protective response elicited by *Anisakis*, where the ingestion of fish infected with this nematode results in violent emesis and diarrhoea in individuals possessing anti-*Anisakis* IgE antibodies [34]. In general, IgE-mediated immediate (Type 1) hypersensitivity responses are considered to have a direct anti-nematode protective function [35], particularly during challenge infections [9]. IgE-mediated immunity acts relatively rapidly, and can be thought of as having two distinct protective effects, one immune, the other reflexive.

The Immune protective effect has three components [30]: (1) protection mediated by immune effectors (histamine, serotonin, cytokines, lipid inflammatory mediators, etc.) released by mast cells/platelets upon activation, which recruit to site of insult and activate specific and non-specific immune effector cells; (2) increased lymph flow from site of antigen accumulation to lymph nodes, where naïve lymphocytes are activated; and (3) induction of muscular contraction, leading to physical expulsion of pathogens from the lungs or GI tract.

Reflexive protective effect: some compounds released by immune cells upon IgE-mediated activation, such as 5-HT, might be picked up by the nervous system and induce protective reflexes. One such reflex would be the induction of scratching at an itching site when an ectoparasite or other pathogen has crossed the epithelial barriers. A second protective reflex would be the initiation of emesis (retching and vomiting) and diarrhoea to get rid of orally-acquired pathogens before they cross the GI mucosa.

Conclusion

The functional overlap of disgust and immunity is limited to those responses that occur during a limited period following exposure to the infectious insult: disgust deals with behaviours preceding exposure and incorporation of the threat, and with those immediately following exposure (which may be a matter of hours in the case of the GI tract); immunity takes over the control of threats that persisted despite disgust-based responses, such as avoidance or emesis. We therefore propose there is a defensive continuum between disgust and immunity, with 5-HT serving as a mechanical link mediating the crosstalk: ''disgust-related'' 5-HT released in the gut by mechanical/chemical/emotional stress might be picked up by immune cells, whose functions could be modulated and/or which could amplify the signal; conversely, ''immune-related'' 5-HT secreted by immune cells could initiate a disgust reflex (e.g., emesis); thus, a single signal, 5-HT, might simultaneously activate both defensive systems, and in the case of the GI tract, provide a link to aversive learning via the enteric nervous system. Detection of exogenous 5-HT might also induce both reflexive and disgust-based emetic reactions, which could be protective considering that 5-HT is found in numerous venoms [13] and is secreted by leeches (and probably other parasites) upon detection of a host [36]. Perhaps particular types or subtypes

of 5-HT receptor in the GI tract and other body surfaces specialize in the detection of exogenous 5-HT. And, also indirectly, in the association, or not, of 5-HT to infectious threats, which could lead to learned aversion.

We propose that the functional position of disgust acting prior to immunity mirrors the evolution of the interrelationship between them: the protective reflex arose first and was complemented later in the evolution of animals by a more complex defensive mechanism, immunity, that builds upon it and in part uses the same regulatory pathways. Adaptive immunity in higher vertebrates similarly builds upon ancient invertebrate defence mechanisms [37,38]. By analogy, we hypothesize that the disgust-related behaviours we see in phylogenetically diverse animals have a common origin and that the human disgust system is built upon these foundations. We further suggest that the various forms of disease avoidance we have discussed are not only linked by a shared function and evolutionary history, but are linked physiologically by a shared mechanism: the workings of serotonin.

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