

The immune system as the sixth sense

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One of the truly remarkable discoveries in modern biology is the finding that the nervous system and immune system use a common chemical language for intra- and inter-system communication. This review will discuss some of the pivotal results that deciphered this chemical language. Specifically the nervous and immune systems produce a common set of peptide and nonpeptide neurotransmitters and cytokines that act on a common repertoire of receptors in the two systems. The paper will also

review more recent studies that have delineated hardwired and humoral pathways for such bidirectional communication. This is discussed in the context of the idea that the sharing of ligands and receptors allows the immune system to serve as the sixth sense that notifies the nervous system of the presence of entities, such as viruses and bacteria, that are imperceptible to the classic senses. Lastly, this review will suggest ways to apply the newfound knowledge of the sixth sense to understand a placebo effect and to treat human disease.

Keywords: immune neuroendocrine interactions, immunosensor, interferon alpha, neuropeptides, neurotransmitter receptors.

Introduction

It is now 20 years since the proposition was put forth that the immune system also serves as a sensory organ that acts as a 'sixth' sense [1]. In this review, we revisit the major foundations of this idea, discuss the progress in establishing the idea's veracity and speculate on its future. The path leading to this sixth sense concept had its beginnings almost a decade earlier. In the 1970s, the suspected mode of action of what are now termed cytokines, such as interferon (IFN) was based on that known to be employed by certain hormones. The ubiquity of the IFN receptor on many different cell types together with the aforementioned notion that IFN might share second messengers, such as cAMP and cGMP, with hormones led to the

hypothesis that IFN and perhaps other cytokines would have multiple hormonal actions resulting in a myriad of physiological changes. Work from my laboratory as well as others proved this to be the case by demonstrating that IFN could mimic the actions of a number of substances such as α -adrenergic agents, neurotransmitters and peptide hormones (adrenocorticotropin, ACTH) by increasing the beat frequency of cardiac myocytes, increasing the firing rate of neurones and causing steroidogenesis in adrenal cells respectively [for review see 2]. Parenthetically, interleukin (IL)-1 was also shown to function as a hypothalamic-releasing factor [3]. This review will initially discuss the history, development and controversies of the early idea that molecular crosstalk exists between the nervous and immune systems.

Discovery of neuropeptides/ neurotransmitters in the immune system

An early important issue was whether the corticotropic and analgesic activities were intrinsic to IFN- α itself or due to some other entity in IFN- α preparations. As our initial studies preceded the routine use of molecular biologic techniques, and as IFN- α had been neither cloned nor sequenced when we began these studies, routine biochemical means (such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis) available at the time were used to address this issue [4, 5]. We reasoned that as IFN- α bioactivity is sensitive to the action of pepsin, whereas ACTH-mediated steroidogenesis is not, if ACTH activity remained after pepsin digestion of IFN- α preparations, this would provide evidence that a molecule other than intact IFN- α was likely responsible. The results show that pepsin destroyed IFN- α antiviral activity, but not the associated ACTH activity [4, 5]. From this we postulated that the IFN- α molecule: (i) contained an ACTH sequence, (ii) was tightly but noncovalently associated with an ACTH-like molecule, or (iii) copurified with a lymphocyte-derived precursor for ACTH and endorphins termed pro-opiomelanocortin (POMC). This

prediction constituted our first major controversy, but it was partially resolved when human IFN- α was cloned in the very same year. Analysis of the DNA showed that the IFN- α molecule did not contain ACTH or β -endorphin [6]. Thus, the first of our three possibilities was untenable. Although we favoured the second, it turns out that the third was ultimately proved correct. Subsequent finding that POMC (31 kDa) as well as its biosynthetic intermediate are produced by activated lymphocytes [7], led us to conclude that in all likelihood, we had been studying a copurified 22-kD biosynthetic intermediate of POMC together with the 23-kD form of natural IFN- α (Fig. 1). This finding, of course, did not exclude the possibility that a noncovalent, perhaps nonspecific, association of POMC peptides with IFN- α , for which we also had evidence.

With regard to the analgesic properties associated with IFN- α preparations, it turned out that the opiate receptor-mediated activity was in part due to an intrinsic property of IFN- α as well as to β -endorphin from immune cell-derived POMC. Although Epstein *et al.* [6] had failed to find a β -endorphin sequence in human IFN- α , our finding demonstrated that it nonetheless bound opiate receptors [5]. This was confirmed 11 years later by

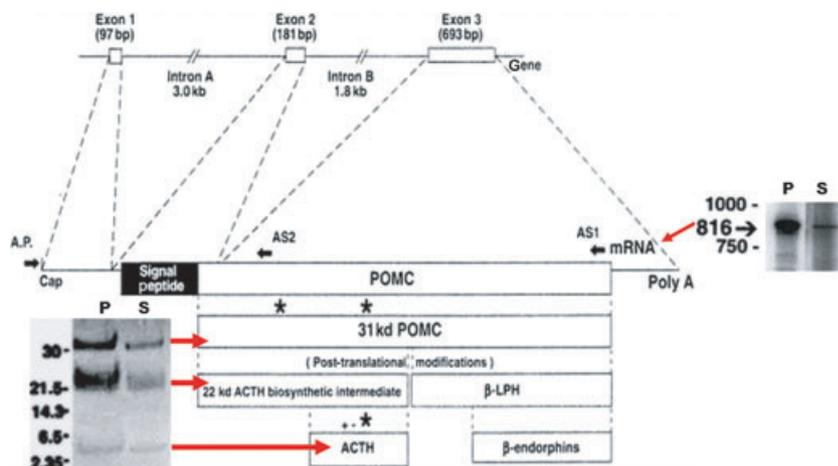


Fig. 1 Structure of the rat POMC gene and schematic of POMC mRNA and protein processing. The translated part of the mRNA is shown as an open box. Cleavage of the signal peptide generates the POMC prohormone, encoded by exons 2 and 3. The major peptide hormone in the anterior pituitary are ACTH which is derived from the 22 kDa biosynthetic intermediate and β -lipotropic hormone (β -LPH), β -endorphin is the major cleavage product from β -LPH in the intermediate lobe of the pituitary, along with other proteolytic products not shown. Asterisks designate sites of N-linked glycosylation. Inset gel to right shows that the expected 816 nucleotide mRNA for POMC is present in the anterior pituitary (P) and in mitogen-activated splenocytes (S). Inset Western blot to left with antibody to ACTH 1-24 demonstrates that the POMC mRNA is translated and processed similarly in the anterior pituitary and in mitogen-activated splenocytes (modified with permission from Lyons and Blalock (1997) [7]).



Fig. 2 Catatonic state caused 5 min after intracerebroventricular injection of human IFN- α (500 U). This mouse remained in this position for 20 min. Human IFN- α mediated catatonia can be prevented or reversed with the opiate antagonist naloxone (reprinted with permission from Blalock and Smith (1981) [9]).

Menzies *et al.* [8]. Indeed, when highly purified IFN- α was injected intracerebroventricularly into mice, it caused naloxone reversible analgesia and catatonia [9] (Fig. 2). These and other actions of IFN- α on the CNS seemed to be via the μ opiate receptor [10]. Most recently, Wang *et al.* provided a truly interesting molecular explanation for this finding [11]. Specifically, when Tyr129 of human IFN- α 2b is mutated to Ser, its antiviral activity is eliminated but the naloxone inhibitable analgesic activity is retained. However, when Tyr122 was

changed to Ser, the analgesic activity was completely lost whilst the mutant IFN- α retained 34% of its antiviral activity. Thus distinct domains of IFN- α are responsible for the immune and analgesic effects (Fig. 3). Furthermore, Wang *et al.* suggested that IFN- α may contain a 3-D Tyr XX Phe motif found in endogenous opioid peptides that is centred around Tyr122 [11].

The idea that immune cells were a source of neuropeptides was viewed by many as heretical as is often the case when fundamental and unexpected discoveries are made. As neuropeptides/neurotransmitters had never been observed in the immune system, immune cells were considered an entirely unlikely source. Fortunately, individuals such as Kavelaars *et al.* reproduced and extended many of our initial findings [12]. Moreover, Westly *et al.* [13] together with John Funder's group [14] and others [15, 16] provided a firm molecular foundation for our initial observations and predictions by demonstrating POMC mRNA is present in immune cells. In spite of these findings, the authenticity of the peptides continued to be questioned [17]. The identity between pituitary and leucocyte POMC peptides was eventually unequivocally established by demonstrating the presence of full length POMC mRNA in lymphocytes. Furthermore, the amino acid and nucleotide sequences of splenic and pituitary ACTH and POMC are identical [7, 18–20] (Fig. 1). Thus, ACTH and endorphins were the first

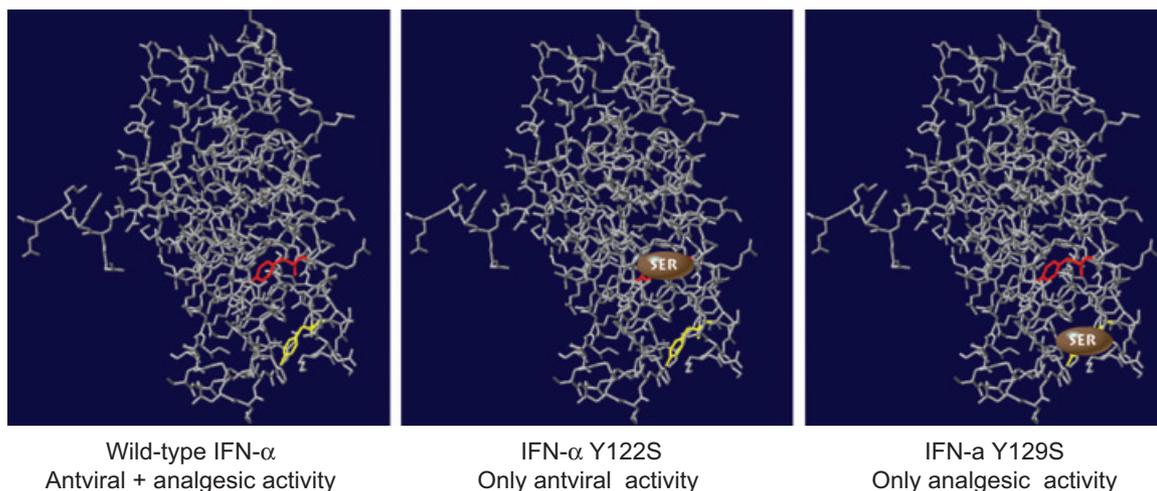


Fig. 3 Distinct domains of human IFN- α are responsible for analgesic and antiviral activities. IFN- α 2b is antiviral and causes analgesia. Mutation of tyrosine 129 to serine results in an IFN- α 2b with analgesic but not antiviral activity. Mutation of tyrosine 122 to serine results in an IFN- α 2b with antiviral but not analgesic activity.

Table 1 Cellular sources of peptide hormones and neurotransmitters in the immune system

Source	Hormone/neurotransmitters
Peripheral blood lymphocytes	Acetylcholine, melatonin
T lymphocytes	ACTH, endorphins, TSH, chorionic gonadotropin, GH, PRL, [Met]enkephalin, parathyroid-hormone-related protein, IGF-1, VIP
B lymphocytes	ACTH, endorphins, GH, IGF-1
Macrophages	ACTH, endorphins, GH, substance P, IGF-1, atrial natriuretic peptide
Splenocytes	LH, FSH, CRH, adrenaline, endomorphins
Thymocytes	CRH, LHRH, AVP, OT, adrenaline
Mast cells and PMN cells	VIP, somatostatin
Megakaryocytes	Neuropeptide Y

ACTH, adrenocorticotropic hormone (corticotropin); AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; LHRH, luteinizing-hormone-releasing hormone; OT, oxytocin; PMN, polymorphonuclear; PRL, prolactin; TSH, thyroid-stimulating hormone; VIP, vasoactive intestinal peptide.

neuroendocrine peptides to be identified as being synthesized *de novo* in the immune system. Today an entire constellation of peptide [for review see 21] as well as nonpeptide, such as acetylcholine (ACh) [for review see 22] and adrenaline [for review see 23], neurotransmitters, and neuroendocrine hormones, recently including melatonin [24] and endomorphins [25] are known to be endogenously produced by the immune system (Table 1). Therefore, our original findings with ACTH and endorphins proved generally correct. Moreover, the prediction that 'in the future it will be difficult to distinguish the receptors and signals that are used within and between the neuroendocrine and immune systems' [1] was demonstrated when it was shown that IL-1 was endogenously expressed in neurones [26]. Of course, for the nervous system, this was the reciprocal of our findings of neurotransmitters in the immune system and presently many ILs as well as IFNs are found both in the central and peripheral nervous system.

Discovery of neuropeptide/neurotransmitter receptors in the immune system

Concurrent with early observations on production of neuropeptides by immune cells emerging studies

reported that neuropeptide/neurotransmitter receptors were present on these same cells. These initial reports relied on functional assays [27, 28] but were quickly confirmed and extended through functional and radioreceptor assays [29]. Arguably the best studied receptor family for this discussion is that of opioids. In large measure, opioid receptors are of the μ , or δ class and each class can be found on immune cells. Specifically, using a δ class-selective ligand ($[^3\text{H}]cis-(+)-3\text{-methyl-fentanyl-isothiocyanate}$), a binding site with a molecular weight of 58 kDa was specifically labelled on murine lymphocytes [30] and the P388d₁ macrophage cell line [31]. The μ -selective, site-directed acylating agent, $[^3\text{H}]2-(\Delta\text{-ethoxybenzyl})-1-[N,N\text{-diethylamino}]\text{ethyl-5-isothiocyanato-benzimidazole}$, labelled a lymphocyte membrane protein, exhibiting μ -class selectivity, that also had a molecular weight of 58 kDa. In addition to the μ - and δ -opioid-receptors, the κ -selective, site-directed acylating agent, $[^3\text{H}](1s, 2s)-(-)\text{-trans-2-isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide}$, labelled a protein on lymphocytes that exhibited κ -class selectivity and had a molecular weight of 38–42 kDa [32].

The cloning of brain δ - [33, 34], κ - [35] and μ -opioid [36, 37] receptors provided probes to demonstrate that opioid receptor transcripts reside in cells of the immune system. The open reading frame for each of the opioid receptor types were found to be 98–99% identical to the brain opioid receptor sequences. A full-length κ -opioid receptor mRNA ordinarily expressed by cells of the nervous system was also found in the T-cell lymphocyte line, R1.1 [38]. These results confirmed previous pharmacological studies showing the existence of a κ -opioid receptor on the R1.1 cells that was coupled to guanine nucleotide-binding protein [38, 39]. A full-length δ -opioid receptor mRNA in thymocytes has also been identified and its cDNA sequenced [40]. In 1996, a nonopioid 'orphan' receptor, having sequence homology (60%) with the 'classic' opioid receptors, was cloned and sequenced in murine lymphocytes [41]. Furthermore, this receptor is up-regulated following cell activation with mitogens. The receptor also has functional significance in mitogen-induced lymphocyte proliferation and polyclonal antibody production, but not in IL-5 production, as determined by antisense experiments [41, 42]. A similar 'orphan' opioid receptor, whose

ligand was also isolated in 1995 [43, 44], has also been observed in activated human lymphocytes [45].

The earliest evidence for ACTH receptors on immune cells also were shown to be functional. For example, ACTH stimulated B-cell growth [46, 47] and antibody synthesis [48] at low concentrations. Interestingly at high concentrations ACTH suppressed cytokine synthesis [49] and antibody synthesis [29]. These findings were followed by others demonstrating ACTH-binding sites on B- and T-lymphocytes but not on thymocytes, by means of a radioreceptor assay [50]. Relatively early in the study of the ACTH receptor, 'experiments of nature' firmly established that the receptor on the classic ACTH target, the adrenal gland, is one in the same with that on immune cells. Specifically, individuals who are congenitally insensitive to ACTH-mediated steroidogenesis due to an aberrant adrenal ACTH receptor, also exhibit a total lack of high-affinity ACTH-binding sites on their peripheral blood leucocytes as compared with normal individuals [51].

The discoveries of virtually all neuropeptide, neurotransmitter and neuroendocrine hormone receptors on cells of the immune system have now been reported [for review see 52].

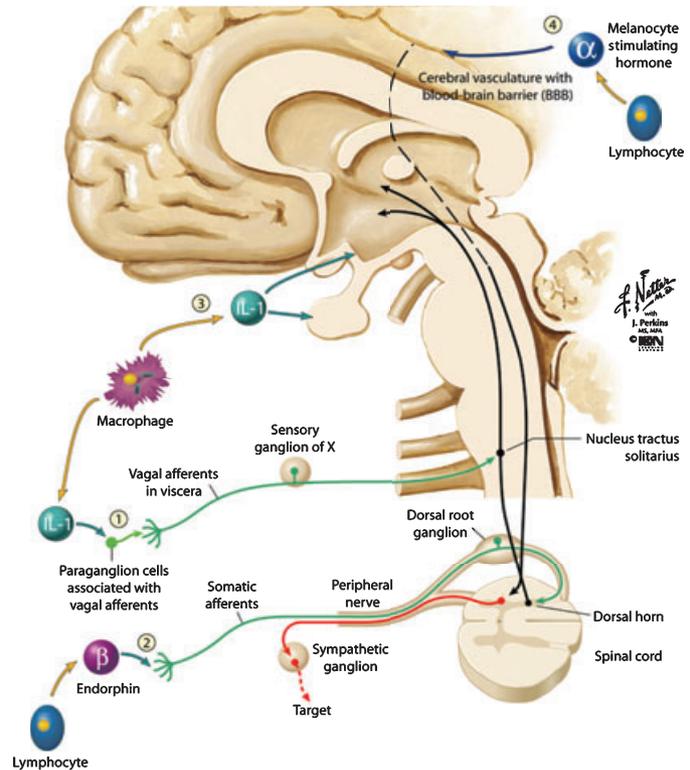
The immune system as a sixth sense

It is now recognized that the nervous system and immune system speak a common biochemical language and communicate via a complete bidirectional circuit involving shared ligands such as neurotransmitters, neuroendocrine hormones, cytokines and the respective receptors. We believe this is one of the truly remarkable discoveries in modern biology. This concept, in turn, provided answers for questions about extremely interesting yet puzzling observations that had long begged for explanation. In particular, the ancient anecdotal-based notion that the mind can influence the body during health and disease has long been on the fringes of mainstream science. Amongst the first experimental evidence for this idea was the demonstration by Metal'nikov and Chorine in 1926 that an immune response can be conditioned in a classic Pavlovian fashion [53]. The paradigm is to repeatedly pair administration of an immunoregulatory compound as an unconditioned stimulus with an external event which is the conditioned stimulus

(i.e. in the case of Pavlov, a ringing bell). With sufficient association, the conditioned stimulus alone is able to cause immunoregulation. This finding, in various forms, has stood the test of time with numerous replications beginning most recently in 1975 [for a review, see 54]. Other convincing evidence for the mind/immune system concept is found in the numerous effects of stress on immune function [for a review, see 55] and the observation that behavioural characteristics can predict susceptibility to autoimmunity in an animal model of multiple sclerosis [56]. Collectively, findings such as these left little doubt that the mind is capable of influencing the immune system. That stimulation or ablation of various regions of the brain could, depending on the region, inhibit or enhance immune responses strongly suggested that immunoregulatory entities resided in the CNS [for a review, see 57]. This being the case, how were these centrally located immunoregulatory entities operating? Two observations seem to have set the stage for solving the mystery. First, it was established that peripheral immune responses could alter the firing rate of neurones in the CNS [58]. Thus, information can flow not only from the CNS to the immune system but also in the opposite direction. Further, innervation of immune tissues and organs provided a conduit for such information [for a review, see 59]. But what is the nature and source of the information, and how and by what means is it received? This was clarified by the second observation that, as previously discussed, immune cells can produce neuropeptides such as β -endorphin and other neurotransmitters and neurons can make cytokines such as IL-1 [60]. Furthermore, cells of the immune system and the CNS each have receptors for both cytokines as well as neuropeptides and neurotransmitters. Thus neurotransmitters, neuropeptides and cytokines represent the signalling molecules relaying chemical information and depending on the stimulus either neurons or immune cells can be the initial source. The chemical information in turn can be received by both neurons and immune cells as they share receptor repertoires.

It has long been our contention that this complete biochemical information circuit between neurons and immune cells allows the immune system to function as a sensory organ [1, 21, 61]. A sixth sense, if you will, that completes our ability to be cognizant not only of the universe of things we can

Fig. 4 Examples of afferent nerve pathways used for immunosensing. (1) Cytokines-like IL-1 act on the vagus nerve to cause behavioural changes and illness symptoms. (2) Lymphocyte-derived neuropeptides such as β -endorphin modulate pain sensations by acting on peripheral sensory nerves. (3) IL-1 can act on the hypothalamus and pituitary to produce CRH and ACTH respectively. (4) Leukocyte-derived hormones like α -melanocyte-stimulating hormone cross the BBB and affect signalling on the sympathetic nervous system.



see, hear, taste, touch and smell but also the other universe of things we cannot. These would include bacteria, viruses, antigens, tumour cells and other agents that are too small to see or touch, make no noise, have no taste or odour. Recognition of such 'nonscognitive stimuli' by the immune system would result in transmission of information to the CNS via the aforementioned shared signal molecules to cause a physiological response that is ultimately beneficial to the host and detrimental to the infectious agent. Should we speculate that this sensory system may be responsible for our relatively frequent premonitions of illness in advance of frank disease? This notion of the immune system as a component of our senses has been borne out by more recent findings (Fig. 4). An example of such communication involving cytokines is the finding that upon peritoneal infection with bacteria or their products the constellation of changes such as fever that are associated with sickness behaviour come about as a direct result of immune cell-derived proinflammatory cytokines signalling the CNS via the subdiaphragmatic vagus nerve [62, 63]. An example involving a neuropeptide is also found by the demonstration that an antinociceptive/analgesic system can be activated by

inflammation. This apparently results from the *in vivo* production of β -endorphin by immune cells at the site of inflammation. Such β -endorphin in turn acts on local sensory nerve fibres to cause analgesia [64]. Thus the informational loop is closed.

Contrariwise, the CNS alerts the immune system to environmental changes using the shared neuropeptide, neurotransmitter and cytokine receptors that reside on immune cells (Fig. 5). An example of this is the effect of stress to dampen immune function. This apparently occurs via the effects of products of the hypothalamic-pituitary-adrenal axis on immune cells. An impairment of this axis to appropriately respond to inflammatory stressors, as in deficient corticotropin-releasing hormone (CRH) release, has been shown to predispose an animal to autoimmune disease as a result of lack of regulation of the immune system [65].

A second and particularly exciting pathway involves the efferent vagus nerve. In elegant studies by Tracey *et al.*, ACh was shown *in vitro* to suppress lipopolysaccharide-induced human macrophage production of proinflammatory cytokines (TNF, IL-16, and IL-6), but not an anti-inflammatory cytokine (IL-10). This inhibitory effect on proinflammatory

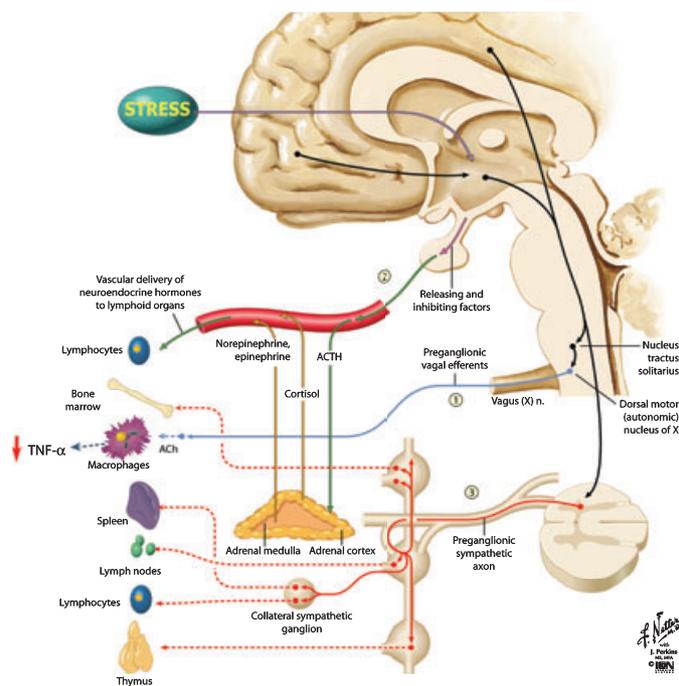


Fig. 5 Examples of efferent nerve pathways that modulate immune function. (1) Vagal acetylcholine acts on macrophages to blunt pro-inflammatory cytokine synthesis. (2) Hormones from the hypothalamic-pituitary-adrenal axis modulate lymphocyte function. (3) Sympathetic outflow can regulate the function of immune tissues and their cells.

cytokines could be recapitulated *in vivo* by electrical stimulation of the efferent vagus during exposure to endotoxin [66]. Suppression of such cytokine synthesis and remarkably the prevention of endotoxic shock was observed *in vivo* and resulted from vagal release of ACh acting on nicotinic ACh receptors known to reside on macrophages and other cells of the immune system [for a review, see 67]. In this particular instance, the nicotinic ACh receptor $\alpha 7$ subunit was essential for the anti-inflammatory effect [68]. Parenthetically, mononuclear leucocytes also express choline acetyltransferase and synthesize ACh [69, 70].

Future directions for the sixth sense

It is truly ironic that in the history of studies on organ systems physiology similarities have often been noted between the immune system and nervous system in terms of total numbers of cells, combinatorial complexity and memory; analogies have even been drawn between the immune system, grammar and language [71]. Yet definitive experiments were lacking. Now it is clear, as borne out by solid findings, that there is a powerful interconnected language between these two systems. Indeed, the term 'immunologic synapse' has entered today's biologic lexicon, and the eminent immunologist

Mark M. Davis recently rephrased the now familiar idea with the statement 'Lymphocytes and natural killer cells can be viewed as cell-sized sensory organs, continuously sampling the internal environment for things that don't belong there or for cellular stress or aberrations. Just as rod cells in the eye can detect even a single photon, cytotoxic T cells can kill on the advice of only three peptide-MHC ligands' [72]. In the context of the present discussion, it appears that the immune/nervous system metaphor has become reality. What was previously viewed as outside the realm of sensory perception, and therefore metaphysical, is now seen as otherwise.

But beyond the basic knowledge, will there be utility and practical benefits to society resulting from our new understanding of the sixth sense? It is our opinion that there is a wealth of opportunities on a not too distant horizon. Although it is beyond the scope of this review to cover them all, a few come immediately to mind. The identification of separate sites on IFN- α that are required for immunoregulatory/antiviral activity as opposed to its analgesic/pyrogenic effects sets the stage for 'designer cytokines' [11]. Mutation of a single amino acid residue can totally eliminate one of the principal side-effects (i.e. pyrogenicity and perhaps flu-like symptoms) [73] whilst retaining immunoregulatory/antiviral

potency. This should allow for a higher maximum tolerated dose of IFN- α and a dramatic change in the treatment schedule and indications of the many diseases for which IFN- α is clinically employed. Mutation of a residue that is essential for the immunoregulatory/antiviral activity leaving opiate receptor binding intact is also tenable. This form of IFN- α might be developed as a new analgesic. Similarly, a form of erythropoietin was recently described that has lost haematopoietic activity but retains potent ability to confer neuroprotection in many pathologic states [74]. Thus erythropoietin may be useful in the treatment of stroke and other neurological disorders.

There are also novel uses for known drugs and medical devices. For instance, an opioid receptor antagonist was shown to be more effective than ciclosporin in prolonging rat renal allograft survival [75]. The discovery of a cholinergic anti-inflammatory pathway whereby ACh released from the efferent vagus nerve can act on macrophages *in vivo* to block inflammation and shock has identified a new therapeutic approach for patients in septic shock [76]. Vagus nerve stimulation with small pacemaker-like devices is a safe, effective and approved therapy for epilepsy and depression [77]. Perhaps these devices will also prove useful in controlling inflammatory diseases via activation of the cholinergic anti-inflammatory pathway. Similarly, the tetravalent guanidylhydrazone CNI-1493, has been found to cross the blood barrier and specifically activate this cholinergic pathway and inhibit inflammation [78]. This compound is currently undergoing testing in phase II clinical trials for Crohn's disease and may be useful in many other inflammatory disorders [for review see 79]. Further proof in principle is the efficacy of the ACh receptor agonist, nicotine, in reducing the severity of ulcerative colitis [80]. In a related vein, some of the anti-inflammatory actions of α -melanocyte-stimulating hormone and some of those for salicylates are via a sympathetic efferent route which may be further exploited [81, 82]. In terms of diagnosis, there is the interesting possibility that neuropeptide, neurotransmitter and hormone receptors on peripheral blood cells might serve as surrogates to evaluate structure and function of those receptors at more central and inaccessible sites. In fact, the ability to detect a form of ACTH insensitivity syndrome by testing for the lack of high-affinity ACTH receptors

on peripheral blood mononuclear cells may represent the first example [51, 83].

A common theme seems to be rapidly emerging as we better understand the sixth sense. Put most simply, an understanding of the physiology and pathophysiology that results from this circuitry will have a revolutionary impact on the understanding and practice of medicine that is not unlike what has resulted from deeper insight into other components of the nervous system, which has led to circuit specific drugs like those now used for Parkinsons disease, depression, etc. It may well reveal many of the deep secrets of our everyday experiences, observations and anecdotes that have defied explanation. A case in point concerns a new interpretation of our

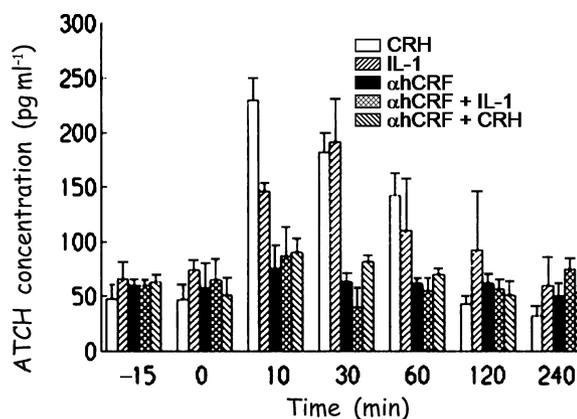


Fig. 6 Prior treatment with the CRH antagonist, α helical (h) corticotropin-releasing factor (CRF) 9-41 blocks the ACTH-releasing activity of CRH as well as IL-1. To determine whether responses to CRH or IL-1 could be blocked by α hCRF [9-41], rats were subjected to a double i.v. injection protocol via indwelling cardiac catheters. Two i.v. injections were spaced 15 min apart. Groups of animals were subjected to one of two treatments. One group was injected first with the vehicle PBS (100 μ L) followed 15 min later by the peptides (CRH 10 μ g 100 μ L⁻¹, IL-1 250 ng 100 μ L⁻¹) or PBS (100 μ L). Another group was pre-treated by first injecting α hCRF [9-41] (100 μ g 100 μ L⁻¹) followed 15 min later with either CRH, IL-1, or PBS. Blood samples (0.5 mL) were withdrawn from the catheter just 15 min prior to, immediately before the CRH, IL-1 or PBS injections (time 0) and 10, 30, 60, 120 and 240 min postinjection. CRH (open bars) and IL-1 (hatched bars) stimulate ACTH secretion. Significant differences were observed as early as 10 min after treatment with CRH or IL-1 and continued to 60 min. The peak with CRH occurred at 10 min whilst the IL-1 effect peaked at 30 min. CRH-induced ACTH secretion and IL-1-induced ACTH secretion were blocked by α hCRF [9-41] (crosshatched bars and hatched bars respectively). The effects of α hCRF [9-41] alone (solid bars) and PBS control (data not shown) were not statistically different. Data represent ACTH concentration \pm SEM with $n = 5$. These results are representative and are from one of five independent experiments (reprinted with permission from Payne *et al.* (1994) [84]).

earlier observation [84]. Beginning with the original demonstration of IL-1-induced ACTH release from a corticotroph cell line [3], AtT20, and subsequent confirmations and demonstrations of this response with primary pituitary cell cultures [85–90], a controversy ensued as to whether *in vivo* IL-1-mediated ACTH increases originated at the level of the hypothalamus or pituitary gland [91]. Although

the consensus of studies concluded that ACTH release occurs as a result of IL-1 eliciting hypothalamic CRH release and that the CRH is entirely responsible for pituitary ACTH secretion [92–95], these findings were difficult to reconcile with the abundance of IL-1 receptors in the pituitary gland when compared with the hypothalamus [96, 97]. The observation that CRH-upregulated-IL-1 receptors on AtT20 cells suggested a solution to this paradox [98]. That is, any direct pituitary response to IL-1 might be CRH-dependent perhaps through an effect on the IL-1 receptor. This, of course, would explain the complete abrogation of ACTH release when antiserum to CRH or the CRH antagonist, α -helical (α h) CRF [9–41] [99], is administered with IL-1 [92–95]. These substances would not only block CRH-mediated ACTH production but would also prevent sensitivity of the pituitary gland to a direct effect of IL-1.

As had been observed by others [92–95], Fig. 6 shows that CRH or IL-1 cause the expected time-dependent increase in circulating ACTH levels in cannulated rats, and these increases are completely abrogated by pretreatment with α hCRF [9–41]. In contrast, a 4-h pretreatment with a dose of CRH below the amount required to directly elicit pituitary ACTH release was sufficient to prompt an ACTH response to IL-1 even in the context of α hCRF

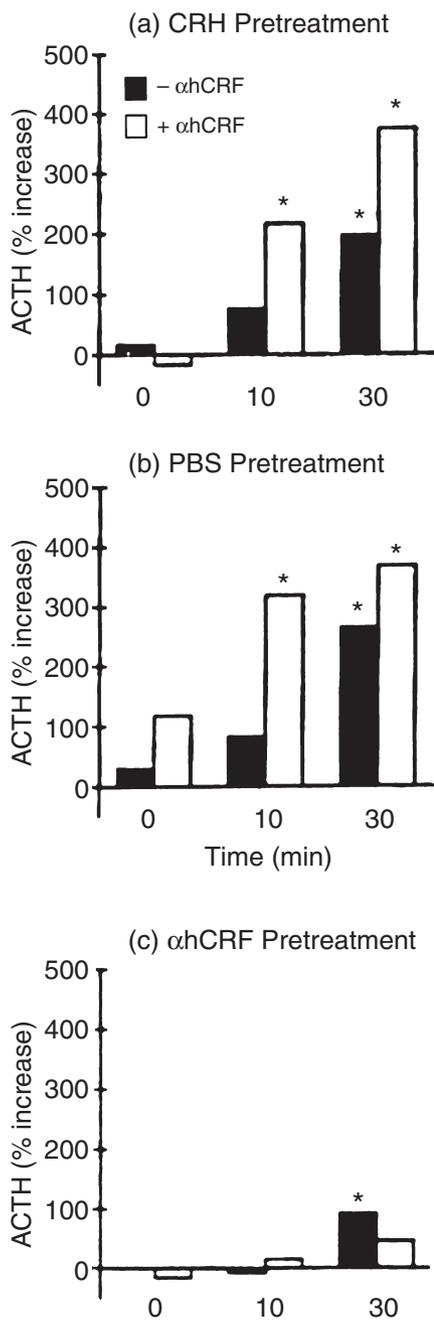
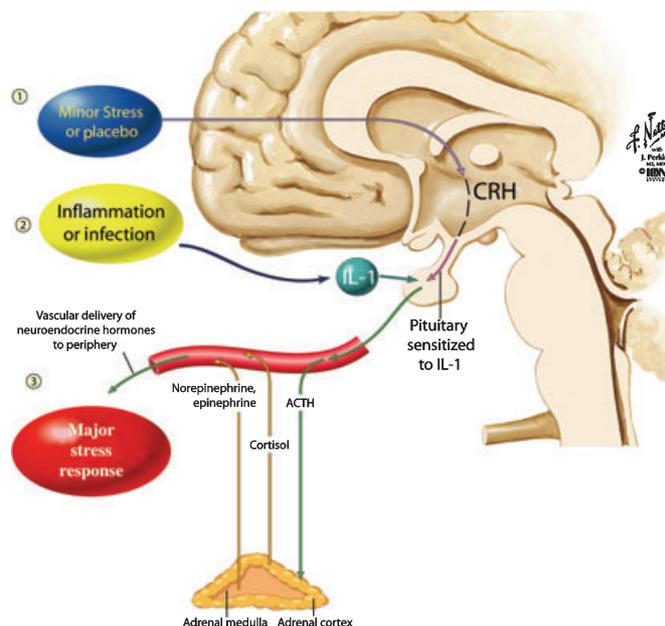


Fig. 7 Low levels of exogenous or endogenous CRH sensitizes the pituitary to direct IL-1 dependent ACTH release. Animals were pretreated with PBS (100 μ L) or suboptimal doses of CRH (1 μ g 100 μ L⁻¹) or α hCRF [9–41] (10 μ g 100 μ L⁻¹). Blood samples (0.5 mL) were withdrawn from the catheter immediately before the i.v. injections (time 0). Four hours after the initial priming, animals were subjected to the same double i.v. injection protocol described in Fig. 6. Blood samples were taken 10 and 30 min postinjection. The effects of i.v.-injected IL-1 (250 ng 100 μ L⁻¹) in the absence (solid bars) or presence (open bars) of α hCRF [9–41] (100 μ g 100 μ L⁻¹) on ACTH secretion after a 4-h pretreatment with an i.v.-injected suboptimal dose of CRH (a) PBS (b), or a suboptimal dose of α hCRF [9–41] (c). Data points represent the percentage of increase from control ACTH levels (48.45 ng mL⁻¹) in PBS-treated animals. IL-1 significantly stimulated ACTH secretion after priming with PBS and suboptimal doses of α hCRF [9–41] and CRH. α hCRF [9–41] was unable to attenuate or block IL-1-induced ACTH secretion after priming with PBS or suboptimal doses of CRH. However, after priming with a suboptimal dose of α hCRF [9–41], α hCRF [9–41] was able to block IL-1-induced ACTH secretion. Statistical analyses were performed by paired two-tailed Student's *t*-test. * $P < 0.05$ from control with PBS injection. Results of IL-1 alone are from 10 rats and all other groups had an $n = 6$. These results are representative and are from one of six independent experiments (reproduced with permission from Payne *et al.* (1994) [84]).

Fig. 8 Crossroads of the classic senses and the sixth sense. (1) Mild, perhaps imperceptible, stress, or a placebo cause the hypothalamus to release CRH in quantities too low to evoke pituitary ACTH release but sufficient to upregulate pituitary IL-1 receptors. (2) Coincident or subsequent inflammation or infections elicits IL-1 which acts directly on the pituitary to cause ACTH release and (3) a stress response that is above and beyond what would be expected for the level of inflammatory stress.



(Fig. 7a). In fact, the animals showed an enhanced ACTH response in the presence of IL-1 and α hCRH [9–41]. To our surprise, administration of phosphate-buffered saline (PBS) caused the same insensitivity of IL-1-induced ACTH release to α hCRF [9–41] inhibition and the combination of PBS then IL-1 followed by α hCRF showed an enhanced ACTH response relative to IL-1 alone (Fig. 7b). To determine whether this effect was due to endogenous CRH release caused by the PBS injection, we pretreated the animals with PBS containing α hCRF [9–41] to block any endogenous CRH. Such treatment not only caused the IL-1-mediated ACTH response to be susceptible to blockade by α hCRF [9–41] (Fig. 7c) but blunted the ACTH response to IL-1 alone. This latter effect suggested that endogenous CRH may be essential for optimal IL-1 activity by sensitizing the pituitary to IL-1.

In essence, observing the same phenomenon with PBS as low dose CRH would be considered a placebo effect. Yet this placebo effect now has a molecular explanation with important ramifications (Fig. 8). Specifically, a seemingly imperceptible stressor (i.v. injection of PBS) that by itself elicits no ACTH or glucocorticoid response can via minute amounts of CRH greatly amplify a coincident or subsequent exposure to mediators of inflammatory stress. Thus, depending on the individual an event that might be perceived by the population at large as mildly stressful or completely lacking psychological or physical stress

could have a profound physiological effect with respect to that individual's coincidental or subsequent inflammatory response associated with illness and infection. With that in mind, it does not seem particularly farfetched that an individual's personality and outlook might have a real and explainable impact on their susceptibility to disease.

Conflict of interest statement

No conflict of interest was declared.

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