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ECOLOGICAL IMMUNOLOGY

Neuroendocrine-immune crosstalk in vertebrates and invertebrates: implications for host defence

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Summary

1. Communication among cells, tissues and organ systems is essential for survival. Vertebrate and invertebrate animals rely primarily on three physiological systems for intra-organismal communication: the nervous, endocrine and immune systems. Rather than acting independently of one another, these systems communicate in an integrated fashion to coordinate suites of species-appropriate physiological and behavioural responses.

2. Our understanding of how these three systems are coordinated remains incomplete, in part because the importance of the immune system as part of this regulatory network has only recently been recognized. In contrast to the well-established integrative approach to the study of the endocrine and nervous systems, the study of immunity has traditionally occurred in relative isolation from other physiological systems. Immunity was typically considered to be largely buffered from environmental perturbations.

3. In the last several decades, however, this simplistic view has changed dramatically; we now know that a wide variety of extrinsic and intrinsic factors can affect immune responses (reviewed in: Ader, Felten & Cohen 2001). This altered perspective has led to the development of new scientific disciplines including psychoneuroimmunology (Ader & Cohen 1981) and ecological immunology (Sheldon & Verhulst 1996).

4. These new research fields focus on the connections among the endocrine, nervous and immune systems. These fields also examine how environmental factors influence interactions among the three systems, and the implications of these interactions for behaviour and host defence. A comparative approach will benefit the search for the adaptive functions of these interactions.

Key-words: ecological immunology, energetics, stress, octopapine, glucocorticoids, leptin, disease ecology, sickness

Neuro-endocrine-immune crosstalk in vertebrates and invertebrates

Functional interactions between the neuroendocrine and immune systems are well-established in both vertebrate and invertebrate animals (Elenkov *et al*. 2000; Ottaviani *et al*. 2008). There is an extensive literature supporting the notion of bi-directional communication or 'crosstalk' between the nervous and immune systems (reviewed in: Elenkov *et al*. 2000; Ottaviani & Franceschi 1996), and it has been suggested that the brain and immune systems represent two major 'super-systems' within the body (Tada 1997; Elenkov *et al*. 2000). In vertebrates, the brain and immune systems are linked by two primary pathways: (i) the sympathoadrenal system (SAS), via either direct neural innervation (primarily via sympathetic nerves) of lymphoid tissue or catecholamine (e.g. epinephrine, norepinephrine) release from the adrenal medulla and (ii) the hypothalamo-pituitary-adrenal (HPA) axis and subsequent release of glucocorticoids (e.g. cortisol, corticosterone) from the adrenal cortex.

Analogous neuro-endocrine-immune connections can also be found in invertebrate phyla (Adamo 2006). Some of these connections appear to be evolutionarily ancient, such as the interaction been the stress and immune responses (Ottaviani & Franceschi 1996; Adamo 2008a). For example, in oysters,

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clamping the shell shut (Norberg & Tedengren 1995) is the bivalve equivalent of a 'flight-or-fight' response, and results in the release of the catecholamine norepinephrine into the circulation (i.e. hemolymph) from chromaffin-like cells located in the heart (Lacoste *et al*. 2001a). Norepinephrine binds to receptors located on the oyster's circulating immune cells, the hemocytes (Lacoste *et al*. 2001b), resulting in a decline in cellular immune responses (e.g. Lacoste *et al*. 2001a) and an increase in mortality after a standard bacterial challenge (Lacoste *et al*. 2001c). In insects such as the flying orthopterans (e.g. crickets, grasshoppers and locusts), 'flight-or-fight' behaviour activates the octopaminergic system within the insect's central nervous system (Orchard *et al*. 1993). Octopamine is the insect equivalent of norepinephrine (Roeder 2005) and these two neuromodulatory systems are thought to have evolved from a single ancestral form (Caveney *et al*. 2006). Octopamine has many of the same functions in insects that norepinephrine does in vertebrates (see Roeder 2005) and mollusks (Lacoste *et al*. 2001a). Octopamine is released into the general circulation (i.e. hemolymph) as a neurohormone in response to 'flight-or-fight' behaviour (Roeder 2005). Insect hemocytes have receptors for octopamine (R. Easy & S.A. Adamo, unpublished results) and octopamine influences insect immune responses (Adamo 2008a,b, 2010). Therefore, in both vertebrates and invertebrates, norepinephrine (or its chemical cousin, octopamine) mediates a connection between the neural/endocrine system and the immune system (Adamo 2008a).

In another interesting parallel between insects and vertebrates, insects follow the release of octopamine with the release a second 'stress' hormone (Orchard *et al*. 1993), just as vertebrates follow the release of norepinephrine/epinephrine with the release of glucocorticoids (Sapolsky 1992). In insects, the second 'stress' hormone is not a steroid, but the peptide adipokinetic hormone (AKH), released by the corpora cardiaca (Orchard *et al*. 1993). The corpora cardiaca, and the brain region innervating it, is loosely analogous to the pituitary gland and hypothalamus in vertebrates (Kodrik 2008). AKH is primarily involved with the mobilization of energy-rich compounds such as lipids (Orchard *et al*. 1993). However, AKH also enhances the activity of the immune enzyme phenoloxidase during an immune challenge (Goldsworthy *et al*. 2002). Therefore, both the insect stress hormone (AKH) and neurohormone (octopamine) interact with the immune system. However, unlike the situation in mollusks, in insects the direct effects of stress hormones are mostly immunoenhancing (Adamo 2008a). Similarly, stress hormones in vertebrates can have both immunoenhancing and immunosuppressing effects on immune function. For example, in macrophages exposed to LPS, norepinephrine can both inhibit the production of TNF-alpha (via beta-adrenergic receptors) as well as stimulate it (via alpha-adrenergic receptors) (Nance & Sanders 2007).

In vertebrates, multiple neuroendocrine systems have been shown to play an important role in the regulation of immune responses in vertebrates. For example, the reproductive neuroendocrine axis [i.e. hypothalamo-pituitrary-gonadal (HPG) axis] and the subsequent release of gonadal steroid hormones (i.e. testosterone, estradiol) exert marked effects on immune responses. Both laboratory and field studies have demonstrated that testosterone can alter specific immune responses (reviewed in: Grossman 1985; Muehlenbein & Bribiescas 2005; Roberts *et al*. 2004). Another example is the pineal indolamine melatonin. Melatonin has been shown to enhance some immune responses (Carrillo-Vico *et al*. 2005). More recently, the adipose tissue hormone leptin, which regulates energy balance and adiposity, has been shown to exert marked influences in immune responses across vertebrate taxa (see Energetics and Immunity section below). Whether leptin is present in all vertebrate taxa, including birds and reptiles, remains controversial (Sharp *et al*. 2008) but appears increasingly likely.

In invertebrates too, multiple neuro-endocrine systems can modulate immune function (see Adamo 2008b). For example, hormones important for development and adult reproduction (e.g. juvenile hormone and ecdysteroids) also influence immune function (Flatt *et al*. 2008).

In both vertebrates (e.g. Dantzer 2004) and invertebrates (insects: Adamo 2008b); mollusks: de Jong-Brink, 1997) the immune system both receives and sends signals to the neuroendocrine system. Immune-derived factors can alter neural activity and behaviour (Adamo 2006; Dantzer & Kelley 2007; de Jong-Brink, 1997; Hart 1988). Interestingly, the changes in behaviour that occur during an immune challenge [i.e. sickness behaviour (e.g. decreased feeding], Hart 1988) are similar in both vertebrates and invertebrates (Adamo 2006).

Implications of neuro-endocrine-immune cross-talk for animal life histories

Environmental influences, whether they be biotic or abiotic, can have profound influences on neuro-endocrine-immune interactions within an animal and changes in these systems can, in turn, have important implications for life history strategies. While it is not possible to cover all areas of research relating to these interactions, we will focus on two areas of research that have garnered significant experimental attention in the fields of psychoneuroimmunology and eco-immunology in both vertebrate and invertebrate study systems: (i) the energetics of immunity and (ii) stress and immunity. By highlighting research in these important areas we hope to demonstrate the many commonalities in how physiological systems regulate immune responses in response to changes in environmental conditions across diverse taxa. A greater understanding of the precise nature of these mechanisms will allow for a more complete appreciation of the physiological regulation of the immune response and its implications for host defence.

Energetics and immunity

As the adages 'feed a cold, starve a fever' or 'an apple a day keeps the doctor away' suggest, there is an important biological link between energy/nutrient balance and immune function and thus, disease susceptibility. Immunity, like all other

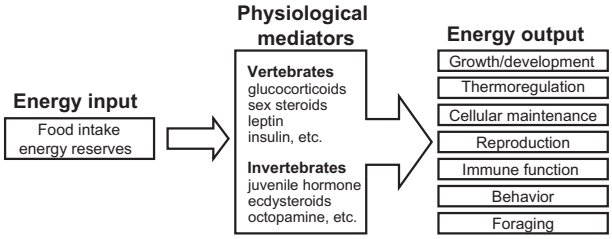

Fig. 1. Both vertebrate and invertebrate organisms have a limited amount of energy and resources available (energy input) to allocate to a wide range of physiological and behavioural processes (energy output). Investing resources in one process can therefore reduce investment into the remaining processes. These energetic trade-offs are mediated by changes in several neuroendocrine factors (physiological mediators), a subset of which are listed here. Changes in these and other factors plate a key role in coordinating optimal responses to match changing energetic budgets.

physiological processes, requires adequate energy to sustain optimal functioning. Despite this fact, the importance of energy availability as a modulator of immune function has only recently been considered. Within the last decade there has been an ever-growing body of research aimed at examining the energetic costs of mounting an immune response and the role of these costs in mediating energetic trade-offs among potentially competing physiological and behavioural systems in both vertebrate and invertebrate species (Fig. 1).

MAMMALS

Seminal studies in mammals conducted early in the last century suggested that mounting an immune response results in a substantial increase in energy use. For example, for every 1 °C increase in body temperature due to the induction of fever there is a 7–13% increase in oxygen consumption (Barr *et al*. 1922). Furthermore, the production of acute phase proteins and cytokines, as well as the proliferation of lymphocytes, requires a large quantity of amino acids and proteins as building blocks (Maier *et al*. 1994; Lee & Klasing 2004). It is well established that prolonged energy restriction can suppress immune responses and increase the risk of infections (Lochmiller & Deerenberg 2000). Even modest infections can result in greater than 200% increases in gluconeogenesis, whereas more severe infections typically cause dramatic increases in metabolic rates, losses in body weight in excess of 20%, as well as substantial mobilization of protein via breakdown of muscle (Maier *et al*. 1994). The potentially large energy demands associated with inflammatory and febrile responses likely require marked shifts in an animal's energy budget. However, only recently has a more precise quantification of the energetic costs of mounting an immune response been demonstrated (reviewed in: Lochmiller & Deerenberg 2000; Nelson *et al*. 2002). Mice immunized with keyhole limpet hemocyanin display ~20–30% increases in oxygen consumption and metabolic heat production compared with pre-immunization baseline values (Demas *et al*. 1997). More recently, the effects of an immune challenge on metabolic rate were assessed in mice selected for either high or low basal metabolic rate (BMR) (Ksiazek *et al*. 2003). Antibody production in response to immunization with sheep red blood cells was significantly reduced in mice with high BMR relative to low BMR mice. Animals in both lines, however, displayed comparable increases in both food intake and digestive efficiency in response to the immune challenge (Ksiazek *et al*. 2003). Furthermore, a similar immunization of wild-caught white footed mice (*Peromyscus maniculatus*) lead to decreases in dry masses of the small intestines and testes by 74% and 22%, respectively, suggesting that the costs of immune activation are met via re-allocation of energy away from other costly physiological systems (Derting & Compton 2003). Lastly, activation of an immune response can alter growth rates in rodents. Specifically, immune activation via injections of lipopolysaccharide, a bacterial mimetic that induces cytokine release and fever, impaired growth and decreased both lean and fat mass in laboratory mice (Laugero & Moberg 2000). Collectively, these and other studies suggest that immune function is energetically costly and that mounting an immune response can decrease energy investment into other physiological responses, including reproduction and growth.

Despite the apparent link between energy balance and immunity, relatively little is known regarding the precise physiological mechanisms by which energy availability regulates immune function. Unlike research in other taxa where studies have focused on energetic trade-offs between reproduction and immune function, most of the work in mammals has focused on the metabolic contributions to immunity. For most mammalian species, white adipose tissue (WAT) depots represent the storage of a substantial portion of the total energetic budget and thus WAT likely plays an important role in maintaining energetically expensive physiological processes, including immune function. Surgical removal of body fat (lipectomy; LIPx) impairs specific antibody responses in response to antigenic challenge in both Siberian hamsters and prairie voles (Demas *et al*. 2003). Furthermore, impaired immunity in LIPx animal is restored following compensatory increases in non-excised fat pads, suggesting that immune responses track total body fat (Demas *et al*. 2003).

The traditional view of adipose tissue has that of a passive reservoir for the storage of excess energy; however, research conducted over the last decade has proven this view inaccurate. In addition to its role as an energy depot, adipose tissue also serves as an important endocrine organ responsible for the production and secretion of a wide range of biochemicals, including bioactive peptides known as adipokines (Kershaw & Flier 2004). Among the many adipokines now identified, one obvious endocrine candidate linking available energy stores with immune function is the hormone leptin. Leptin is synthesized and secreted almost exclusively by adipose tissue (Ahima & Flier 2000). Circulating concentrations of leptin are directly proportional to adipose tissue mass such that high levels of the hormone indicate the existence of adequate energy stores to coordinate necessary physiological responses, whereas low circulating levels of leptin suggest an energy deficit. A wide variety of diverse actions

within the immune system are influenced by leptin. Ob/ob mice that are unable to synthesize leptin experience atrophy of specific lymphoid tissues (e.g. spleen, thymus), accompanied by decreases in the number of circulating lymphocytes and increases in the number of circulating monocytes (Lord *et al*. 1998). Furthermore, db/db mice that have a mutation of the leptin receptor display similar immunological deficits (Fernandes *et al*. 1978). Leptin also appears to differentially regulate the proliferation of naïve and memory T cells (Lord *et al*. 1998).

Exogenous leptin has been shown to increase lymphocyte proliferation (Demas 2010) in hamsters. Moreover, exogenous treatment with leptin can reverse experimentally-induced immune deficits. For example treatment of either short-day hamsters (Drazen *et al*. 2001) or long-day hamsters receiving lipectomies (Demas & Sakaria 2005) with exogenous leptin via osmotic minipumps attenuates the immunological deficits created by reduced adiposity in these animals. Although high affinity leptin receptors have been localized on lymphoid tissues, leptin appears to act indirectly via the sympathetic nervous system (SNS) to mediate changes in immunity. For example, intracerebroventricular administration of leptin to mice alters mitogen-stimulated splenocyte proliferation and is abolished by surgical denervation of the spleen (Okamoto *et al*. 2000). In hamsters, *in vivo*, but not *in vitro*, leptin enhances splenocyte proliferation (Demas 2010). Furthermore, the ability of exogenous leptin to enhance antibody production is blocked via either surgical (Demas *et al*. 2003) or chemical (i.e. 6-OHDA) (Demas 2010) sympathectomy of lymphoid tissues, suggesting that the actions of leptin are mediated, at least in part, by the SNS.

More recently, research has demonstrated that leptin can mediate energetic trade-offs between reproduction and immune function across generations (French *et al*. 2009). Specifically, pregnant Siberian hamsters treated with exogenous leptin invested more in offspring, giving birth to larger litters and inhibiting pup cannibalism compared with control mothers. Interestingly, however, this increased maternal reproductive investment came at a cost to the mother's own immune function; bacterial killing ability, an innate immune parameter, was reduced in pregnant, leptin-treated hamsters (French *et al*. 2009). These preliminary results suggest that leptin helps regulate energetic trade-offs with immunity across generations.

BIRDS, AMPHIBIANS AND REPTILES

Unlike in mammalian systems, the effects of many protein hormones on immune function are poorly characterized in avian, reptilian, and amphibian species. Instead, most energetic studies centred around immune function focus on single system trade-offs. Similar to mammals and invertebrates, we see significant metabolic costs for mounting immune responses in avian species. For example, mounting a cell-mediated immune response against the mitogen phytohaemagglutinin in house sparrows (*Passer domesticus)*, and a acquired immune response against an antigen (sheep red blood cells) in great tits (*Parus major*) significantly elevates metabolic energy expenditure (Ots *et al*. 2001; Martin *et al*. 2003), suggesting significant energetic investment in the different humoral responses.

Indirect evidence is available through studies that manipulate resource availability, whereby limiting required resources can suppress immunity. For example, resource restriction and reproductive investment are both known to reduce fat stores and important endocrine mediators in tree lizards (French *et al*. 2007a). Migration and parental care reduces body condition and fat stores multiple different avian species (Blem 1976; Roland & Moore 1996; Golet & Irons 1999). Similar, natural resource restriction via incubation-induced fasting in female eiders (*Somateria mollissima*) results in suppressed cell-mediated and humoural responses (Bourgeon *et al*. 2006). Further, experimental food restriction suppresses cell-mediated immunity and wound healing in yellow-legged gulls (*Larus cachinnans*) and tree lizards (*Urosuarus ornatus*) respectively (Alonso-Alvarez & Tella 2001; French *et al*. 2007a). It is important to note that immunological resource requirements are precise; specific proteins and nutrients are required, not just calories. For example, restricting protein alone decreases delayed-type hypersensitivity responses in chickens, and cell-mediated responses, lymphocyte yields, and lymphoid organ mass in Northern Bobwhite chicks (*Colinus virginianus*) (Glick *et al*. 1983; Lochmiller *et al*. 1993).

Historically, seasonal immune profiles in many species have been tied to environmental resource availability such as individual fat stores and endocrine mediators of energy availability (e.g. leptin, insulin, glucocorticoids), emphasizing the association between immunity and energy/resource availability. For example, there are marked seasonal changes in blood thyroxine levels and humoural immunity in the toad, *Bufo regularis* (Saad & Ali 1992). Similar seasonal changes are present in male song sparrows, where males in breeding condition have a suppressed sickness response relative to non-breeding males in the winter (Owen-Ashley & Wingfield 2006). Seasonal changes in circulating testosterone levels are directly related to the seasonal immune profiles of the lizard, *Chalcides ocellatus* (Saad *et al*. 1990). Further, melatonin, a hormonal mediator of seasonal changes in many species, can alter diurnal variation in both cellular and humoural immune in Japanese Quail, suggesting that it may also regulate seasonal changes in immunity (Siopes & Underwood 2008).

Seasonal changes in other physiological processes other than immune function may also play an important role in energy-dependent variations in immunity. In particular, investing energetic resources in alternate physiological processes, such as reproduction, can limit or suppress immunity, providing indirect evidence for the high energetic costs of immunity. Many of the effects on immunity are mediated via hormonal regulators of reproduction. For example, experimentally elevating testosterone in male sand lizards (*Lacerta agilis*) results in significant increases in parasite load (Olsson *et al*. 2000). Further studies in a myriad of avian species dem-

onstrate that treating males and females with testosterone results in both suppressed cell-mediated and humoural immunity (Duffy *et al*. 2000; Casto *et al*. 2001; Zysling *et al*. 2006). In the turtle *Mauremys caspica* injections of testosterone induce lymphoid mass involution and lymphopenia (Saad *et al*. 1991). However, the effects of testosterone appear context-dependent, where it is immunosuppressive in the laboratory but not in the field in superb fairy-wrens (*Malurus cyaneus*) (Peters 2000), while others studies find no association between testosterone and immunity (Hasselquist *et al*. 1999; Roberts *et al*. 2004). Alternatively, other studies show that alternate sex steroid hormones, including estradiol, DHT, and corticosterone have significant effects on immunity (Al-Afaleq & Homeida 1998; Mondal & Rai 1999; Owen-Ashley *et al*. 2004). Multiple sex steroid hormones are therefore important immune-modulators, regulating immune function and potentially resource allocation during reproduction and reproductive-related behavioural responses, such as territorial aggression and mate-guarding.

INSECTS

Maintaining and activating the immune system has significant energetic costs for insects too (Siva-Jothy *et al*. 2005). Freitak *et al*. (2003) showed that pupating white cabbage butterflies increase their standard metabolic rate by 8% during an immune challenge. To put this increase in perspective, vigorous physical activity in insects (e.g. crickets) raises their metabolic rate by 20 to 40% (Hack 1997b). Therefore, activating an immune response appears to be less energetically expensive than an extended bout of intense activity. On the other hand, some insects (e.g. crickets) spend 78% of their daily energy budget on metabolic maintenance (Hack 1997a). If this amount were significantly increased by an immune challenge, it would reduce the energy available for reproduction. In fact, there are examples of immune activation suppressing reproduction (reviewed in: Siva-Jothy *et al*. 2005). Similarly, reductions in energy availability (e.g. starvation) decrease immune function (Siva-Jothy & Thompson 2002) and increase disease susceptibility (Donegan & Lighthart 1989). In *Drosophila melanogaster*, an immune challenge reduces insulin signalling (DiAngelo *et al*. 2009). This reduced signalling suppresses growth as well as the movement of nutrients into storage (DiAngelo *et al*. 2009), supporting the concept that immune activation is costly.

If activating an immune response is energetically costly, then an immune challenge should result in the mobilization of energy reserves. In fact, this occurs in both vertebrates (Wellen & Hotamisligil 2005) and invertebrates (Mullen *et al*. 2004). Maier (2003) argues that the need for energy by both flight-or-fight behaviours and the immune response explains why both responses induce some of the same effects. However, AKH, the hormone that is most important for the liberation of energy stores, is not released during an immune response in insects (Mullen *et al*. 2004), making this hypothesis less likely in insects. How an immune response liberates lipid remains unknown in insects.

ENERGETICS AND IMMUNITY – A COMPARATIVE APPROACH

Some general patterns emerge when examining immune costs across phyla. (i) Immune activation results in energetic costs. Currently, however, it is difficult to rigorously compare energetic costs across phyla. The evidence suggests that mammals pay a particularly high price metabolically for their immunity (Malagoli & Ottaviani 2010). Why mammals have a proportionately more expensive immune system than other animals remains an outstanding question. (ii) An immune challenge can reconfigure intermediate metabolism, with ramifications for other behaviours (e.g. reproduction).

Stress and immunity

Another predominant area of active investigation within both psychoneuroimmunology and eco-immunology is the effects of stressors on immune function (Biondi 2001). Ever since Hans Selye first reported that exposure to extreme heat, cold or handling leads to alterations in immune organ masses and cell numbers in laboratory animals (Seyle 1936), there has been a well-established link between stress and immunity across phyla. The precise nature of this relationship, however, has remained equivocal with many studies reporting stress-induced immunosuppression whereas other have reported stress-induced immunoenhancement, depending on the duration of stress, the type of stressor, and the specific immune measure assessed. Thus, we still lack a comprehensive understanding of how and why stress alters immunity.

MAMMALS

Stress has traditionally been a nebulous concept within vertebrate biology and studies of stress-induced immunomodulation have employed a wide range of both physical and psychosocial stressors, often yielding different results. Stress has the ability to affect immune responses via two primary pathways, the sympathoadrenal [SA system (via catecholamines)] and the HPA neuroendocrine axis [via glucocorticoids (GCs)] (Fig. 2). It is well known that prolonged activation of the HPA axis in response to stress, and the subsequent release of GCs, triggers a wide range of physiological responses including suppression of virtually all aspects of immune function (Rook *et al*. 2000; (Biondi 2001). Stress-induced glucocorticoid release can cause marked changes in cellular trafficking, proliferation, antibody production and cytokine secretion. In addition, both *in vivo* and *in vitro* administration of exogenous GCs suppresses immune function in a wide variety of species (Rook *et al*. 2000; Biondi 2001). Accordingly, synthetic GCs are commonly prescribed for immunosuppressive therapy in cases of autoimmunity and local inflammation (e.g. Cohn 1997; Koski & Hermunen 2001). The exact actions of stress-induced glucocorticoid secretion on immune function, however, depend on whether the stressor is acute or chronic in nature. More recently, it has been demonstrated that GCs actually enhance immune functions during the

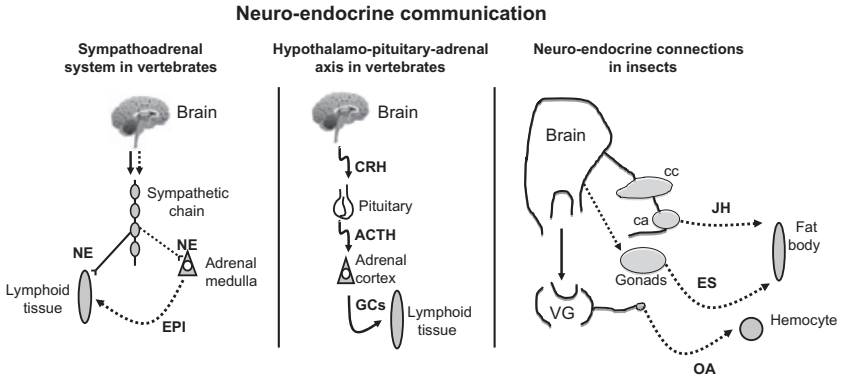

Fig. 2. Stress alters vertebrate physiology and behaviour primarily via: (i) the sympathoadrenal system and subsequent activation of sympathetic nerves directly innervating lymphoid tissue and/or via release of catecholamines (e.g. epinephrine, EPI, norepinephrine, NE) from the adrenal medulla or (ii) via the hypothalamo-pituitary-adrenal axis. Activation of the HPA axis triggers corticotropin releasing hormone (CRH) release from the hypothalamus, which in turn stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, and finally glucocortcioids (e.g. cortisol, corticosterone) release from the adrenal cortex. (iii) In insects, at least three hormones/neurohormones influence immune function. The corpora allata (ca) releases juvenile hormone (JH). After stimulation by a brain-derived peptide, the gonads release ecdysteroids (ES). During acute stress, octopamine (OA) is released from neurons into the hemolymph. Figure adapted from Loher and Zaretsky, 1989 and Nijhout, 1994. Abbreviations: corpora cardiaca (cc), ventral ganglion (VG). The dashed lines represent movement through the hemolymph.

short-term. For example, mice undergoing acute (2 h) stress experience an enhanced skin delayed-type hypersensitivity (DTH) response (i.e. increased proliferation of immune cells and swelling at the site of inflammation) compared with unstressed control mice (Dhabhar 2002a). Furthermore, adrenalectomy eliminates stress-induced immunoenhancement whereas treatment with exogenous GCs enhance the DTH response, suggesting an important role for GCs in mediating this response (Dhabhar 2002b). Acute stress triggers a state of 'immunoredistribution', with important immune components migrating from centrally-located lymphoid organs and blood to peripheral cites of inflammation. Consistent with this, acute stress reduces blood leukocyte numbers but increases erythema, the number of infiltrating leukocytes, as well as elevating proinflammatory cytokine gene expression (Dhabhar 2002b). Chronic stress, in contrast, leads to prolonged GC secretion and subsequent immunosuppression. It has been suggested that immunosuppression resulting from chronic stress prevents excessive immune activation, preventing the onset of autoimmune disorders (Dhabhar 2009).

Because of the rather extensive literature demonstrating suppression of immunity by glucocorticoids, as well as the robust effects of these hormones on immune function, SNS contributions to immune regulation have been understudied (Felten *et al.* 1998). Certain forms of stress-mediated immune suppression, however, are independent of the adrenal gland (i.e. experimental adrenalectomy fails to inhibit immune suppression), suggesting an important role for other neural/endocrine candidate(s) such as the SNS. For example, previous evidence suggests the SNS regulates both humoural and cell-mediated immunity (Josefsson *et al.* 1994; Madden 2001). An inhibitory role of the SNS in the regulation of immune function has been suggested in several species following denervation of a variety of lymphoid tissue, including the spleen (Madden 2001). Specifically, global chemical SNS denervation of adult animals enhances antibody responses to T-cell dependent antigens, but has no effect on T-cell independent antibody production (Miles *et al.* 1981). In contrast, it has been reported that chemical denervation reduces antibody responses to T-cell dependent antigens in adult rats (Livnat *et al.* 1985; Fuchs *et al.* 1988), whereas surgical denervation of the splenic nerve enhances antibody production in neonatal animals (Besedovsky *et al.* 1979; Williams *et al.*, 1981). More recently it has been demonstrated that chemical denervation results in a modest increase in anti-KLH antibody production in young rats, but a more marked increase in antibodies in older animals (Bellinger *et al.* 1990). In addition, B cells produce increased levels of Immunoglobulin G1 (IgG1) when exposed to norepinephrine (NE), the primary post-ganglionic sympathetic neurotransmitter, during antigen processing (Kasprowicz *et al.* 2000). Interestingly, NE increases IgG2a levels only after 12 h of NE exposure in culture; exposure of antibody secreting plasma cells to NE at a later time decreases antibody production (Melmon *et al.*, 1978). These findings suggest that the common dogma that catecholamines released by the SNS suppress immunity is too simplistic; adrenergic effects on both humoural and cell-mediated immunity are likely more complex than originally believed. A comparative and evolutionary perspective on these connections may help demonstrate their functional significance.

BIRDS, AMPHIBIANS AND REPTILES

While SNS and catecholamine-mediated effects of stress on immunity are significant in the mammalian literature, there is limited evidence of similar effects in non-traditional systems. Studies have characterized stress-related changes in catecholamines in tree lizards (*Urosaurus ornatus*) (Matt *et al.* 1997)

and various avian species (el-Halawani *et al*. 1973; McNeill *et al*. 1975; Le Maho *et al*. 1992). Although a few studies in chickens demonstrate catecholamine mediated effects on macrophages and humoral immune responses (Ali, Qureshi & McCorkle 1994; Denno *et al*. 1994), there is a relative paucity of evidence for what effects catecholamines exert on immunity in avian, reptilian and amphibian species. The majority of stress-related work across these taxa has focused primarily on GC associated interactions. Experimental studies involving treatment with adrenal steroids directly test effects of specific hormones on immune system. For example, hydrocortisone treatment altered lymphoid tissue masses in the ocellated skink (*Chalcides ocellatus)* (Saad *et al*. 1984a,b), and the bursa of Fabricius, spleen, and thymus in various avian species (Siegel 1971). Similarly, corticosterone treatment decreased a myriad of circulating immune cells in juvenile alligators (*Alligator mississippiensis)* (Morici *et al*. 1997).

Similar effects are present in studies examining immune function at the response level. Cortisol and aldosterone treatment inhibit lymphocyte proliferation in the South African clawed frog (*Xenopus laevis*) (Rollins-Smith & Blair 1993) and the effects are reversed by treatment with GC receptor antagonist RU486 and mineralocorticoid receptor antagonist RU26752 (Rollins-Smith *et al*. 1997). In the leopard frog (*Rana pipiens*) the immunosuppressive effects of hydrocortisone cause a normally sublethal infection with *Mycobacterium marinum* to become acute and lethal (Ramakrishnan *et al*. 1997). Functional studies altering HPA activity via external stressors (i.e. restraint, predator stress) also demonstrate elevations in HPA activity and downstream corticosterone levels to suppressed immune parameters. Restraint stress increases circulating corticosterone and decreases wound healing in tree lizards (*Urosuarus ornatus*) (French *et al*. 2006). However, GCs are not inevitably immunosuppressive.

As in mammalian and invertebrate systems the interactions between the HPA axis and immune system are complex. Specifically, the interactions are context-dependent, where GCs suppress immunity in temperate but not tropical populations of house sparrows (Martin *et al*. 2005). In tree lizards corticosterone treatment only suppresses wound healing when an individual is energetically compromised (French *et al*. 2007b). In certain cases the immunosuppressive effects of GCs may even be adaptive. For example, increases in corticosteroids during metamorphosis help decrease lymphocyte numbers, presumably to help reorganize the immune system and prevent autoimmune responses against newly developing tissues (Rollins-Smith *et al*. 1997; Rollins-Smith 1998).

It is important to note that these effects, as with mammals, can be trans-generational. Even though the amniotic egg would seem a fairly impenetrable barrier to developing young, the pre-hatching effects of maternal stress and GCs are profound. For example, offspring of corticosterone-implanted mothers experienced decreased T-cell-mediated immunity but not humoural immunity in yellow-legged gulls (*Larus michahellis*) (Rubolini *et al*. 2005).

INSECTS

As in vertebrate studies, 'stress' has a wide meaning in the entomological literature (Brey 1994). Various forms of stress, such as pesticide poisoning and extreme heat, depress immune function and disease resistance (Brey 1994). However, as these types of stressors cause a number of pathological changes in multiple physiological systems, it is not surprising that they have negative effects on immunity. In this section we focus on those studies that examine the effect of acute 'flight-or-fight' stress on immune function.

The production of 'flight-or-fight' behaviours results in a transient decline in disease resistance, as do injections of the stress neurohormone, octopamine (Adamo & Parsons 2006), or the stress hormone, AKH (Goldsworthy, Opoku-Ware & Mullen 2005). These studies suggest that the effects of stress hormones contribute to the decline in disease resistance observed after acute stress (Adamo & Parsons 2006). A parallel phenomenon exists in vertebrates. For example, humans show a transient decline in disease resistance (at least to upper respiratory tract infections) after intense exercise stress (e.g. Pedersen & Hoffman-Goetz 2000). Stress hormones are thought to be involved in this immunosuppression (Webster *et al*. 2002).

In another parallel between vertebrates (Karrow 2006) and invertebrates (Adamo 2010), both release stress hormones (octopamine in insects) during an immune response. In vertebrates, this release is thought to prevent an immune response from damaging host tissue (Karrow 2006). This explanation does not fit the data for insects, as octopamine tends to upregulate individual immune responses such as phagocytosis (Baines, DeSantis & Downer 1992). In fact, in insects, both stress hormones, octopamine and AKH, have predominantly positive effects on individual immune functions (Goldsworthy *et al*. 2002; Adamo 2008a, 2010).

The decline in disease resistance during acute stress in insects appears to be due, at least in part, to a physiological conflict between immune function and lipid transport (Adamo *et al*. 2008). During 'flight-or-fight' behaviours, the protein apolipophorin III loses its immune surveillance function (Adamo *et al*. 2008) and is co-opted into lipid transport in order to increase energy supply to the muscles (Weers & Ryan 2006). The result is decreased resistance to bacteria (Adamo 2008a). In insects, stress hormones may depress immune function indirectly, by redirecting resources (e.g. molecules like apolipophorin III) towards 'flight-or-fight' requirements. The direct immune enhancing effects of octopamine and AKH may act to reconfigure the immune system so that it can maintain functionality even though some of its molecules (e.g. apolipophorin III) are redistributed to flight-or-fight tasks (Adamo 2010). The effects of octopamine on immune function vary depending on the presence or absence of pathogens, suggesting that the effects of stress hormones on immune function also depend on physiological context in invertebrates (Adamo 2010), just as they do in vertebrates (Nance & Sanders 2007).

STRESS AND IMMUNITY – A COMPARATIVE APPROACH

As shown in the previous sections, the effects of stress hormones on immune function are multifaceted, especially in mammals. Work on invertebrates such as insects suggests that stress impacts immune function because the response to stress requires a reshuffling of resources (e.g. DiAngelo *et al*. 2009). To minimize the effects of the immune system's altered access to resources, stress hormones may reconfigure the immune system to maintain maximal functionality under different physiological conditions. Such a framework, borrowed from insights from simpler invertebrate systems, might explain the seemingly paradoxical and maladaptive connections between stress and immune function in more complex vertebrate systems.

Conclusions and implications for disease resistance

Connections between the neuro-endocrine systems and the immune system require a large number of specializations [e.g. signalling molecules with appropriately placed receptors in specific cells, as well as mechanisms to transmit peripheral immune signals across a blood-brain barrier (Dantzer 2004)]. It seems unlikely that such an array of molecules and physiological mechanisms would evolve in animals across phyla unless bidirectional communication between the neuro-endocrine and immune systems serves important adaptive functions. In fact examining these connections from an evolutionary and comparative perspective may be necessary to make sense of their complex interactions. Current research suggests that these interactions allow animals to both reallocate resources depending on the animal's environmental and physiological conditions (Ottaviani *et al*. 2008) and to reconfigure the immune system in order to maintain functionality despite the reallocation of resources (e.g. vertebrates, Dhabhar 2009; invertebrates, Adamo 2010). Why would animals in all taxa sometimes divert resources away from immune function? Although maintenance of a competent immune system is critical for resistance to disease, immune function is not solely responsible for survival and successful reproduction. Animals are selected to maximize fitness, not necessarily lifespan, and, as this review demonstrates, immune function does not always have unrestricted access to resources (Viney *et al*. 2005). Immunity fluctuates depending on the demands of other physiological systems, such as reproduction, and these demands will vary depending on environmental conditions such as nutrient availability and the need for 'flight-or-fight' behaviour. Although shifting resources away from immune function may maximize fitness, it can also result in periods of relative immunosuppression. Because of the potential costs of immunosuppression, selection will favour mechanisms that reduce its severity during these shifts in resources. Such selection pressure may explain why hormones/neurohormones tend to have both positive and negative effects on immune function in both invertebrates and vertebrates (vertebrates, Dhabhar 2009; invertebrates Adamo 2010). The varied effects allow organisms to limit the decline in immune system function even while shifting resources away from immunity (e.g. Adamo 2010), thereby fine tuning responses.

Such periods of immunosuppression may play a role in the pronounced temporal fluctuations in the prevalence of a wide variety of infectious diseases (e.g. AIDS, influenza, West Nile virus). Although some of this variation is undoubtedly due to changes in pathogen abundance (e.g. 'flu season'), changes within the host's immune system may also contribute to changes in patterns of disease transmission. In diseases such as West Nile Virus, the effect of immune function variability on disease transmission could be compounded by changes in resistance to the virus in both the insect vector and the vertebrate host. Furthermore, changing environmental conditions such as global warming is likely to increase the reproductive rate of mosquitoes (e.g. Afrane *et al*. 2006). Reproductive-induced immunosuppression is commonplace across species, and can lead to seasonal susceptibility to natural pathogens. Therefore, increased mosquito reproduction could lead not only to more mosquitoes, but to more mosquitoes susceptible to diseases like West Nile Virus. Despite the potential importance of fluctuating immune function on disease transmission, the majority of research investigating variation in disease prevalence has focused on monitoring pathogen abundance. Much less is known about how changes in host immune function affect disease susceptibility.

Complex interactions between different physiological systems can result in changes in disease transmission. The strength of psychoneuroimmunology is the focus on proximate control underlying neuro-endocrine and immunological interactions, providing a fairly comprehensive understanding of complex mechanisms. However it is often difficult to apply these findings to natural populations, where the environment including energy availability, stressors, and pathogen abundance are not static. For example, hormonal leptin signals provide key information regarding available resources, and are therefore vital for animals to properly allocate resources among systems, especially in the face of changing physiological states and external environments. However, its role may not be easy to determine under constant lab conditions. Therefore, more work is needed to understand how various alterations in immune function correspond to disease susceptibility in natural populations. A comprehensive knowledge of both the regulatory biology underlying neuro-endocrine-immune interactions and how these relate to resistance is critical for a fundamental understanding of the prevalence and transmission of infectious diseases.

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