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26. THE GUT MICROBIOME AND THE NERVOUS SYSTEM

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INTEROCEPTION AND THE SENTIENT SELF

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INTRODUCTION

Langley was the first person to describe the autonomic nervous system (ANS) (*Langley*, 1903). Near the end of his report, he noted explicitly that he had omitted a description of the sensory inputs that are required for the ANS to function efficiently; he explained that he had not been able to unequivocally distinguish the necessary elements anatomically. As Cannon later emphasized, the neural processes (autonomic, neuroendocrine and behavioural) that maintain an energy-efficient, physiological balance across conditions in the body, i.e., homeostasis, must receive modality-selective afferent inputs that report the condition of the tissues of the body (*Cannon*, 1939). Finally, Prechtl and Powley suggested that the missing category of homeostatic afferents could be identical to the anatomically well-defined small, dark B-cells in the mammalian dorsal root ganglia (*Prechtl* and *Powley*, 1990); unfortunately, their proposal was rejected by nearly all of the discussants of their publication. Had they been aware of the findings described in the present report, they would have recognized the central continuation of the pathway they had envisioned and the unmistakable confirmation of their proposal.

The sensory pathway that is described in the following sections provides not only the sensory inputs required for organotopic homeostatic control of the body's condition, but also the basis for human feelings from the body, such as cool, warm, pricking pain, burning pain, itch, sensual touch, muscle ache, bowel distension, urge to urinate, vascular flush, hunger, taste, thirst and "air hunger". These feelings, which are all related to the condition of the body and underlie mood and emotional state, are all associated with strong affective motivations that are the correlates of behavioural responses needed to maintain the health of the body. Thus, these feelings can all be viewed as homeostatic emotions, a concept which emphasizes their essential autonomic role. The anatomical pathways described below underpin the homeostatic nature of these feelings, and conversely, reveal a fundamental relationship with the ANS that explains why the affectively charged feelings from the body all have strong autonomic sequelae. So, pain is accompanied by autonomic changes because it is the perceptual correlate of a behavioural motivation generated in response to a condition which the homeostatic system cannot rectify automatically.

In the conventional view, the well-discriminated feelings of temperature, itch and pain are associated with a somatosensory system that maps the sense of touch to a recognized map of the body (homunculus) in Rolandic cortex (Figure 1). In contrast, the less distinct visceral feelings of vasomotor activity, hunger, thirst and internal sensations are said to be associated with a separate visceral system in more archaic regions. That conceptualization obscures fundamental discrepancies, such as the fact that stimulation of Rolandic somatosensory cortices almost

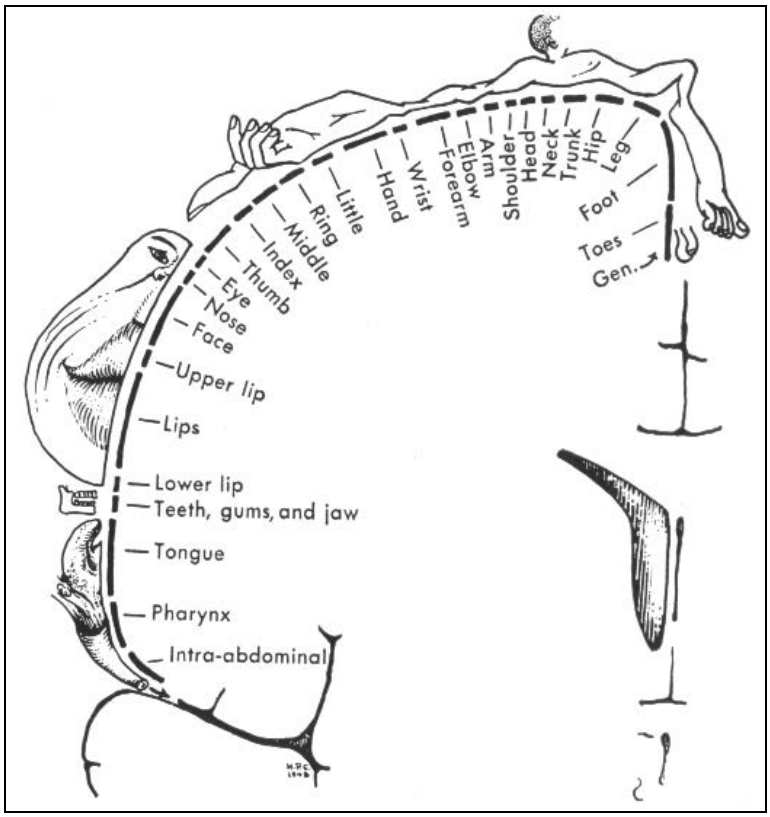

Figure 1: Human homunculus (*Penfield* and *Rasmussen*, 1950).

never produces feelings of pain or temperature, or that lesions of Rolandic cortex have no effect on temperature or pain sensations. It also leaves unexplained the inherent emotional (affective/motivational) qualities and reflexive autonomic effects that all feelings from the body share, which distinguish them from tactile mechanoreception and from the sense of limb position (proprioception). In the following sections it is shown that all feelings from the body are represented in a phylogenetically new system in primates that evolved from the afferent limb of the evolutionarily ancient, hierarchical homeostatic system that maintains the integrity of the body, that is, the sensory complement of the ANS. These feelings thus represent a sense of the physiological condition of the entire body, providing a broad redefinition of the term 'interoception'. More importantly, from the clinical perspective, these findings reveal that feelings from the body, such as pain, are inherently linked with autonomic conditions, such as plasma extravasation or cardiac rhythmicity, because they are, respectively, sensory and motor aspects of the same homeostatic system. In humans, further processing builds a meta-representation of primary interoceptive activity, engendered in the anterior insula, which seems to provide the basis for the subjective image of the material self as a feeling (sentient) entity, that is, emotional awareness. More detailed reviews of the evidence for these views are available elsewhere (*Craig*, 2002, 2003a, 2009).

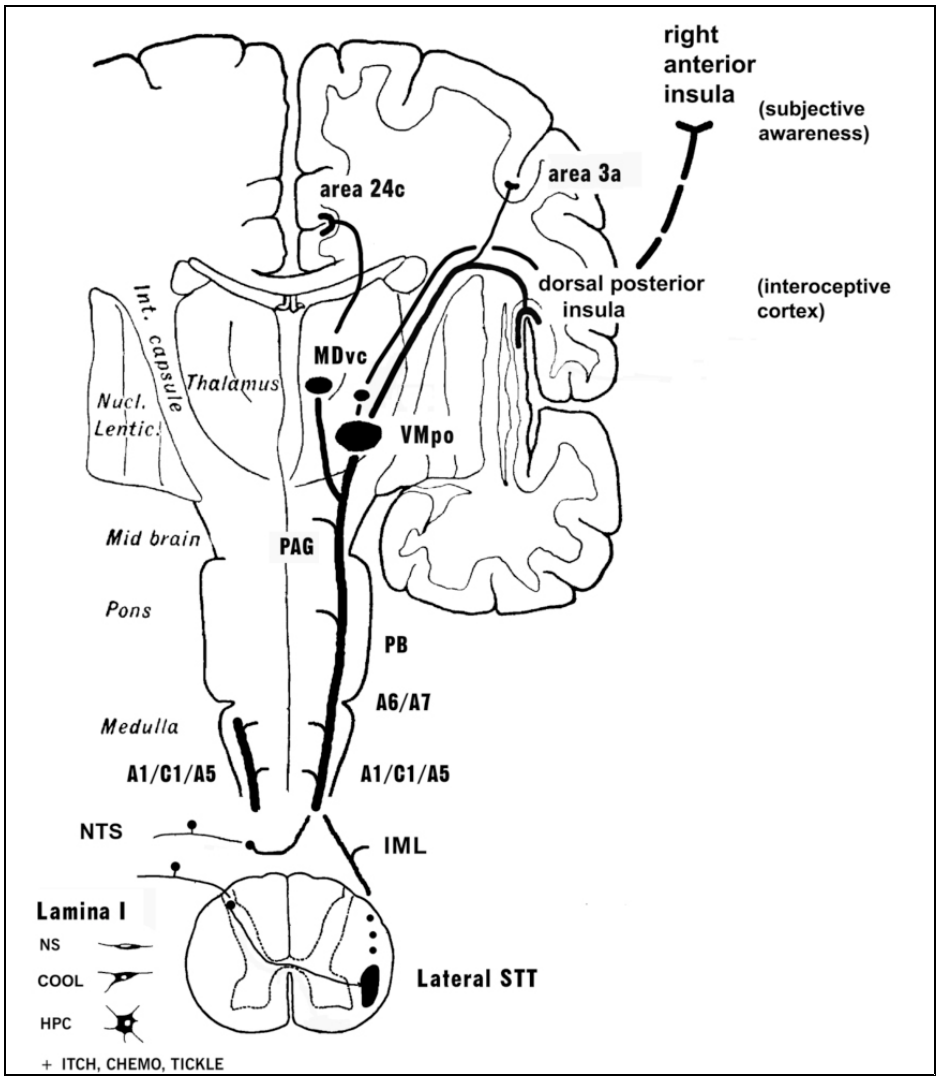

Figure 2: The overall organization of lamina I projections in the primate.

THE ASCENDING PATHWAY AND ITS PRINCIPLES OF ORGANIZATION

Spinal cord

In the periphery, the small-diameter A-delta and C primary afferent fibres that emerge late in development from small dorsal root ganglion cells (small, dark B-cells) innervate every tissue of the body, and they project to the superficial dorsal horn of the spinal cord or to the medullary nucleus of the solitary tract (NTS). The output neurons from these two regions (i.e., lamina I and the NTS) project to the homeostatic integration sites and pre-autonomic motor regions in the brainstem. Lamina I neurons also project heavily to the spinal autonomic nuclei, where sympathetic

pre-ganglionic neurons are found. Altogether, these substrates provide the sensory and motor components of the hierarchically organized homeostatic (autonomic) nervous system. This conclusion is underscored by the observation that the descending projections from the hypothalamus (which many view as a master autonomic control centre) target exactly these sites. Indeed, the superficial dorsal horn that is part of the homeostatic afferent system is easily distinguishable in myelin-stained transverse sections of the human spinal cord from the deep dorsal horn, where large neurons receive input from large-diameter primary afferents (which emerge early in development from large dorsal root ganglion cells, or A-cells) and project to motorneurons in the ventral horn and to motor control sites centrally. Thus, the terms interoception and exteroception can be redefined (*Craig*, 2002) to differentiate these two systems (i.e., one that controls smooth muscle, as distinct from one that receives large-diameter mechanoreceptive and proprioceptive inputs and controls striate muscle). An important principle of the hierarchical homeostatic system is that it has identifiable sensory and motor components at each level that are tightly interconnected centrally.

Brainstem

Lamina I and NTS neurons project densely and selectively to pre-autonomic sites in the brainstem, thus extending the afferent limb to the next rungs of the homeostatic hierarchy (Figure 2) and generating spino-bulbospinal loops for somato-autonomic reflexes (*Sato* and *Schmidt*, 1973; *Craig*, 1995). In all mammals, the highest level of this homeostatic hierarchy in the upper brainstem consists of the parabrachial nuclei (PB) and the periaqueductal gray (PAG). These sites can be viewed as the lowest level of the so-called limbic system that controls emotional behaviour (*Heimer* and *Van Hoesen*, 2006), because together they organize whole-body behaviours that serve life-supporting functions (cardiorespiratory control, ingestion, elimination, reproduction, etc.), as discovered in studies of chronic decerebrate and decorticate animals at the end of the 1800's (e.g., *Sherrington*, 1900).

More recent experiments that revealed approach/avoidance columns in the PAG with correlative, opposing cardiorespiratory actions (*Bandler* et al., 1991) supply the fundamental pattern for a combined behavioural and autonomic opponent organization that many authors have envisaged in the forebrain of mammals and all vertebrates (*Solomon*, 1980; *Craig*, 2005; *Uvnas-Moberg*, 2005; *Macneilage* et al., 2009). The principle of opponent organization is found throughout physiology, e.g., in colour vision, antagonist muscles, hormones controlling water balance, and cardiac function, probably because it provides an energy-efficient method for precise control. Indeed, opponent interaction is present between the lamina I (i.e., "sympathetic") afferent pathway and the NTS (i.e., vagal, or "parasympathetic") afferent pathway already in the medulla and spinal cord (*Chandler* et al., 2002; *Potts*, 2006), and a similar behavioural/autonomic opponent organization is present in the medial and lateral portions of the hypothalamus (*Swanson*, 2000). Thus, the PB and PAG can be viewed as identifiable, complementary sensory and motor regions, respectively, that support homeostasis with a coordinate behavioural/autonomic opponent organization.

Forebrain

In mammals other than primates, the ascending pathway from lamina I

and the NTS generates widely scattered projections, and the main homeostatic afferent pathway to their forebrains conveys integrated activity from PB to hypothalamus, amygdala, and, by way of the thalamus, to striatum and insular and cingulate cortices (*Saper*, 2002). Accordingly, multimodal context-dependent responses have been recorded in these regions in the rat. The insular cortex can be viewed as limbic sensory cortex, because it provides descending control of brainstem homeostatic integration in PB, and the cingulate cortex can be viewed as limbic motor cortex, because it projects densely to the behavioural/autonomic columns of the PAG. Lesions at cingulate cortex and PAG disrupt homeostatic behaviour in rodents (*Johansen* et al., 2001; *Saper*, 2002). The emotional behaviour of non-primate mammals suggests the anthropomorphic inference that they experience feelings from the body in the same way that humans do. However, the neuro-anatomical evidence indicates that they cannot, because the phylogenetically new pathway that underlies feelings from the body in humans is either rudimentary or absent in non-primates (*Craig*, 2002).

In primates, lamina I neurons and NTS neurons extend such pathways by projecting topographically to a pair of relay nuclei in the postero-lateral thalamus, the posterior ventral medial nucleus (VMpo) and the adjoined basal ventral medial nucleus (VMb). The lamina I axons ascend in the lateral spinothalamic tract, precisely where lesions can selectively interrupt the feelings from the body in human patients (*Craig* et al., 2002). The VMpo / VMb is organized antero-posteriorly, orthogonal to the medio-lateral topography of the main somatosensory ventral posterior (VP) nuclei, to which it is connected at the representation of the mouth. The VMb receives direct input from NTS in addition to the integrated input that it receives from PB in all mammals (*Beckstead* et al., 1980). The VMpo is small in macaque monkeys, but in the human thalamus it is almost half as large as the VP (*Blomqvist* et al., 2000).

The VMpo and VMb project topographically to interoceptive cortex in the dorsal margin of the insula (a cortical 'island' buried within the lateral sulcus that has intimate connections with the ACC, amygdala, hypothalamus, and orbitofrontal cortex). The projections of VMpo and VMb extend over the entire posterior-to-anterior extent of the insula in the macaque monkey (*Beckstead* et al., 1980; *Craig* and *Zhang*, 2005; *Ito* and *Craig*, 2008), approximately 6-8 mm. However, in humans, the insula extends approx. 50-60 mm antero-posteriorly, and functional imaging studies indicate that lamina I input (e.g., pain, temperature, or itch stimuli) first activates the most posterior 15-20 mm, while vagal and gustatory input (e.g., gastric distension, salty intensity) activates the next 10 mm or so (*Craig*, 2009, 2010; *Small*, 2010). In other words, primary interoceptive cortex occupies the entire dorsal insula in monkeys, but only the posterior third in humans. This pathway contains modality-selective components that each generate a distinct "feeling" from the body in humans, including first (pricking) pain, second (burning) pain, cool, warm, itch, muscle ache, gastric distension, vasomotor flush, sweet, salty, and so on. PET imaging studies of innocuous cool sensation (*Craig* et al., 2000) showed that activation in the dorsal posterior insula, which is linearly related to objective stimulus intensity, is accompanied by activation of the mid-insula and the anterior insula, where activity correlates much more strongly with subjective feelings of cool. Indeed, activation in the anterior insula is

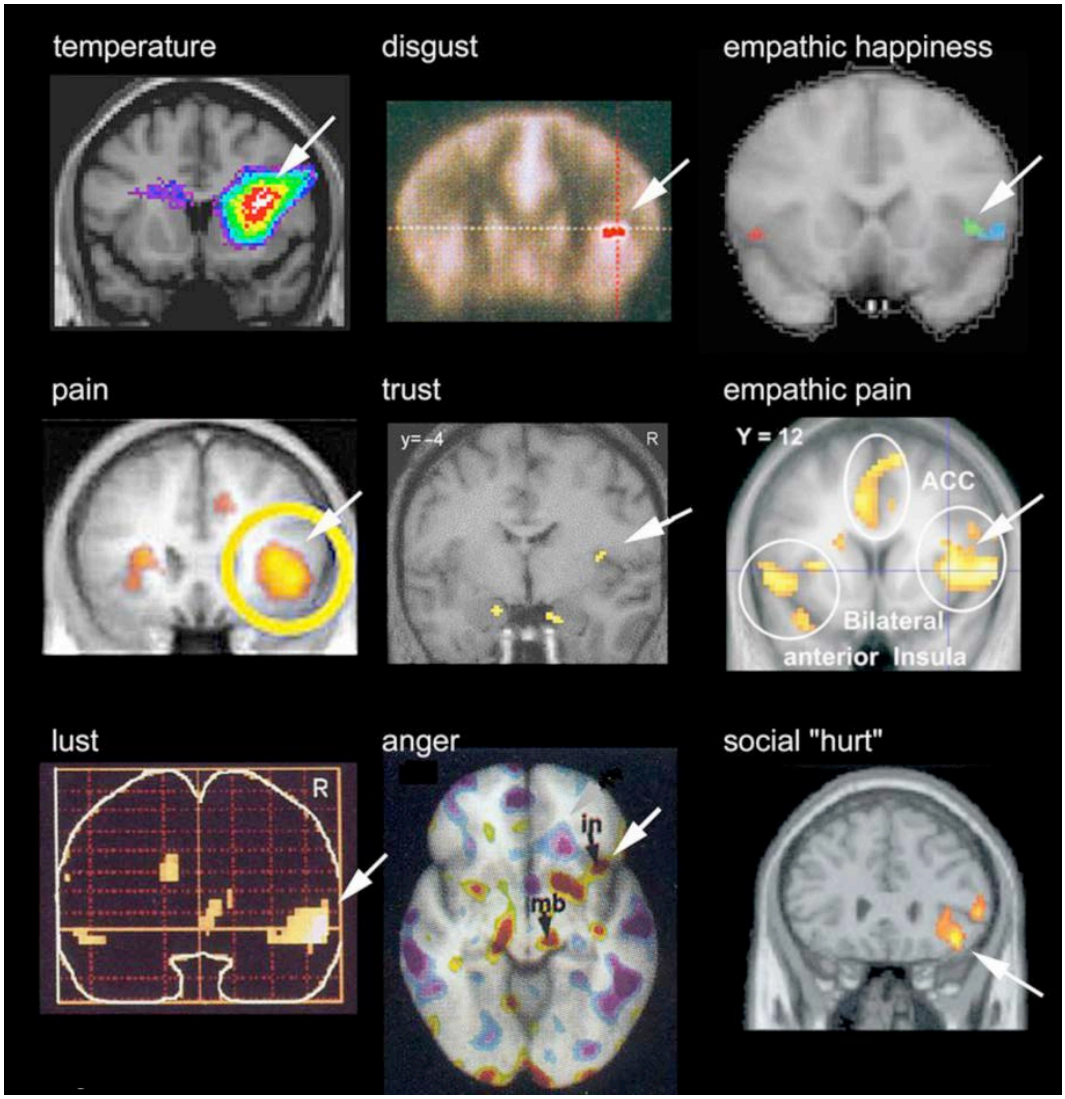

Figure 3: Activation of the right anterior insula by subjective feelings (*Craig*, 2002).

uniquely associated with subjective feelings of all kinds (Figure 3) (*Craig*, 2002, 2009). This pattern suggests a posterior-to-anterior processing gradient in the human insular cortex, which fits with considerable evidence (e.g. *Schweinhardt* et al., 2006) and that subjective feelings are based directly on homeostatic sensory integration, which is consistent with the James-Lange theory of emotion and the "somatic marker" hypothesis (*James*, 1890; *Damasio*, 1993). This pattern also suggests that integration within the insula generates the template for a "feeling", namely a neural representation of homeostatic sensori-motor conditions that can valuate or quantify energy utilization, thus providing a metric for amodal computation of homeostatic efficiency (a "common currency") (*Rainville* et al., 2006; *Duncan* and

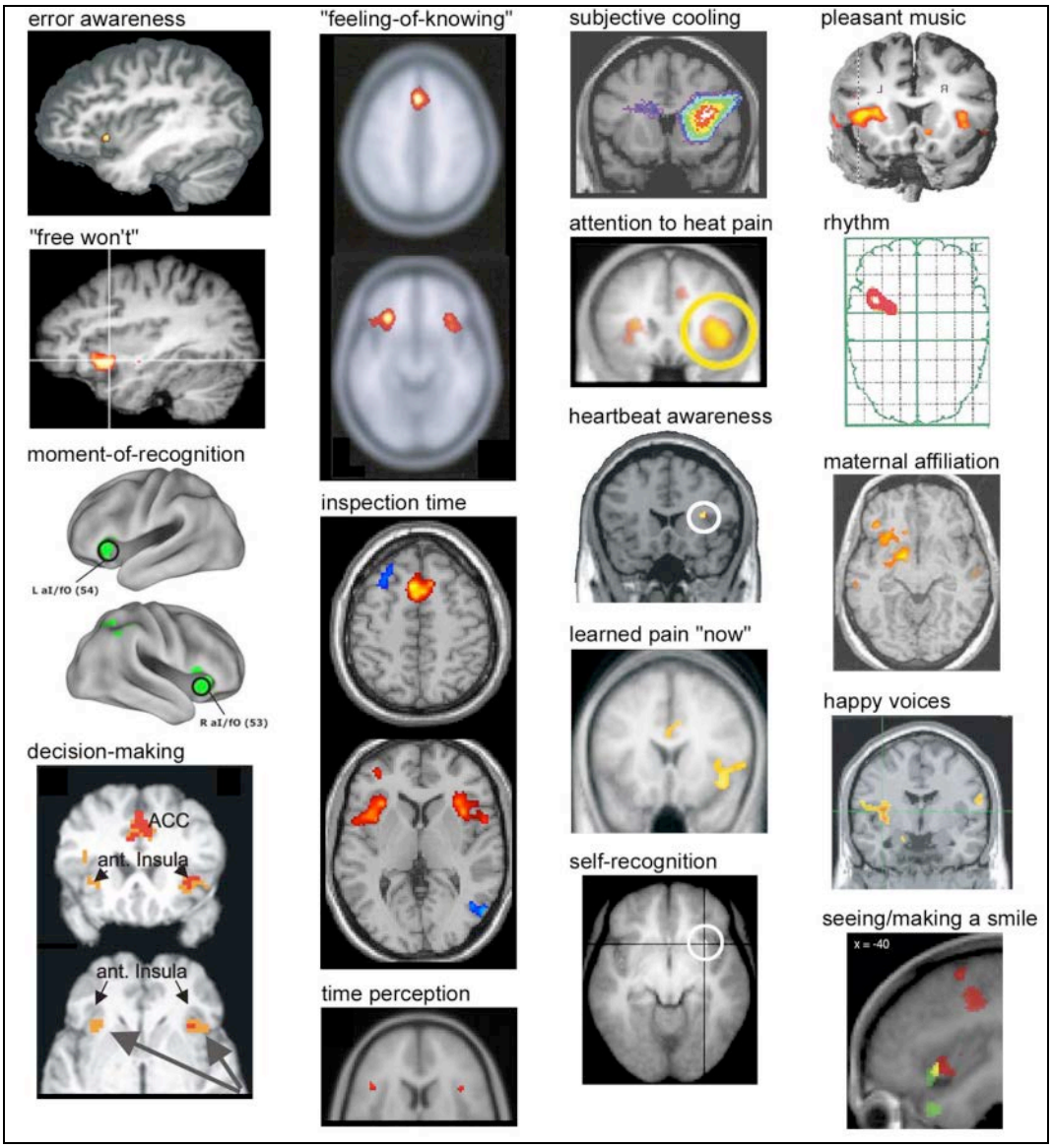

Figure 4: The anterior insula and human awareness (*Craig*, 2009).

Barrett, 2007; *Harrison* et al., 2010). From an evolutionary perspective, it makes sense that an integrated representation of the activity in all brain networks was needed in order to improve behaviour from the perspective of homeostatic efficiency. The goal of homeostatic efficiency (with respect to both the individual and the species) provides a plausible explanation of the progressive posterior-to-anterior integration in the insula.

A corollary VMpo projection to area 3a in sensorimotor cortex may relate cutaneous pain to (exteroceptive) somatic motor activity and to cortical control of spinal viscero-somatic reflex activity. In addition, a direct lamina I

pathway to the ACC is also present in primates by way of a topographic projection to the ventral caudal portion of the medial dorsal nucleus (MDvc). In non-primates, in contrast, the ACC (i.e. limbic motor cortex) receives integrated homeostatic information from the PB by way of the medial thalamus, and lamina I activity is relayed instead to ventrolateral orbitofrontal cortex through the submedial nucleus. Physiological and behavioural studies validate the primordial role of the ACC in homeostatic behaviour in rats (*Johansen* et al., 2001) and in the affective/motivational component of human pain by way of the direct lamina I path to MDvc.

The direct activation of both the interoceptive cortex and the ACC by the distinct homeostatic modalities corresponds with the simultaneous generation of both a sensation and a motivation. In humans, an emotion can be defined as a feeling and a motivation; this directly supports the view of these feelings from the body as homeostatic emotions that reflect the survival needs of the body. Pain, temperature, and itch are homeostatic emotions that drive behaviour, just as hunger and thirst do (Craig 2002, 2003a, 2009). Convergent functional imaging data indicates that the anterior insula is associated with subjective feelings from the body and with virtually all human emotions, and so it seems to provide an image of the physical self as a feeling (sentient) entity (Figure 4). The association of the anterior insula with the subjective perception of pain, the anticipation of pain, the subjective reduction of pain (placebo analgesia), and the subjective generation of pain (hypnotic psychogenic pain) underscores the importance of the meta-representation of interoceptive state in the anterior insula for clinical understanding of the effects of emotion and belief on health. Furthermore, the recognition that sensual touch is included in the interoceptive system emphasizes the need to incorporate the neurobiological basis of conspecific human contact in therapies for emotional and physical health (*Björnsdotter* et al., 2009).

PAIN AS AN ASPECT OF HOMEOSTASIS

Pain is a homeostatic feeling. It has characteristics exactly comparable to other physical sensations (feelings) from the body. Pain normally originates with a change in the condition of the tissues of the body, a physiological imbalance that automatic (subconscious) homeostatic systems alone cannot rectify. It comprises a sensation, an affective behavioural drive and autonomic adjustments. Pain generates characteristic reflexive motor patterns, as do itch, hunger and rectal distension. The behavioural motivation of pain is normally correlated with the intensity of the sensory input, but this can vary under different behavioural, autonomic and emotional conditions, so that pain can become intolerable or it can disappear, similar to any other homeostatic emotions (e.g., hunger). However, unremitting pain that outlasts its homeostatic role is pathological.

Viewing pain as a homeostatic emotion provides a ready explanation of the interactions of pain with other homeostatic conditions (including temperature, blood glucose, blood pressure, level of arousal), because homeostasis is an integrated, dynamic process, and it explains the modulation of pain by homeostatic mechanisms. This conceptual perspective also provides a firm basis for explaining the interac-

tions of pain with emotional status or attention (i.e., the psychological dimension of pain), and it unifies the different conditions that can cause different types of pain from different tissues under a common homeostatic function - the maintenance of the integrity of the body. *Darwin* (1872) recognized that all animals respond with emotional behaviour to stimuli that in humans cause a feeling of pain. The new data reveal that noxious stimuli are represented in an evolutionarily ancient neural pathway that has the primary purpose of driving homeostatic mechanisms at spinal and brainstem levels and generating integrated behavioural motivation at the forebrain level. In primates, novel thalamo-cortical projections have emerged from this basic homeostatic system that provide encephalized cortical mechanisms for highly resolved sensations and motivations. Notably, sub-primates have the sub-cortical mechanisms that drive integrated homeostatic (emotional) behaviour, but they do not have these direct telencephalic pathways, and so they do not have the neuroanatomical capacity to feel pain in the same way that humans do.

The concept that human pain sensation is underpinned by a distinct and separate pathway for nociceptive-specific lamina I neurons to the primary interoceptive cortex in the dorsal posterior insula contradicts the conventional view that spinal lamina V ("wide dynamic range") neurons are "necessary and sufficient" for pain (*Price* et al., 2003). Evidence to the contrary has accumulated for years (*Craig*, 2003b), and recently the conventional view was definitively refuted by evidence that almost all such lamina V neurons convey Group II muscle afferent activity, respond tonically to limb position, and project directly onto ventral horn motorneurons and other motor-related sites. That is, such lamina V neurons are an integral component of the skeletal motor system (*Craig*, 2008). Recent functional imaging results on so-called "windup" (temporal summation of "second pain"; *Staud* et al., 2008) in fact corroborates strongly the fundamental role of lamina I spino-thalamo-cortical projections in pain. Nevertheless, in light of the fundamental role of the lamina I pathway in homeostasis, it would be more appropriate to call it a homeostatic afferent pathway than simply a "pain pathway" (*Craig*, 2006).

CONCLUSION

It is important to recognize that, contrary to the conventional textbook view, feelings from the body, such as pain or temperature, are neurologically distinct from tactile mechanoreception and proprioception at all levels. Such feelings from the body represent homeostatic afferent activity, that is, the sensory input representing the physiological condition of the body, which drives the central network that controls the ANS. The spinal and brainstem levels of the hierarchical homeostatic network are present in all mammals, but in humans there is a high-resolution representation of the condition of the body in primary interoceptive cortex in the posterior insula, which generates an energy-efficient map of all brain activity in the anterior insula that underpins the subjective "material me." Thus, there is a fundamental relationship between the physiological condition of the body and subjective feelings of all kinds.

LITERATURE

Bandler, R., Carrive, P., and Zhang, S.P.: Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: Viscerotopic, somatotopic and functional organization. In: Progress in brain research, vol. 87 (Ed.: Holstege, G.). Elsevier, New York, 269-305 (1991).

Beckstead, R.M., Morse, J.R., and Norgren, R.: The nucleus of the solitary tract in the monkey: Projections to the thalamus and brain stem nuclei. J. Comp. Neurol. 190, 259-282 (1980).

Björnsdotter, M., Löken, L., Olausson, H., Vallbo, A., and Wessberg, J.: Somatotopic organization of gentle touch processing in the posterior insular cortex. J. Neurosci. 29, 9314-9320 (2009).

Blomqvist, A., Zhang, E.T., and Craig, A.D.: Cytoarchitectonic and immunohistochemical characterization of a specific pain and temperature relay, the posterior portion of the ventral medial nucleus, in the human thalamus. Brain 123, 601-619 (2000).

Cannon, W.B.: The wisdom of the body. Norton & Co., New York (1939).

Chandler, M.J., Zhang, J.H., Qin, C., and Foreman, R.D.: Spinal inhibitory effects of cardiopulmonary afferent inputs in monkeys: Neuronal processing in high cervical segments. J. Neurophysiol. 87, 1290-1302 (2002).

Craig A.D.: Distribution of brainstem projections from spinal lamina I neurons in the cat and the monkey. J. Comp. Neurol. 361, 225-248 (1995).

Craig, A.D., Chen, K., Bandy, D., and Reiman, E.M.: Thermosensory activation of insular cortex. Nat. Neurosci. 3, 184-190 (2000).

Craig, A.D.: How do you feel? Interoception: The sense of the physiological condition of the body. Nat. Rev. Neurosci. 3, 655-666 (2002).

Craig, A.D., Zhang, E.T., Blomqvist, A.: Association of spinothalamic lamina I neurons and their ascending axons with calbindin-immunoreactivity in monkey and human. Pain 97, 105-115 (2002).

Craig, A.D.: Interoception: The sense of the physiological condition of the body. Curr. Opin. Neurobiol. 13, 500-505 (2003a).

Craig, A.D.: Pain mechanisms: Labeled lines versus convergence in central processing. Annu. Rev. Neurosci. 26, 1-30 (2003b).

Craig, A.D.: Forebrain emotional asymmetry: A neuroanatomical basis? Trends Cogn. Sci. 9, 566-571 (2005).

Craig, A.D. and Zhang, E.T.: Cortical projections of VMpo, a specific pain and temperature relay in primate thalamus. Society for Neuroscience 21, 1165. 2005.

Craig, A.D.: Evolution of pain pathways. In: Evolution of nervous systems, Volume 3 (Ed.: Kaas, J.H.). Academic Press Oxford, 227-236 (2006).

Craig, A.D.: Retrograde analyses of spinothalamic projections in the macaque monkey: Input to the ventral lateral nucleus. J. Comp. Neurol. 508, 315-28 (2008).

Craig, A.D.: How do you feel - now? The anterior insula and human awareness. Nat. Rev. Neurosci. 10, 59-70 (2009).

Craig, A.D.: The sentient self. Brain Struct. Funct. 214, 563-577 (2010)

Damasio, A.R.: Descartes' error: Emotion, reason, and the human brain. Putnam, New York (1993).

Duncan, S. and Barrett, L.F.: Affect is a form of cognition: A neurobiological analysis. Cogn. Emot. 21, 1184-1211 (2007).

Harrison, N.A., Gray, M.A., Gianaros, P.J., and Critchley, H.D.: The embodiment of emotional feelings in the brain. J. Neurosci. 30, 12878-12884 (2010).

Heimer, L. and Van Hoesen, G.W.: The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. Neurosci. Biobehav. Rev. 30, 126-147 (2006).

Ito, S.I. and Craig, A.D.: Thalamocortical projections of the vagus-responsive region of the basal part of the ventral medial nucleus in monkeys. Soc. Neurosci., Abstr. Online 364.10 (2008).

James, W.: The Principles of Psychology

(1890). eBook, The University of Adelaide: http://ebooks.adelaide.edu.au/j/james/william/principles/index.html

Johansen, J.P., Fields, H.L., and Manning, B.H.: The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. Proc. Natl. Acad. Sci. USA 98, 8077-8082 (2001).

Langley, J.N.: The autonomic nervous system. Brain 26, 1-26 (1903).

Macneilage, P.F., Rogers, L.J., and Vallortigara, G.: Origins of the left and right brain. Sci. Am. 301, 60-67 (2009).

Penfield, W.G. and Rasmussen, T.: The cerebral cortex of man: A clinical study of localization of function. Macmillan, New York (1950).

Potts, J.T.: Inhibitory neurotransmission in the nucleus tractus solitarii: Implications for baroreflex resetting during exercise. Exp. Physiol. 91, 59-72 (2006).

Prechtl, J.C. and Powley, T.L.: B-afferents: A fundamental division of the nervous system mediating homeostasis? Behav. Brain Sci. 13, 289-331 (1990).

Price, D.D., Greenspan, J.D., and Dubner, R.: Neurons involved in the exteroceptive function of pain. Pain 106, 215-219 (2003).

Rainville, P., Bechara, A., Naqvi, N., and Damasio, A.R.: Basic emotions are associated with distinct patterns of cardiorespiratory activity. Int. J. Psychophysiol. 61 , 5-18 (2006).

Saper, C.B.: The central autonomic nervous system: Conscious visceral perception and autonomic pattern generation. Annu. Rev. Neurosci. 25, 433-469 (2002).

Sato, A. and Schmidt, R.F.: Somatosympathetic reflexes: Afferent fibers, central pathways, discharge characteristics. Physiol. Rev. 53, 916-947 (1973).

Sherrington, C.S.: Cutaneous sensations. In: Text-book of physiology (Ed.: Schäfer, E.A.). Pentland, Edinburgh, 920-1001 (1900).

Small, D.M.: Taste representation in the human insula. Brain Struct. Funct. 214, 551-561 (2010).

Solomon, R.L.: The opponent-process theory of acquired motivation: The costs of pleasure and the benefits of pain. Am. Psychol. 35, 691-712 (1980).

Staud, R., Craggs, J.G., Perlstein, W.M., Robinson, M.E., and Price, D.D.: Brain activity associated with slow temporal summation of C-fiber evoked pain in fibromyalgia patients and healthy controls. Eur. J. Pain 12, 1078-1089 (2008).

Swanson, L.W.: Cerebral hemisphere regulation of motivated behavior. Brain Res. 886, 113-164 (2000).

Schweinhardt, P., Glynn, C., Brooks, J., McQuay, H., Jack, T., Chessell, I., Bountra, C., and Tracey, L.: An fMRI study of cerebral processing of brush-evoked allodynia in neuropathic pain patients. Neuroimage 32, 256-265 (2006).

Uvnas-Moberg, K., Arn, I., and Magnusson, D.: The psychobiology of emotion: The role of the oxytocinergic system. Int. J. Behav. Med. 12, 59-65 (2005).

MICROBIAL ENTERIC NEUROPHYSIOLOGY

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SUMMARY

The myenteric plexus contains a type of neuron that innervates the mucosa, and projects to all other classes of myenteric neurons. This neuron has a specific multipolar morphotype and possesses a characteristic action potential whose firing frequency is moderated a potassium current dependent post-spike slow after-hyperpolarisation. The neuron, termed AH cell, is functionally an intrinsic intestinal primary afferent neuron (IPAN) with chemosensory and mechano-sensory responses to adequate stimuli. With about 80% of sensory neuropeptide containing nerve fibres in the mucosa belonging to IPANs they are ideally positioned to respond to signals from commensal or probiotic gut bacteria. Application of *Lactobacillus rhamnosus* JB-1 or *Bacteroides fragilis* to the mucosal epithelium evokes sensory action potentials in the IPANs within a few seconds of administering the bacteria. In the case of *B. fragilis*, a capsular polysaccharide A is necessary and sufficient to carry the signal from microbe to neuron. This sensory activation leads to a longer-term increase in the intrinsic excitability of the IPAN sensory neuron network caused at least in part by a decrease in a calcium dependent potassium conductance. We have identified a molecular and neuronal target of commensal signalling. The neuron's rapid sensory responses may activate the neuronal circuits that mediate some of the motility and central nervous system effects of ingesting beneficial microorganisms.

INTRODUCTION

It may be time to consider the idea of probiotic or microbial neurophysiology. This notion derives from observations that microbes, especially symbiotic, commensal or probiotic bacteria, can change how the host nervous system functions. The concept has parallels to other constructs such as microbial endocrinology (*Lyte*, 2010, 2011; *Roshchina*, 2010) or microbial immunology (*Artis*, 2008; *O'Hara* and *Shanahan*, 2006) that seek to elucidate how the host systems interact with the microflora. It is tacitly recognised that within the whole animal, or even for individual organs, there must be reciprocal interplay between these systems. A simple example of this is if behavioural stress (nervous system) and increased stress hormones (endocrine system) co-exist to alter the constituents of the microbiome (see *Lyte*, 2011). Within the enteric nervous system, neuronal behaviour may be changed by luminal microbes, mast cell products or inflammation or paracrine

hormones (*Nurgali* et al., 2011; *Furness* and *Poole*, 2011; *Buhner* and *Schemann*, 2012; *Sundler* et al., 1989; *Ahlman* and *Nilsson*, 2001).

The gut microbiome forms a complex ecosystem with its associated "metabolome" producing myriads of metabolites including chemical messengers and foodstuffs. The totality of this microbial organ has profound effects on the host during development and adult life. Such effects include education of the immune system and the normal development of the gut-brain axis. Much of this knowledge has been deduced from work with germ-free animals or alterations of the microbiome using antibiotics (*Collins* and *Bercik*, 2009; *Bienenstock* and *Collins*, 2010; *Heijtz* et al., 2011). Such major experimental perturbations cannot unaided explain the remarkable observation that ingesting about 1 billion cells of a single probiotic strain (that may or may not be a commensal) can have a therapeutic effect (*Moayyedi* et al., 2010) on an adult who at the same time carries 100 trillion or more gut commensal organisms.

The FDA defines beneficial bacteria that are intended to prevent or help treat disease as drugs (*Hoffman*, 2008). Homeostatic organ responses to repeated dosing with probiotics may mask the underlying physiological mechanisms responsible for the direct neuronal action of active probiotic derived ligand(s) (*Dykstra* et al., 2011). In addition, pharmacological tolerance (*Koch* and *Höllt*, 2008), sensitisation and priming (*Kostrzewa*, 1995) are potential additional confounding factors. To clarify the mechanisms of action of beneficial bacteria, quantitative measurements of their acute actions on individual neurons and other target cells should be made. In the same way, the mechanisms of action of opium, antidepressant or anxiolytic plants, or other biologics could not have been deduced solely from their long-term chronic actions.

DO COMMENSALS, PROBIOTICS ACT ON THE NERVOUS SYSTEM?

Even in the absence of overt inflammation, probiotics may influence behaviour or alter brain chemistry. Some of the symptoms of chronic fatigue syndrome have been reported to be decreased by ingestion of a Shirota strain of *Lactobacillus casei* (*Rao* et al., 2009). Ingestion of a *Lactobacillus rhamnosus* strain (JB-1) had antidepressive and anti-anxiety effects (*Bravo* et al., 2011). On the other hand, *Citrobacter rodentium*, when given at a dose that produces no inflammation or increase in plasma inflammatory cytokines, induced anxiety in mice (*Lyte* et al., 2006). A formulation of *Lactobacillus helveticus* plus *Bifidobacterium longum* reduced anxiety in a rat model and decreased anxiety scores in humans (*Messaoudi* et al., 2011).

Probiotics can modulate physiological parameters controlled by the autonomic nervous system. Intra-duodenal injection of *Lactobacillus johnsonii* (La1) has been shown to reduce systemic and renal blood pressure in rats (*Yamano* et al., 2006; *Tanida* et al., 2005) within 15 min of injection. Feeding milk fermented with *Lactobacillus helveticus* appeared to decrease ambulatory blood pressure in hypertensive patients (*Jauhiainen* et al., 2005; *Aihara* et al., 2005). There is also evidence that certain probiotic strains moderate gut migrating motor complexes (MMCs) (see below).

It is possible that the behavioural and autonomic effects ascribed to pro-

biotic ingestion could be caused by alterations in the immune status of peripheral tissue or the release of circulating hormones (*Bansal* et al., 2010; *Bercik* et al., 2012). Another explanation, not mutually exclusive, is that primary afferent neurites are activated within the gut wall and these then transmit synaptically to the higher order autonomic neurons whose processing modulate peripheral autonomic reflexes or limbic system function centrally. To resolve this question, it will be important to determine if neurons in the intestine are in fact direct or early targets of probiotic application. Early perturbations of primary afferent neuron firing by commensals could also underlie later longer-term effects on immune, endocrine systems (*Logan* and *Katzman*, 2005; *Lyte*, 2011).

SPINAL PRIMARY AFFERENTS

Spinal primary afferent neurons have their soma in the dorsal root ganglia that lie on either side of the spinal cord. Gut spinal primary afferents have sensory terminals that ramify throughout the width of the gastrointestinal wall where they are activated by mechanical or chemical stimuli, generally of a nociceptive nature (*Blackshaw* et al., 2007).

Some probiotic strains attenuate afferent pain signals. Feeding a non-absorbable antibiotic to decrease colon *Lactobacillus* species has been reported to increase thresholds of pseudo-affective pain responses to colorectal distension (CRD) (*Verdu* et al., 2006), suggesting a possible role of these species in visceral pain transmission or transduction. In a more strain-specific study, *Kamiya* et al. (2006) showed that 9 day feeding of JB-1 to rats decreased pain responses to CRD, and that this was paralleled by a reduction of CRD evoked increases in spinal dorsal root single unit firing. It is important to realise that the anti-nociceptive action of JB-1 occurred in the absence of experimentally induced or a detectable peripheral inflammation. Therefore, even if the bacterium exerted anti-inflammatory actions on the host, this may not explain its pain-suppressing ability. Similarly, feeding *L. acidophilus* (NCFM) reduced pseudo-affective responses to CRD in rats, and this was accompanied by an increase in opioid and cannabinoid receptor expression in mucosal epithelial cells (*Rousseaux* et al., 2007). It is not clear how such receptors in epithelial cells could alter firing in nociceptive neurons.

Probiotic bacteria may be able to block activity-dependent sensitisation of pain pathways. Afferent pain pathways can be sensitised by repeated painful stimuli (*Woolf* and *Salter*, 2000). Such sensitisation represents a form of cellular memory of pain, and is implicated in maintaining pathological pain states (*Woolf* and *Salter*, 2000). CRD induces greater than normal substance P expression in rat DRG neurons that persists at least 24 h after the distensions (*Lu* et al., 2005). Functionally, the substance P overexpression was accompanied by hyper-excitability in the pseudo-affective response to CRD (*Ma* et al., 2009). It is noteworthy that this type of sensitisation occurred in the absence of histological or chemical markers for inflammation. Prior feeding of JB-1 was able to block this nociceptive sensitisation (*Ma* et al., 2009).

INTESTINAL PRIMARY AFFERENT NEURONS

The putative intestinal primary afferent neuron (IPAN) is a plausible target for beneficial microbes. IPANs are part of the enteric nervous system, which, long regarded as a part of the parasympathetic division of the autonomic nervous system, is now considered to be a third independent division (*Gershon*, 1999). It has been acknowledged that the gut can perform its motor and secretory roles in the absence of connections with nervous systems extrinsic to the gut. This has led to the proposition (*Furness* et al., 1998) that there are neurons (IPANs), with somata within the wall of the intestine, whose activation by chemo- or mechano-sensory stimuli lead to propulsive reflexes. Patterned on-going activity in networks of IPANs contributes to motor or secreto-motor programs that produce propulsive MMCs, and possibly stationary motor complexes that result in mixing (*Gwynne* and *Bornstein*, 2007; *Bornstein* et al., 2002, 2004). Yet, whether IPANs truly exist, or what their identity might be, and if the apparent autonomy of the gut results from axon reflexes of extrinsic primary afferent fibres embedded in the gut wall is still under discussion.

IPANs were first anticipated because of the morphology and physiology of a specific type of myenteric neuron. Such neurons have a large flattened oval soma and multiple long neurites that innervate adjacent myenteric ganglia and the epithelial cell layer of the mucosa (Dogiel Type II morphology). This morphotype led *Dogiel* (1899) to propose that the neurons were sensory in function. David Hirst (*Hirst* et al., 1972; *Hirst* and *Spence*, 1973; *Hirst* et al., 1974) found that the action potentials of Dogiel type II neurons were broad with a calcium hump on their repolarising phase. Significantly, because of its effects on firing patterns, the action potential was followed by a slow inhibitory afterhyperpolarisation (sAHP) lasting several seconds (*Hirst* et al., 1985, 1974). Such action potential characteristics are very similar to those of unmyelinated spinal DRG neurons (*Hay* and *Kunze*, 1994; *Schild* et al., 1994). These putative IPANs, termed "AH cells" by Hirst, did not appear to receive the classical fast (inotropic) synaptic input that characterises ganglionic inter- and motor- or relay-neurons (*Hirst* et al., 1974). The absence of synaptic input from other neurons suggested that the neurons are by default sensory. Hirst argued that if AH cells were not activated by other neurons they must be the first or primary afferent neuron whose activation arises from sensory stimuli (*Hirst* et al., 1974). Interneurons or motorneurons would necessarily receive synaptic input from sensory or other interneurons. Later, *Kirchgessner* et al. (1992) blocked nicotinic transmission in the submucosal plexus and then mechanically stimulated the mucosa. They reported enhanced c-fos expression in some neurons, even under nicotinic receptor blockade. Lack of nicotinic synaptic input has been taken as proof that some submucosal neurons are primary afferent by *Furness* (2006a). Against this, there remained the possibility of non-nicotinic synaptic transmission in the submucosal plexus.

SENSORY RESPONSES TO CHEMICAL LUMINAL AND MECHANICAL STIMULI

The “classic schema” has been that luminal chemicals and bacterial products are detected by the immune system which then signals via the release of mediators to the enteric nervous system (*Cooke*, 1994). Experimental data, however, suggest that myenteric AH cells can respond directly to luminal chemicals with varying degrees of involvement enteroendocrine cells (EECs) playing the role of "taste cells" (*Bertrand*, 2009). Orthodromic action potentials can be recorded from guinea pig myenteric AH cells in response to applying brief puffs of HCl to the mucosal epithelium (*Kunze* et al., 1995). "Orthodromic" means travelling in the "right" or normal direction; for a peripheral sensory neuron action potential this is from the receptive field towards the soma and, in the case an AH cell, from the mucosal epithelium to the myenteric plexus. AH cells were shown to be IPANs when the response to HCl persisted even if all synaptic transmission was blocked by removing extracellular calcium and raising extracellular magnesium tenfold (*Kunze* et al., 1995). Since the response could not have come from other neurons, it either must have been generated by sensory transduction occurring in the neuronal endings near the lumen or in closely associated mucosal entero-endocrine "taste" cells (*Bertrand*, 2003, 2009). Sensory responses have also been recorded in myenteric AH cells in response to epithelial applications of short chain fatty acids for mouse small intestine (*Mao* et al., 2006) and rat colon (*Kunze* et al., 2009).

AH cells are additionally mechano-sensitive neurons responding to tension. Generator and action potentials have been recorded from myenteric AH cells when their processes within the ganglia are distorted. In contrast, compression of the soma inhibits firing (*Kunze* et al., 2000). Such responses were recorded during total synaptic blockade establishing their primary afferent nature. Active muscle contraction was required for these responses to occur, and passive stretch with paralysed muscle could not evoke them (*Kunze* et al., 1998). Thus, tensions sensitive mechano-sensitive IPANs are also Dogiel type II AH cells (*Kunze* et al., 1999, 2000). Sub-modalities and multimodal properties have been ascribed to IPANs (*Mayer*, 2011); so far, experimental evidence for this is lacking.

Mechano-sensory responses have also been recorded from significant proportions of myenteric S cells (so named because they receive prominent fast synaptic input) (*Schemann* and *Mazzuoli*, 2010; *Mazzuoli* and *Schemann*, 2009; *Spencer* and *Smith*, 2004), which have been previously been assumed to be inter-or motor-neurons within the enteric circuits (*Nurgali* et al., 2004; *Furness* et al., 1998, 2004; *Kunze* et al., 1999). These responses occurred even when the muscle was paralysed with nicardipine, suggesting that these S cells are stretch rather than tension receptors. Since MMCs require active muscle contraction for their initiation (*Lüderitz*, 1891) it is not easy to know what might be the functional role of these mechano-sensory S cells.

The promiscuous proliferation in the literature of putative intrinsic gut primary afferent neurons has not gone without criticism (see: *Wood*, 2008). It is argued that intramural vagal or spinal axon reflexes are sufficient to account for the functional independence of the gut (*Christofi* and *Wood*, 1993; *Wood*, 2008). Peristaltic reflexes that persist in

ex vivo gut segments are proposed to be mediated by the severed stumps of extrinsic sensory fibres that remain in the organ wall. Also, the idea that the enteric nervous system contains its own primary afferent neurons has been rejected on principles of evolutionary parsimony (*Christofi* and *Wood*, 1993; *Wood*, 2008). It is suggested that extrinsic vagal and spinal primary afferents would suffice for all the sensory innervation of the gut. However, the idea that in biology evolution designs the simplest system for a particular function (ontological parsimony) has been refuted many times (for a discussion of the general issue and a distinction between ontological and methodological parsimony see for example: *Crisci*, 1982). Occam's razor (in the form of ontological parsimony) cannot reasonably be used as an argument against the existence of IPANs. Finally, *Wood* (2008) has argued that since chemosensitive AH cells appear to require sensory transduction from specialised entero-endocrine cells, they cannot be primary afferent neurons. If this argument is granted, then the lingual nerve, for example, which appears to require type 4 taste cells for some of its responses would not be primary afferent.

RESPONSES TO LUMINAL PROBIOTIC OR COMMENSAL BACTERIA

A key function of chemosensory IPANs may be to monitor and respond to the microbiome and its metabolome. There are 500 million enteric neurons in humans and 500,000 in mice. Dogiel Type II/AH cells make up 15 to 20% of the total neurons (*Kunze* et al., 1999; *Furness* et al., 1998). An important teleological question arises; why would the gut have so many chemosensory neurons all of which innervate the mucosa (*Song* et al., 1994)? The total numbers of cells contributing to the microbiome is not known with certainty, but the concentrations of dominant commensal genera can reach 8 log cfu/ml in the small, and 11 log cfu/ml in the large intestine (*Tappenden* and *Deutsch*, 2007; *Reuter*, 2001). By far the richest innervation (compared to spinal or vagal afferents) for mucosal epithelial layer cells derives from the myenteric plexus, which provides in excess of 90% of sensory neuropeptide containing fibres to the mucosal layer (*Ekblad* et al., 1987; *Keast* et al., 1984). Each enteric AH neuron innervates 80-120 villi (*Kunze* et al., 1999) and there are about 500,000 neuropeptide (calcitonin gene related peptide, CGRP) containing AH cells in the mouse (*Furness*, 2006b). Therefore, IPANs are ideally positioned to sample signals from the microbiome and ingested beneficial bacteria; possibly having evolved just so, to monitor this large source of metabolites and foreign DNA.

There is experimental evidence that probiotics influence myenteric IPANs. Using a modified Trendelenburg *ex vivo* gut segment preparation to record intraluminal pressure, MMCs were reduced 50% in amplitude 9-16 min after 7.7 log cfu/ml (*Wang* et al, 2010a) were introduced into the lumen. Nine day ingestion 9 log cfu JB-1 similarly reduced MMC amplitudes (*Wang* et al., 2010b). Since the MMC were blocked by the neuron sodium channel blocker tetrodotoxin (TTX) (*Wang* et al., 2010a, 2010b), these results suggest that the probiotic acted on enteric neurons. Slow wave related contractions persisted when all neural activity was blocked with tetrodotoxin (*Wang* et al.,

2010a, 2010b) were not altered by JB-1. Kamm and colleagues fed 7.3 log cfu/g of *Saccharomyces boulardii* per day to pigs for 9 days, after which jejunal myenteric neurons were assayed histochemically for chemical markers known to correlate with major functional subpopulations within the enteric nervous system (ENS) (*Kamm* et al., 2004). The authors reported decreased expression of the vitamin D dependent calcium binding protein, calbindin, in myenteric Dogiel Type II neurons (*Kamm* et al., 2004; *Jungbauer* et al., 2006). They also assayed for choline acetyltransferase, substance P, calcitonin gene-related peptide, vasoactive intestinal polypeptide, nitric oxide synthase and the calcium binding protein calretinin. These chemicals identify AH cell/Dogiel Type II neurons or each of the major classes of inhibitory and excitatory motor neurons or interneurons (S cells). For none of these chemicals (except for calbindin) was there a change in the amount expressed in the ENS, demonstrating what appears to be a high functional selectivity in perturbation of the ENS by the fungus. (*Kamm* et al., 2004; *Jungbauer* et al., 2006). Nine day feeding of 9 log cfu per day of JB-1 increased the intrinsic excitability (decreased firing thresholds and increased number of action potentials fired during a standard stimulus current pulse) of rat colon myenteric IPANs. S cell (inter- and motor-neuron) excitability was unaffected (*Kunze* et al., 2009). Since myenteric IPAN\Dogiel Type II cells, but not S cells, innervate the mucosa (*Furness* et al., 1998; *Bertrand*, 2004; *Song* et al., 1994) it is likely that for each ingestion, IPANs are the first type of neuron to be activated (*Furness* et al., 1999; *Kunze* et al., 1995). Then, the increase in IPAN firing is transmitted to S cells by metabotropic synaptic transmission (*Kunze* et al., 1993). Such daily activation of IPANs would cause them to evoke transient slow postsynaptic potentials and firing in their target S cells (*Kunze* et al., 1993). IPANs (*Clerc* et al., 1999) but not S cells (*Alex* et al., 2002) have a form of activity dependent long-lasting memory that is entrained by their repeated, frequency dependent, excitation. This may be why only IPANs were found to have heightened intrinsic excitability after 9 day feeding with JB-1. That is, inter- or motor-neurons may have been activated during and after presentation of the bacteria to the mucosal epithelial surface but this effect would have waned when the intestine was excised for subsequently electrophysiological analysis. IPANs have the ability to induce long-term potentiation of their excitability many hours beyond the duration of their sensory stimulation (*Clerc* et al., 1999). How long such potentiation could ultimately last, and what are the optimal intervals between probiotic ingestion periods, are questions that require further research and may well be specific for individual probiotic strains and host species and gut regionalisation.

IPAN ION CHANNEL TARGETS FOR PROBIOTIC ACTION

IPANs excitability and discharge properties are determined by a complex interaction of membrane ion channel currents. In general, and with the exception of the pig, myenteric AH cells have electrophysiological properties that are well conserved from mouse to man (*Mao* et al., 2006). The action potential upstroke, which is generated by transient and persisting Na^+ (*Zholos* et

al., 2002; *Rugiero* et al., 2002, 2003), and N (and R) -type Ca^{2+} currents (*Rugiero* et al., 2002; *Bian* et al., 2004), is followed by a fast (fAHP) and then a slow (sAHP) after-hyperpolarisation. The fAHP is generated by a mixture of voltage sensitive K^+ currents including the delayed rectifier, an A current (*Starodub* and *Wood*, 2000) and a Ca^{2+} dependent large conductance K^+ conductance (*Kunze* et al., 2000; *Vogalis* et al., 2002). The sAHP is produced by the generation an intermediate conductance Ca^{2+} dependent K^+ (IK_{Ca}) current and is opposed by a coincident hyperpolarisation activated cationic current (I_h) (*Mao* et al., 2006). The duration and frequency of action potential firing are determined by the sAHP and fAHP, I_h and the inactivation characteristics of the Na^+ currents. Action potential firing thresholds depend on the activation characteristics of Na^+ currents and on the total plasmalemma leak conductance. The resting membrane potential is determined by various background or leak conductances, which include I_h , IK_{Ca} current, Na+ window currents and at least one type of tandem pore K^+ channel current (*Matsuyama* et al., 2008).

The IK_{Ca} current underlying sAHP in IPANs is at present the most plausible target for JB-1 although other bacteria might act on any other or combination of other IPAN ion channels. The reduction in MMC amplitude by JB-1 (see above) was reproduced within 5-15 min (see for example Figure 4 in *Wang* et al, 2010a and Figure 3 in *Wang* et al, 2010b) of adding the specific intermediate conductance Ca^{2+} dependent K^+ (IK_{Ca}) channel blocker TRAM-34 to the Krebs buffer superfusing segments of rat colon or mouse small intestine (*Wang* et al., 2010b). In the absence of overt inflammation, only IPANs (AH cells) but not inter- or motor-neurons (S cells) express functional IK_{Ca} channels. We deduce that the probiotic altered motility by altering IPAN function, probably by decreasing the IK_{Ca} dependent inhibitory slow after-hyperpolarisation current (*Wang* et al., 2010a, 2010b). It is important to note that a similar effect on MMCs was produced for rat colon after log 9 cfu JB-1 was fed to the animals daily for 9 days (*Wang* et al., 2010b), suggesting IK_{Ca} may be a probiotic target for both acute and repeated probiotic applications and for more than one host species.

LITERATURE

Ahlman, H. and Nilsson, O.: The gut as the largest endocrine organ in the body. Ann. Oncol. 12 (Suppl. 2), S63-S68 (2001).

Aihara, K., Kajimoto, O., Hirata, H., Takahashi, R., and Nakamura, Y.: Effect of powdered fermented milk with *Lactobacillus helveticus* on subjects with high-normal blood pressure or mild hypertension. J. Am. Coll. Nutr. 24, 257-265 (2005).

Alex, G., Clerc, N., Kunze, W.A., and Furness, J.B.: Responses of myenteric S neurones to low frequency stimulation of their synaptic inputs. Neuroscience 110, 361-373 (2002).

Artis, D.: Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nat. Rev. Immunol. 8, 411-420 (2008).

Bansal, T., Alaniz, R.C., Wood, T.K., and Jayaraman, A.: The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proc. Natl. Acad. Sci. USA 107, 228-233 (2010).

Bercik, P., Collins, S.M., and Verdu, E.F.: Microbes and the gut-brain axis. Neurogastroenterol. Motil. 24, 405-413 (2012).

Bertrand, P.P.: ATP and sensory transduction in the enteric nervous system. Neuroscientist 9, 243-260 (2003).

Bertrand, P.P.: Bursts of recurrent excitation in the activation of intrinsic sensory neurons of the intestine. Neuroscience 128, 51-63 (2004).

Bertrand, P.P.: The cornucopia of intestinal chemosensory transduction. Front. Neurosci. 3, 48 (2009).

Bian, X., Zhou, X., and Galligan, J.J.: R-type calcium channels in myenteric neurons of guinea pig small intestine. Am. J. Physiol. Gastrointest. Liver Physiol. 287, G134-G142 (2004).

Bienenstock, J. and Collins, S.: 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Psychoneuroimmunology and the intestinal microbiota: Clinical observations and basic mechanisms. Clin. Exp. Immunol. 160, 85-91 (2010).

Blackshaw, L.A., Brookes, S.J., Grundy, D., and Schemann, M.: Sensory transmission in the gastrointestinal tract. Neurogastroenterol. Motil. 19 (1 Suppl), 1-19 (2007).

Bornstein, J.C., Furness, J.B., Kunze, W.A.A., and Bertrand, P.P.: Enteric reflexes that influence motility. In: Innervation of the Gastrointestinal Tract (Costa, M. and Brookes, S.J.H., Eds.). Taylor and Francis, London, 1-55 (2002).

Bornstein, J.C., Costa, M., and Grider, J.R.: Enteric motor and interneuronal circuits controlling motility. Neurogastroenterol. Motil. 16 (Suppl 1), 34-38 (2004).

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA 108, 16050-16055 (2011).

Buhner, S. and Schemann, M.: Mast cell-nerve axis with a focus on the human gut. Biochim. Biophys. Acta 1822, 85-92 (2012).

Christofi, F.L. and Wood, J.D.: Effects of PACAP on morphologically identified myenteric neurons in guinea pig small bowel. Am. J. Physiol. 264, G414-421 (1993).

Clerc, N., Furness, J.B., Kunze, W.A., Thomas, E.A., and Bertrand, P.P.: Long-term effects of synaptic activation at low frequency on excitability of myenteric AH neurons. Neuroscience 90, 279-289 (1999).

Collins, S.M. and Bercik, P.: The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology 136, 2003-2014 (2009).

Cooke, H.J.: Neuroimmune signaling in regulation of intestinal ion transport. Am. J. Physiol. 266, G167-G178 (1994).

Crisci, J.V.: Parsimony in evolutionary theory: Law or methodological prescription? J. Theor. Biol. 97, 35-41 (1982).

Dogiel, A.S.: Ueber den Bau der Ganglien in den Geflechten des Darmes und der Gallenblase des Menschen und der Saeugetiere. Arch. Anat. Physiol. (Leipzig), Anat. Abt., 130-158 (1899).

Dykstra, N.S., Hyde, L., Adawi, D., Kulik, D., Ahrne, S., Molin, G., Jeppsson, B., MacKenzie, A., and Mack, D.R.: Pulse probiotic administration induces repeated small intestinal Muc3 expression in rats." Pediatr. Res. 69, 206-211 (2011).

Ekblad, E., Winther, C., Ekman, R., Hakanson, R., and Sundler, F.: Projections of peptide-containing neurons in rat small intestine. Neuroscience 20, 169-188 (1987).

Furness, J.B., Kunze, W.A., Bertrand, P.P., Clerc, N., and Bornstein, J.C.: Intrinsic primary afferent neurons of the intestine. Prog. Neurobiol. 54, 1-18 (1998).

Furness, J.B., Kunze, W.A., and Clerc, N.: Nutrient tasting and signaling mechanisms in the gut. II. The intestine as a sensory organ: neural, endocrine, and immune responses. Am. J. Physiol. 277, G922-G928 (1999).

Furness, J.B., Jones, C., Nurgali, K., and Clerc, N.: Intrinsic primary afferent neurons and nerve circuits within the intestine. Prog. Neurobiol. 72, 143-164 (2004).

Furness, J.B.: Novel gut afferents: Intrinsic afferent neurons and intestinofugal neu-

rons. Auton Neurosci 125, 81-85 (2006a).
Furness, J.B.: The enteric nervous system. Blackwell Publishing, Massachusetts, Oxford, Carlton (2006b).
Furness, J.B. and Poole, D.P.: Involvement of gut neural and endocrine systems in pathological disorders of the digestive tract. J. Anim. Sci. 90, 1203-1212 (2011).
Gershon, M.D.: The enteric nervous system: a second brain. Hosp. Pract. (Minneap.) 34, 31-32, 35-38, 41-32 passim. (1999).
Gwynne, R.M. and Bornstein, J.C.: Mechanisms underlying nutrient-induced segmentation in isolated guinea pig small intestine. Am. J. Physiol. Gastrointest. Liver Physiol. 292, G1162-G1172 (2007).
Hay, M. and Kunze, D.L.: Calcium-activated potassium channels in rat visceral sensory afferents. Brain Res. 639, 333-336 (1994).
Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M., Forssberg, H., and Pettersson, S.: Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. USA 108, 3047-3052 (2011).
Hirst, G.D., Holman, M.E., Prosser, C.L., and Spence, I.: Some properties of the neurones of Auerbach's plexus. J. Physiol. 225, 60P-61P (1972).
Hirst, G.D. and Spence, I.: Calcium action potentials in mammalian peripheral neurones. Nature 243, 54-56 (1973).
Hirst, G.D., Holman, M.E., and Spence, I.: Two types of neurones in the myenteric plexus of duodenum in the guinea pig. J. Physiol. 236, 303-326 (1974).
Hirst, G.D., Johnson, S.M., and van Helden, D.F.: The slow calcium-dependent potassium current in a myenteric neurone of the guinea-pig ileum. J. Physiol. 361, 315-337 (1985).
Hoffman, F.A.: Development of Probiotics as Biologic Drugs. Clin. Infect. Dis. 46 (Suppl. 2), S125-S127 (2008).
Jauhiainen, T., Vapaatalo, H., Poussa, T., Kyronpalo, S., Rasmussen, M., and Korpela, R.: *Lactobacillus helveticus* fermented milk lowers blood pressure in hypertensive subjects in 24-h ambulatory blood pressure measurement. Am. J. Hypertens. 18, 1600-1605 (2005).
Jungbauer, C., Lindig, T.M., Schrödl, F., Neuhuber, W., and Brehmer, A.: Chemical coding of myenteric neurons with different axonal projection patterns in the porcine ileum. J. Anat. 209, 733-743 (2006).
Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y., Tougas, G., and Bienenstock, J.: Inhibitory effects of Lactobacillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. Gut 55, 191-196 (2006).
Kamm, K., Hoppe, S., Breves, G., Schroder, B., and Schemann, M.: Effects of the probiotic yeast *Saccharomyces boulardii* on the neurochemistry of myenteric neurones in pig jejunum. Neurogastroenterol. Motil. 16, 53-60 (2004).
Keast, J.R., Furness, J.B., and Costa, M.: Origins of peptide and norepinephrine nerves in the mucosa of the guinea pig small intestine. Gastroenterology 86, 637-644 (1984).
Kirchgessner, A.L., Tamir, H., and Gershon, M.D.: Identification and stimulation by serotonin of intrinsic sensory neurons of the submucosal plexus of the guinea pig gut: activity-induced expression of Fos immunoreactivity. J. Neurosci. 12, 235-248 (1992).
Koch, T. and Höllt, V.: Role of receptor internalization in opioid tolerance and dependence. Pharmacol. Ther. 117, 199-206 (2008).
Kostrzewa, R.M.: Dopamine receptor supersensitivity. Neurosci. Biobehav. Rev. 19, 1-17 (1995).
Kunze, W.A., Furness, J.B., and Bornstein, J.C.: Simultaneous intracellular recordings from enteric neurons reveal that myenteric AH neurons transmit via slow excitatory postsynaptic potentials. Neuroscience 55, 685-694 (1993).
Kunze, W.A., Bornstein, J.C., and Furness, J.B.: Identification of sensory nerve cells in a peripheral organ (the intestine) of a mammal. Neuroscience 66, 1-4 (1995).
Kunze, W.A., Furness, J.B., Bertrand, P.P., and

Bornstein, J.C.: Intracellular recording from myenteric neurons of the guinea-pig ileum that respond to stretch. J. Physiol. 506, 827-842 (1998).

Kunze, W.A., Clerc, N., Bertrand, P.P., and Furness, J.B.: Contractile activity in intestinal muscle evokes action potential discharge in guinea-pig myenteric neurons. J. Physiol. 517, 547-561 (1999).

Kunze, W.A. and Furness, J.B.: The enteric nervous system and regulation of intestinal motility. Annu. Rev. Physiol. 61, 117-142 (1999).

Kunze, W.A., Clerc, N., Furness, J.B., and Gola, M.: The soma and neurites of primary afferent neurons in the guinea-pig intestine respond differentially to deformation. J. Physiol. 526, 375-385 (2000).

Kunze, W.A., Mao, Y.K., Wang, B., Huizinga, J.D., Ma, X., Forsythe, P., and Bienenstock, J.: *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. J. Cell. Mol. Med. 13, 2261-2270 (2009).

Logan, A.C. and Katzman, M.: Major depressive disorder: Probiotics may be an adjuvant therapy. Med. hypotheses 64, 533-538 (2005).

Lüderitz, C.: Das motorische Verhalten des Magens bei Reizung seiner äusseren Fläche. Pflügers Arch. Gesammte Physiol. Menschen Tiere 49. 158-174 (1891)

Lu, C.L., Pasricha, P.J., Hsieh, J.C., Lu, R.H., Lai, C.R., Wu, L.L., Chang, F.Y., and Lee, S.D.: Changes of the neuropeptides content and gene expression in spinal cord and dorsal root ganglion after noxious colorectal distension. Regul. Pept. 131, 66-73 (2005).

Lyte, M., Li, W., Opitz, N., Gaykema, R.P., and Goehler, L.E.: Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium*. Physiol. Behav. 89, 350-357 (2006).

Lyte, M.: Microbial endocrinology as a basis for improved L-DOPA bioavailability in Parkinson's patients treated for *Helicobacter pylori*. Med. Hypotheses 74, 895-897 (2010).

Lyte, M.: Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. Bioessays 33, 574-581 (2011).

Ma, X., Mao, Y.K., Wang, B., Huizinga, J.D., Bienenstock, J., and Kunze, W.: *Lactobacillus reuteri* ingestion prevents hyperexcitability of colonic DRG neurons induced by noxious stimuli. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G868-G875 (2009).

Mao, Y., Wang, B., and Kunze, W.: Characterization of myenteric sensory neurons in the mouse small intestine. J. Neurophysiol. 96, 998-1010 (2006).

Matsuyama, H., Nguyen, T.V., Hunne, B., Thacker, M., Needham, K., McHugh, D., and Furness, J.B.: Evidence that TASK1 channels contribute to the background current in AH/type II neurons of the guinea-pig intestine. Neuroscience 155, 738-750 (2008).

Mayer, E.A.: Gut feelings: the emerging biology of gut-brain communication. Nat. Rev. Neurosci. 12, 453-466 (2011).

Mazzuoli, G. and Schemann, M.: Multifunctional rapidly adapting mechanosensitive enteric neurons (RAMEN) in the myenteric plexus of the guinea pig ileum. J. Physiol. 587, 4681-4694 (2009).

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J., Rougeot, C., Pichelin, M., Cazaubiel, M., and Cazaubiel, J.: Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. Br. J. Nutr. 105, 755-764 (2011).

Moayyedi, P., Ford, A., Talley, N., Cremonini, F., Foxx-Orenstein, A., Brandt, L., and Quigley, E.: The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut 59, 325-332 (2010).

Nurgali, K., Stebbing, M.J., and Furness, J.B.: Correlation of electrophysiological and morphological characteristics of enteric neurons in the mouse colon. J. Comp. Neurol. 468, 112-124 (2004).

Nurgali, K., Qu, Z., Hunne, B., Thacker, M., Pontell, L., and Furness, J.: Morphological and functional changes in guinea-pig neurons projecting to the ileal mucosa at early stages after inflammatory damage. J. Physiol. 589, 325-339 (2011).

O'Hara, A.M. and Shanahan, F.: The gut flora as a forgotten organ. EMBO Rep. 7, 688-693 (2006).

Rao, A.V., Bested, A.C., Beaulne, T.M., Katzman, M.A., Iorio, C., Berardi, J.M., and Logan, A.C.: A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog. 1, 6 (2009).

Reuter, G.: The *Lactobacillus* and *Bifidobacterium* microflora of the human intestine: Composition and succession. Curr. Issues Intest. Microbiol. 2, 43-53 (2001).

Roshchina, V.V.: Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. In: Microbial endocrinology: Interkingdom signaling in infectious disease and health (Lyte, M. and Freestone, P.P.E., Eds.). Springer, New York, 17-52 (2010).

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., Dubuquoy, C., Merour, E., Geboes, K., Chamaillard, M., Ouwehand, A., Leyer, G., Carcano, D., Colombel, J. F., Ardid, D., and Desreumaux, P.: *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat. Med. 13, 35-37 (2007).

Rugiero, F., Gola, M., Kunze, W.A., Reynaud, J.C., Furness, J.B., and Clerc, N.: Analysis of whole-cell currents by patch clamp of guinea-pig myenteric neurones in intact ganglia. J. Physiol. 538, 447-463 (2002).

Rugiero, F., Mistry, M., Sage, D., Black, J.A., Waxman, S.G., Crest, M., Clerc, N., Delmas, P., and Gola, M.: Selective expression of a persistent tetrodotoxin-resistant Na+ current and NaV1.9 subunit in myenteric sensory neurons. J. Neurosci. 23, 2715-2725 (2003).

Schemann, M. and Mazzuoli, G.: Multifunctional mechanosensitive neurons in the enteric nervous system. Auton. Neurosci. 153, 21-25 (2010).

Schild, J.H., Clark, J.W., Hay, M., Mendelowitz, D., Andresen, M.C., and Kunze, D.L.: A- and C-type rat nodose sensory neurons: Model interpretations of dynamic discharge characteristics. J. Neurophysiol. 71, 2338-2358 (1994).

Song, Z.M., Brookes, S.J., and Costa, M.: All calbindin-immunoreactive myenteric neurons project to the mucosa of the guinea-pig small intestine. Neurosci. Lett. 180, 219-222 (1994).

Spencer, N.J. and Smith, T.K.: Mechanosensory S-neurons rather than AH-neurons appear to generate a rhythmic motor pattern in guinea-pig distal colon. J. Physiol. 558, 577-596 (2004).

Starodub, A.M. and Wood, J.D.: Histamine suppresses A-type potassium current in myenteric neurons from guinea pig small intestine. J. Pharmacol. Exp. Ther. 294, 555-561 (2000).

Sundler, F., Bottcher, G., Ekblad, E., and Hakanson, R.: The neuroendocrine system of the gut. Acta Oncol. 28, 303-314 (1989).

Tanida, M., Yamano, T., Maeda, K., Okumura, N., Fukushima, Y., and Nagai, K.: Effects of intraduodenal injection of *Lactobacillus johnsonii* La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. Neurosci. Lett. 389, 109-114 (2005).

Tappenden, K.A. and Deutsch, A.S.: The Physiological Relevance of the Intestinal Microbiota - Contributions to Human Health. J. Am. Coll. Nutr. 26, 679S-683 (2007).

Verdu, E.F., Bercik, P., Verma-Gandhu, M., Huang, X.X., Blennerhassett, P., Jackson, W., Mao, Y., Wang, L., Rochat, F., and Collins, S.M.: Specific probiotic therapy attenuates antibiotic induced visceral hy-

persensitivity in mice. Gut 55, 182-190 (2006).

Vogalis, F., Harvey, J.R., and Furness, J.B.: TEA- and apamin-resistant K(Ca) channels in guinea-pig myenteric neurons: Slow AHP channels. J. Physiol. 538, 421-433 (2002).

Wang, B., Mao, Y.K., Diorio, C., Pasyk, M., Wu, R.Y., Bienenstock, J., and Kunze, W.A.: Luminal administration ex vivo of a live *Lactobacillus* species moderates mouse jejunal motility within minutes. FASEB J. 24, 4078-4088 (2010a).

Wang, B., Mao, Y.K., Diorio, C., Wang, L., Huizinga, J.D., Bienenstock, J., and Kunze, W.: *Lactobacillus reuteri* ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. Neurogastroenterol. Motil. 22, 98-107 (2010b).

Wood, J.D.: Enteric nervous system: reflexes, pattern generators and motility. Curr. Opin. Gastroenterol. 24, 149-158 (2008).

Woolf, C.J. and Salter, M.W.: Neuronal plasticity: Increasing the gain in pain. Science 288, 1765-1769 (2000).

Yamano, T., Tanida, M., Niijima, A., Maeda, K., Okumura, N., Fukushima, Y., and Nagai, K.: Effects of the probiotic strain *Lactobacillus johnsonii* strain La1 on autonomic nerves and blood glucose in rats. Life Sci. 79, 1963-1967 (2006).

Zholos, A.V., Baidan, L.V., and Wood, J.D.: Sodium conductance in cultured myenteric AH-type neurons from guinea-pig small intestine. Auton. Neurosci. 96, 93-102 (2002).

MICROBIOTA, STRESS AND THE BRAIN

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SUMMARY

Bacterial colonisation of the intestine has a major role in the post-natal development and maturation of the immune and endocrine systems. These processes are key factors underpinning central nervous system (CNS) signalling. Regulation of the microbiota-gut-brain axis is essential for maintaining homeostasis, including that of the CNS. Moreover, there is now expanding evidence for the view that commensal organisms within the gut play a role in early programming and later responsivity of the stress system. Research has focused on how the microbiota communicates with the central nervous system (CNS) and thereby influences brain function. The routes of this communication are not fully elucidated but include neural, humoral, immune and metabolic pathways. This view is underpinned by studies in germ-free animals and in animals exposed to pathogenic bacterial infections, probiotic agents or antibiotic agents which indicate a role for the gut microbiota in the regulation of mood, cognition, pain and obesity. Thus the concept of a microbiota-gut brain axis is emerging which suggests that modulation of the gut microflora may be a tractable strategy for developing novel therapeutics for complex stress-related CNS disorders where there is a huge unmet medical need.

INTRODUCTION

The fields of microbiology and neuroscience in modern medicine have largely developed in distinct trajectories, with the exception of studies focused on the direct impact of infectious agents on brain function, which include early investigations of syphilis to more recent studies of neuroAIDS. In addition to the direct effect of bacteria on CNS function, the field of psychoneuroimmunology has emerged to provide a framework as to how pathogenic bacterial agents can alter brain function. Thus, the role of the CNS in mediating the behavioural responses to infections ranging from "sickness behaviour" to septic encephalopathy has been advanced significantly. More recently, a new concept has emerged which indicates that commensal bacteria can also affect brain function in both health and disease. In this review we discuss the evidence to date and delineate how harnessing such pathways may provide for a novel approach to treat a variety of disorders of the brain-gut axis.

It is increasingly recognized that the brain-gut axis provides a bi-directional homeostatic route of communication which, if dysfunctional, can have important pathophysiological consequences (*Mayer*, 2011). This axis is

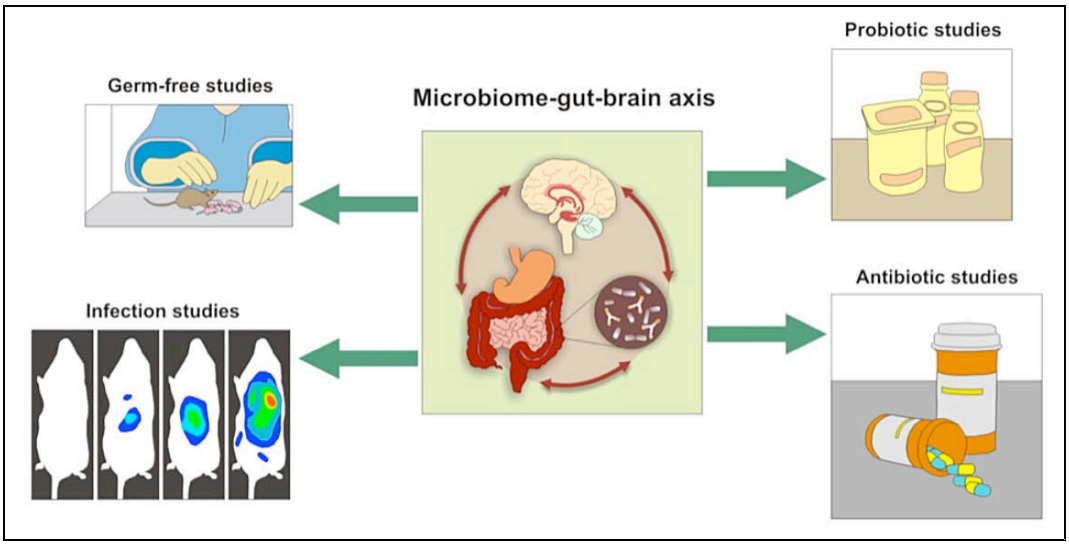

Figure 1: Strategies used to investigate the role of the microbiome gut brain axis in health and disease

regulated at neural, hormonal and immunological levels and alterations in brain-gut interactions are associated with gut inflammation, chronic abdominal pain syndromes and eating disorders (*Mayer*, 2011). Indeed, modulation of brain-gut axis function is associated with specific alterations in the stress-response and overall behaviour (*Rhee* et al., 2009). The high comorbidity between stress-related psychiatric symptoms such as anxiety with gastrointestinal disorders including irritable bowel syndrome (IBS) and inflammatory bowel disorder (IBD) (*Reber*, 2012) is further evidence of the importance of this axis. Thus modulation of the brain-gut axis is being seen as an attractive target for the development of novel treatments for a wide variety of disorders ranging from obesity, mood and anxiety disorders to GI disorders such as IBS (*Mayer*, 2011). In addition, increasing evidence also suggests that the enteric microbiome can greatly influence all aspects of physiology (*Clemente* et al., 2012; *Sekirov* et al., 2010) not least of which is its ability to impact on gut-brain communication.

THE MICROBIOTA

The human gastrointestinal tract (GIT) is inhabited with 10^{13}-10^{14} microorganisms, which is >10 times that of the number of human cells in our bodies and 150 times as many genes as our genome (*Gill* et al., 2006; *Qin* et al., 2010), and thus often referred to as the "forgotten organ" (*O'Hara* et al., 2006). Our appreciation of the relationship between the microbiome and host is changing rapidly and it now can be viewed as being mutualistic (with both partners experiencing increased fitness) (*Backhed* et al., 2005), playing a crucial role in the development and functionality of the innate and adaptive immune responses (*Olszak* et al., 2012; *Round* et al., 2010) regulating gut mo-

tility, intestinal barrier homeostasis, nutrient absorption and fat distribution (*Backhed* et al., 2004; *Bercik* et al., 2012). Over the past 5 years significant advances have been made in the technology for assessing microbiota composition at the genetic level (*Fraher* et al., 2012; *Qin* et al., 2010) that is also having an immense impact on increasing our understanding of host-microbe interactions.

THE MICROBIOTA-GUT BRAIN AXIS AND BEHAVIOUR

Taken together, it is thus perhaps not surprising that there is a growing body of literature focused on assessing the impact of enteric microbiota on brain and behaviour and the concept of the microbiome-gut-brain axis is emerging. The general building blocks of this axis include the CNS, the neuroendocrine and neuro-immune systems, the sympathetic and parasympathetic arms of the autonomic nervous system (ANS) and the enteric nervous system (ENS) along with the intestinal microbiota, which is being increasingly viewed as the cornerstone. These components converge to form a complex reflex network with afferents that project to integrative CNS structures and efferents to smooth muscle (*Mayer*, 2011). Crucially, this axis functions bi-directionally (*Mayer*, 2011). Approaches used to parse the role of gut microbiota on brain function (see Figure 1) include using germ-free animals, assessing the impact of probiotic agents, antibiotic-induced dysbiosis and pathogenic infections (*Cryan* and *O'Mahony*, 2011). In addition, accumulating studies have also shown that manipulations known to impact brain function (e.g. stress) also impacts microbiota composition (*Dinan* et al., 2012).

GERM-FREE ANIMALS

The use of germ-free (GF) animals enables the direct assessment of the role of the microbiota on all aspects of physiology. In these animals surgical delivery replaces the normal birthing process, thus eliminating the opportunity for post-natal colonization of the GIT and allows for direct comparison with their conventionally colonized counterparts. In a landmark study, Sudo and colleagues (*Sudo* et al., 2004) provided direct evidence that intestinal microbiota plays a key role in the development of the hypothalamic-pituitary-adrenal (HPA) axis. In GF mice a mild restraint stress induced an exaggerated release of corticosterone and adrenocorticotrophin hormone compared to the specific pathogen free controls. The stress response in the GF mice was partially reversed by colonization with faecal matter from control animals and fully reversed by mono-association with *Bifidobacterium infantis* in a time dependant manner (*Sudo* et al., 2004). These data clearly demonstrated that the microbial content of the GIT is critical to the development of an appropriate stress response later in life and also that there is a critical window in early life where colonization must occur to ensure normal development of the HPA axis. From a neuronal point of view, a decrease in brain derived neurotrophic factor (BDNF), a key neurotrophin involved in neuronal growth and survival, and decreased expression of the N-methyl-D-asparaginezuur (NMDA)

receptor subunit 2a (NR2a) in the cortex and hippocampus of GF animals compared to controls was also observed. However, it took a further seven years for these findings to be followed up at a behavioural level. Three independent groups have now shown that GF animals (from different strains and genders) have reduced anxiety in the elevated plus maze or light dark box (*Clarke* et al., 2012a; *Heijtz* et al., 2011; *Neufeld* et al., 2010), but see (*Gareau* et al., 2011) The mentioned tests are widely used to assess anxiety related behaviour (*Cryan* and *Sweeney*, 2011). These findings are somewhat puzzling as it is opposite to what one would have been predicted based on the exaggerated HPA axis. Interestingly, Neufeld and colleagues (*Neufeld* et al., 2010) also reported changes in BDNF, NR_{2B} and $5\text{-}HT_{1A}$ receptor mRNA expression in GF mice. However, it is worth noting that the direction of such changes is not in agreement with data reported by *Sudo* et al. (2004) From a cognitive point of view GF mice displayed deficits in simple non-spatial and working memory tasks (novel object recognition and spontaneous alternation in the T-maze). Future studies should focus on enhancing the repertoire of behavioural cognitive assays employed. However, maintaining animals GF and conducting complex behavioural studies is not a trivial logistical hurdle.

More recently, we have shown that GF animals have a significant elevation in the hippocampal concentration of 5-HT and 5-HIAA, its main metabolite, compared with conventionally colonised control animals (*Clarke* et al., 2012a). Concentrations of tryptophan, the precursor of serotonin, are also increased in the plasma of GF animals, suggesting a humoral route through which the microbiota can influence CNS serotonergic neurotransmission. Interestingly, colonisation of the germ-free animals post-weaning is insufficient to reverse the CNS neurochemical consequences in adulthood of an absent microbiota in early-life despite the peripheral availability of tryptophan being restored to baseline values. Gender may play a role in such effects. Indeed, we have recently shown that many of the neurochemical but not endocrine or immune effects of growing up in a germ-free environment are only evident in male animals (*Clarke* et al., 2012a). Further behavioural studies, including the use of other species such as germ-free rats will greatly expand our knowledge of the role of microbiota in stress-related disorders.

PROBIOTICS

Probiotics are live organisms that, when ingested in adequate quantities, exert a health benefit on the host (*Gareau* et al., 2010; *Quigley*, 2008). Probiotics are reported to have a wide variety of effects in both human and animal studies (*Gareau* et al., 2010; Quigley, 2008). Increasing evidence points to beneficial effects of various probiotics in the treatment of the gastrointestinal symptoms of disorders such as IBS (*Clarke* et al., 2012b). Moreover, there is some clinical evidence to support the role of probiotic intervention in reducing the anxiety and stress response as well as improving mood in IBS patients and those with chronic fatigue (*Logan* et al., 2005; et al., 2009). Recently, a study assessing the effect of a combination of *Lactobacillus helveticus* and *B. longum* on both human subjects and rats

demonstrated the ability of this probiotic cocktail to reduce anxiety in animals and had beneficial psychological effects with a decrease in serum cortisol in patients (*Messaoudi* et al., 2011). While the mechanism of action is not known it has been postulated that probiotic-induced effects on pro-inflammatory cytokines, oxidative stress and in nutritional status may contribute to such effects (*Cryan* and *O'Mahony*, 2011; *Logan* et al., 2005).

Recently we have shown that a bacterium of the *Lactobacillus* species *L. rhamnosus* (JB1*),* decreases anxiety and despair-like behaviour as well as reducing the stress-induced increase of corticosterone in mice (*Bravo* et al., 2011). Moreover, this potential probiotic alters the mRNA expression of both $GABA_A$ and $GABA_B$ receptors in the CNS. Alterations in these receptors are associated with anxious and depressive-like behaviours in animal models. Interestingly, these effects are vagus-dependent as vagotomy prevented the anxiolytic and antidepressant effects of this bacterium as well as the effects on the central GABA receptors. This suggests that parasympathetic innervation is necessary for *L. rhamnosus* to participate in the microbiota-brain interaction. Whilst there have been studies showing that potential probiotics can reverse the effects of colitis, infection or stress these data are the first to our knowledge to show beneficial effects in animal assays used to assess anxiolytic or antidepressant activity (*Cryan* and *Sweeney*, 2011).

Indeed, previous studies have shown that the probiotic *B. longum* NCC3001 but not *L. rhamnosus* NCC4007 reversed colitis-induced anxiety and alterations in hippocampal BDNF without impacting gut inflammation or circulating cytokines (*Bercik* et al., 2010; *Bercik* et al., 2011a). The anxiolytic effect of *B. longum* NCC3001 was absent in mice with vagotomy, suggesting a neural mechanism, which was confirmed by *ex vivo* electrophysiological studies, in enteric neurons (*Bercik* et al., 2011b). We have also shown that probiotic agents such as *B. infantis* can modulate antidepressant-like behaviour in a maternal separation model (*Desbonnet* et al., 2010) and modulate peripheral pro-inflammatory cytokine and tryptophan concentrations both of which have been implicated in depression. Finally, exciting studies are emerging showing that brain fatty acid concentrations (including stearic acid, arachidonic acid, and DHA) can be elevated in mice whose diets were supplemented with *B. breve* NCIMB 702258 (*Wall* et al., 2012). Interestingly, this effect was bacterial strain dependent as it was not induced by another B. *breve* strain DPC 6330. Taken together, certain probiotic strains can modulate various aspects of the microbiome-gut-brain axis some of which are vagus dependent. However, it is clear that caution needs to be exercised when generalising such effects from one bacterial strain to another and efforts need to be directed at identifying the mechanism of how each strain induces their effects.

PAIN

Some of the most convincing data on the microbiome-gut-brain axis emerges from the pain field. Visceral pain is a pronounced and, at times, dominant feature of a variety of gastrointestinal disorders, including IBS. Recurrent, episodic but often unpredictable painful events can exert a disabling impact on daily life and result in impairment of several domains of quality of life. Vis-

ceral pain perception is regulated by complex mechanisms. These include peripheral sensitization of sensory nerves whereas the central processing of visceral nociception is mediated at both cortical and subcortical levels by pathways also involved in the processing of psychological stress. Specifically, the prefrontal cortex has been shown to play an integral role in these processes, with imaging studies in humans (*Mayer* et al., 2008; *Mertz* et al., 2000) and animal (*Gibney* et al., 2010; *O'Mahony* et al., 2010; *Wang* et al., 2008) showing activation of the anterior cingulate, pre-limbic and infra-limbic cortices in response to painful and stressful stimuli. Growing evidence suggests that both central and peripheral mechanisms could be affected by intestinal microbiota. In animal studies, probiotics (mostly *Lactobacilli and Bifidobacteria*) have been shown to alleviate visceral pain in stress models (*Ait-Belgnaoui* et al., 2006; *Gareau* et al., 2007; *Johnson* et al., 2011; *McKernan* et al., 2010; *Rousseaux* et al., 2007; *Verdu* et al., 2006). This has also been also the case clinically with many different probiotics showing beneficial effects in abdominal pain (*Bercik* et al., 2012; *Clarke* et al., 2012b). The mechanisms of action of such effects remain unclear currently and as in animal studies may involve a combination of effects on neural, immune and endocrine effects. We have recently demonstrated that *B. infantis* 35624 is effective at increasing the pain threshold in rats and reducing the number of pain behaviours following CRD in both a normo-sensitive and a hypersensitive rat strain (*McKernan* et al., 2010). Recently, it has been shown that *Lactobacillus acidophilus* reduces visceral hypersensitivity in rats by inducing cannabinoid (CB2) and opioid receptor (MOR1) expression in the colonic epithelium (*Rousseaux* et al., 2007). Furthermore, in a number of recent studies, *Lactobacilli* have been shown to affect the excitability of enteric neurons and nerves innervating the gut, which in turn has been shown to have effects on colonic motility (*Kunze* et al., 2009; *Ma* et al., 2009; *Wang* et al., 2010).

MICROBIOTA AND STRESS

It is long known that stress and the HPA axis can influence the composition of gut microbiome (*Tannock* et al., 1974). However, the functional consequences of such changes are now only being unravelled (*Dinan* et al., 2012). Maternal separation, an early life stressor which can result in long-term HPA axis changes (*O'Mahony* et al., 2011), has been shown to cause a significant decrease in faecal lactobacilli on day 3 post separation, which returns to baseline by day seven as assessed by enumeration of total and Gram-negative aerobic and facultative anaerobic bacterial species (*Bailey* et al., 1999). However, we have more recently shown that early life stress can also have long-term effects on the microbiome. Analysis of the 16S rRNA diversity in adult rats exposed to maternal separation for three hours per day from post natal days 2-12 revealed a significantly altered faecal microbiome when compared to the non-separated control animals (*O'Mahony* et al., 2009). A study using deep sequencing methods demonstrated that the community structure of microbiota from mice exposed to chronic restraint stressor was significantly different to that in non-stressed control mice (*Bailey* et al., 2011). More

recently chronic psychosocial stress decreased the relative abundance of *Bacteroides*, while increasing the relative abundance of bacteria in the genus *Clostridium* in the caecum. The stressor also increased circulating levels of IL-6 and MCP-1, which were significantly correlated with stressor-induced changes to three bacterial genera (i.e., *Coprococcus, Pseudobutyrivibrio,* and *Dorea*). These data show that exposure to repeated stress affects gut bacterial populations in a cytokine dependent manner (*Bailey* et al., 2011).

CONCLUSIONS AND PERSPECTIVE

A growing body of experimental data and clinical observations support the existence of the microbiome-gut-brain axis and suggest that it is poised to control canonical aspects of brain and behaviour in health and disease. Future research should focus on deconvoluting the relative contributions of immune, neural, and metabolic pathways to this axis. A better understanding of these relationships will inform our understanding of a host of GI and extra-GI disorders including neuropsychiatric diseases such as depression and anxiety. Much work is needed to tease apart the various constructs at play in this complex communication network. It is not clear how the various microbial strains can differentially affect CNS functioning but metabolite production, polysaccharide actions, structural effects and direct and indirect activation of the immune system are likely factors at play. Indeed, the bacterial metabolism of dietary fibre to short-chain fatty acids is a significant energy source for humans and these metabolites are of importance for gut motility, have a trophic effect on epithelial cells, impact on immune system development and modulate entero-endocrine hormone secretion (*Grenham* et al., 2011). Certain microorganisms including *Lactobacilli* are able to convert nitrate to nitric oxide, a potent regulator of both the immune and nervous systems whilst others can produce neuroactive amino acids such as GABA (*Forsythe* et al., 2010). Elucidating the mechanisms underlying such effects will be crucially important for the development of any microbiota-based therapeutic strategies for CNS diseases.

It is clear that the clinical translation of any animal data especially those in complex areas of anxiety, depression and cognition is now warranted and that this should be done with the same rigour as pharmaceutical drug development. Overall, research to data clearly shows that behaviours, physiology, and neurochemistry can be affected in many ways by modulation of gut microbiota. Whether this translates to microbial-based CNS therapeutics remains a tempting possibility and one that is worthy of much further investigation.

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LITERATURE

Ait-Belgnaoui, A., Han, W., Lamine, F., Eutamene, H., Fioramonti, J., Bueno, L., and Theodorou, V.: *Lactobacillus farciminis* treatment suppresses stress induced visceral hypersensitivity: A possible action through interaction with epithelial cell cytoskeleton contraction. Gut 55, 1090-1094 (2006).

Backhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F., and Gordon, J.I.:. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. USA 101, 15718-15723 (2004).

Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I.: Host-bacterial mutualism in the human intestine. Science 307: 1915-1920 (2005).

Bailey, M.T. and Coe, C.L.: Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Dev. Psychobiol. 35, 146-155 (1999).

Bailey, M.T., Dowd, S.E., Galley, J.D., Hufnagle, A.R., Allen, R.G., and Lyte, M.: Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav. Immun. 25, 397-407 (2011).

Bercik, P., Verdu, E.F., Foster, J.A., Macri, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blenneerhassett, P., Neufeld, K.A., Lu, J., Kahn, W.I., Corthesy-Theulaz, I., Cherbut, C., Bergonzelli, G.E., and Collins, S.M.: Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139, 2102-2112 (2010).

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Veerdu, E.F., and Collins, S.M.: The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. Gastroenterology 141, 599-609 (2011a).

Bercik, P., Park, A.J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P.A., Fahnestock, M., Moine, D., Berger, B., Huizinga, J.D., Kunze, W., McLean, P.G., Bergonzelli, G.E., Collins, S.M., and Verdu, E.F.:The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. Neurogastroenterol. Motil. 23, 1132-1139 (2011b).

Bercik, P., Collins, S.M., and Verdu, E.F.: Microbes and the gut-brain axis. Neurogastroenterol. Motil. 24, 405-413 (2012).

Bravo, J..A, Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA 108, 16050-16055 (2011).

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., and Cryan, J.F.: The microbiome-gut-brain axis during early-life regulates the hippocampal serotonergic system in a gender-dependent manner. Mol. Psychiatry, doi: 10.1038/mp.2012.77, Epub ahead of print (2012a)

Clarke, G., Cryan, J.F., Dinan, T.G., and Quigley, E.M.: Review article: Probiotics for the treatment of irritable bowel syndrome--focus on lactic acid bacteria. Aliment. Pharmacol. Ther. 35, 403-413 (2012b).

Clemente, J.C., Ursell, L.K., Parfrey, L.W., and Knight, R.: The impact of the gut microbiota on human health: An integrative view. Cell 148, 1258-1270 (2012).

Cryan, J.F. and O'Mahony, S.M.: The microbiome-gut-brain axis: from bowel to behavior. Neurogastroenterol. Motil. 23, 187-192 (2011).

Cryan, J.F. and Sweeney, F.F.: The age of anxiety: Role of animal models of anxiolytic action in drug discovery. Br. J. Pharmacol. 164, 1129-1161 (2011).

Desbonnet, L., Garrett, L., Clarke, G., Kiely,

B., Cryan, J.F., and Dinan, T.G.: Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 170, 1179-1188 (2010).

Dinan, T.G. and Cryan, J.F.: Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. Psychoneuroendocrinology 37, 1369-1378 (2012).

Forsythe, P., Sudo, N., Dinan, T., Taylor, V.H., and Bienenstock, J.: Mood and gut feelings. Brain Behav. Immun 24, 9-16 (2010).

Fraher, M.H., O'Toole, P.W., and Quigley, E.M.: Techniques used to characterize the gut microbiota: A guide for the clinician. Nat. Rev. Gastroenterol. Hepatol. 9, 312-322 (2012).

Gareau, M.G., Jury, J., MacQueen, G., Sherman, P.M., and Perdue, M.H.: Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut 56 1522-1528 (2007).

Gareau, M.G., Sherman, P.M., and Walker, W.A.: Probiotics and the gut microbiota in intestinal health and disease. Nat. Rev. Gastroenterol. Hepatol. 7 503-514 (2010).

Gareau, M.G., Wine, E., Rodrigues, D.M., Cho, J.H., Whary, M.T., Philpott, D.J., Macqueen, G., and Sherman, P.M.: Bacterial infection causes stress-induced memory dysfunction in mice. Gut 60, 307-317 (2011).

Gibney, S.M., Gosselin, R.D., Dinan, T.G., and Cryan, J.F.: Colorectal distension-induced prefrontal cortex activation in the Wistar-Kyoto rat: Implications for irritable bowel syndrome. Neuroscience 165, 675-683 (2010).

Gill, S.R., Pop, M., Deboy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., Gordon, J.I., Reiman, D.A., Fraser-Liggett, C.M., and Nelson, K.E.: Metagenomic analysis of the human distal gut microbiome. Science 312, 1355-1359 (2006).

Grenham, S., Clarke, G., Cryan, J., and Dinan, T.G.: Brain-gut-microbe communication in health and disease. Front. Physiol. 2, 94 (2011).

Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M., Forssberg, H., and Pettersson, S.: Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. USA 108, 3047-3052 (2011).

Johnson, A.C., Greenwood-Van Meerveld, B., McRorie, J.: Effects of *Bifidobacterium infantis* 35624 on post-inflammatory visceral hypersensitivity in the rat. Dig. Dis. Sci. 56, 3179-3186 (2011).

Kunze, W.A., Mao, Y.K., Wang, B., Huizinga, J.D., Ma, X., Forsythe, P., and Bienenstock, J.: *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. J. Cell. Mol. Med. 13, 2261-2270 (2009).

Logan, A.C. and Katzman, M.: Major depressive disorder: Probiotics may be an adjuvant therapy. Med. hypotheses 64, 533-538 (2005).

Ma, X., Mao, Y.K., Wang, B., Huizinga, J.D., Bienenstock, J., and Kunze, W.: *Lactobacillus reuteri* ingestion prevents hyperexcitability of colonic DRG neurons induced by noxious stimuli. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G868-G875 (2009).

Mayer, E.A., Bradesi, S., Chang, L., Spiegel, B.M., Bueller, J.A., Naliboff, B.D.: Functional GI disorders: from animal models to drug development. Gut 57, 384-404 (2008).

Mayer, E.A.: Gut feelings: the emerging biology of gut-brain communication. Nat. Rev. Neurosci. 12, 453-466 (2011).

McKernan, D.P., Fitzgerald, P., Dinan, T.G., and Cryan, J.F.: The probiotic *Bifidobacterium infantis* 35624 displays visceral antinociceptive effects in the rat. Neurogastroenterol. Motil. 22, 1029-1035 (2010).

Mertz, H., Morgan, V., Tanner, G., Pickens, D., Price, R., Shyr, Y., and Kessler, R.: Regional cerebral activation in irritable bowel syndrome and control subjects with painful and nonpainful rectal distention. Gastroenterology 118, 842-848 (2000).

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J., Rougeot, C., Pichelin, M., Cazaubiel, M., and Cazaubiel, J.: Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. Br. J. Nutr. 105, 755-764 (2011).

Neufeld, K.M., Kang, N., Bienenstock, J., and Foster, J.A.: Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 23, 255-264 (2010).

O'Hara, A.M. and Shanahan, F.: The gut flora as a forgotten organ. EMBO Rep. 7, 688-693 (2006).

O'Mahony, S.M., Marchesi, J.R., Scully, P., Codling, C., Ceolho, A.M., Quigley, E.M., Cryan, J.F., and Dinan, T.G.: Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol. Psychiat. 65, 263-267 (2009).

O'Mahony, C.M., Sweeney, F.F., Daly, E., Dinan, T.G., and Cryan, J.F.: Restraint stress-induced brain activation patterns in two strains of mice differing in their anxiety behaviour. Behav. Brain Res. 213, 148-154 (2010).

O'Mahony, S.M., Hyland, N.P., Dinan, T.G., and Cryan, J.F.: Maternal separation as a model of brain-gut axis dysfunction. Psychopharmacology 214, 71-88 (2011).

Olszak, T., An, D., Zeissig, S., Vera, M.P., Richter, J., Franke, A., Glickman, J.N., Siebeert, R., Baron, R.M., Kasper, D.L., and Blumberg, R.S.: Microbial Exposure During Early Life Has Persistent Effects on Natural Killer T Cell Function. Science 336, 489-493 (2012).

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.-M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., MetaHIT Consortium, Bork, P., Ehrlich, S.D., and Wang, J.: A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59-65 (2010).

Quigley, E.M.: Probiotics in functional gastrointestinal disorders: what are the facts? Curr. Opin. Pharmacol. 8, 704-708 (2008).

Rao, S., Srinivasjois, R., and Patole, S.: Prebiotic supplementation in full-term neonates: A systematic review of randomized controlled trials. Arch. Pediatr. Adolesc. Med. 163, 755-764 (2009).

Reber, S.O.: Stress and animal models of inflammatory bowel disease--an update on the role of the hypothalamo-pituitary-adrenal axis. Psychoneuroendocrinology 37, 1-19 (2012).

Rhee, S.H., Pothoulakis, C., Mayer, E.A.: Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat. Rev. Gastroenterol. Hepatol. 6, 306-314 (2009).

Round, J.L., O'Connell, R.M., and Mazmanian, S.K.: Coordination of tolerogenic immune responses by the commensal microbiota. J. Autoimmun. 34, J220-J225 (2010).

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., Dubuquoy, C., Merour, E., Geboes, K., Chamaillard, M., Ouwehand, A., Leyer, G., Carcano, D., Colombel, J. F., Ardid, D., and Desreumaux, P.: *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat. Med. 13, 35-37 (2007).

Sekirov, I., Russell, S.L., Antunes, L.C., and Finlay, B.B.: Gut microbiota in health and disease. Physiol. Rev. 90, 859-904 (2010).

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., Kubo, C., and Koga, Y.: Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J.

Physiol. 558, 263-275 (2004).

Tannock, G.W. and Savage, D.C.: Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. Infect. Immun. 9, 591-598 (1974).

Verdu, E.F., Bercik, P., Verma-Gandhu, M., Huang, X.X., Blennerhassett, P., Jackson, W., Mao, Y., Wang, L., Rochat, F., and Collins, S.M.: Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 55, 182-190 (2006).

Wall, R., Marques, T.M., O'Sullivan, O., Ross, R.P., Shanahan, F., Quigley, E.M., Dinan, T.G., Kiely, B., Fitzgerald, G.F., Cotter, P.D., Fouhy, F., and Stanton, C.: Contrasting effects of *Bifidobacterium breve* NCIMB 702258 and *Bifidobacterium breve* DPC 6330 on the composition of murine brain fatty acids and gut microbiota. Am. J. Clin. Nutr. 95, 1278-1287 (2012).

Wang, Z., Bradesi, S., Maarek, J.M., Lee, K., Winchester, W.J., Mayer, E.A., and Holschneider, D.P.: Regional brain activation in conscious, nonrestrained rats in response to noxious visceral stimulation. Pain 138, 233-243 (2008).

Wang, B., Mao, Y.K., Diorio, C., Wang, L., Huizinga, J.D., Bienenstock, J., and Kunze, W.: *Lactobacillus reuteri* ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. Neurogastroenterol. Motil. 22, 98-107 (2010b).

GUT BACTERIA, THE VAGUS NERVE AND THE BRAIN

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SUMMARY

There is now strong evidence from animal studies that gut microorganisms can activate the vagus nerve and that such activation plays a critical role in mediating effects on the brain and behaviour. The vagus can differentiate between commensal and potentially pathogenic bacteria even in the absence of overt inflammation and vagal afferent signals can induce both anxiogenic and anxiolytic effects depending on the nature of the stimulus. In addition to direct afferent signalling to the brain circuitry associated with stress and anxiety there are well described anti-inflammatory efferent responses that may contribute to behavioural effects of vagal stimulation in certain circumstances. Advances in our understanding of the microbiome-gut-brain axis will come from studies of how distinct microbial stimuli activate the vagus and the nature of the signals transmitted to the brain that lead to differential neurochemical changes that subsequently drive distinct behavioural changes.

THE VAGUS NERVE

The vagus (10^{th} cranial nerve) innervates the pharynx, larynx and visceral organs. It contains more afferent than efferent nerve fibres and projects from the medulla oblongata in the brain stem to the colon. Indeed, the vagus nerve is the main afferent pathway from the abdominal cavity to the brain. Information from the heart, lungs, pancreas, liver, stomach and intestines are delivered tonically to the brain via sensory fibres in the vagus nerve (*Browning* and *Mendelowitz*, 2003). Sensory vagal inputs arrive in the nucleus of the solitary tract (NTS), and are thence transmitted to widespread areas of the CNS, including the cerebral cortex and medulla oblongata. Neurones of the rostral ventrolateral medulla oblongata (RVLM) provide one of two major sources of afferent inputs to the locus coeruleus (*Aston-Jones* et al., 1986), which in turn projects to areas of the cortex that are associated with stress-related behaviour and affective disorders. The locus coeruleus is also considered a major site for integrating stress-responses (*Aston-Jones* et al., 1996). Following repeated activations, a feed-forward system between noradrenergic locus coeruleus neurones and areas of the forebrain that produce corticotropin-releasing factor (CRF) can lead to altered behavioural responses (*Ziegler* et al., 1999). Chronic activation of this system induces changes in neuronal activity that underlies anxiety, panic disorders and depression (*Arborelius* et al., 1999). The concept of interoception and experimental data suggesting that changes in visceral sensation can affect the perception and interpretation of external inputs (*Crucian* et al., 2000) has

lead to the suggestion that altered sensory vagal inputs can influence our attitude to the outside world and that pathological changes in sensory vagal inputs may increase the risk of affective behavioural disorders. It has been proposed that chronic sensory vagal inputs could act as 'natural' breaks for augmentation of stress-related behavioural responses via tonic modulation of the neuronal activity in the locus coeruleus and in turn the forebrain (*Zagon*, 2001).

THE VAGUS AND SICKNESS BEHAVIOUR

Some of the earliest indication of the role of the vagus in modulating behaviour came from studies of animals exposed to endotoxin. Sickness behaviour is a term used to describe the drastic changes in behaviour that occur in physically ill patients and animals, and is a motivational state responsible for re-organizing perceptions and actions to enable ill individuals to cope better with infection (*Dantzer* et al., 2000). The associated behaviours include lethargy, depression, anxiety, and loss of appetite, sleepiness, hyperalgesia, and reduction in grooming. These behavioural changes are mediated by pro-inflammatory cytokines particularly IL-1β and TNF (*Dantzer* et al., 2000).

The role of vagal afferents in the induction of sickness behaviour following intraperitoneal administration of the cytokine inducer lipopolysaccharide (LPS) or IL-1 β has been assessed in laboratory animals that have been submitted to subdiaphragmic vagotomy (*Luheshi* et al., 2000; *Konsman* et al., 2000). In these experiments carried out in rats and mice, sickness behaviour was measured by decreased social exploration and by depressed operant responding for a food reward in mice that had been trained to repeatedly poke their noses into a hole for getting a food pellet. After recovery from surgery, a dose of LPS or IL-1 β that induced consistent sickness behaviour in sham-operated animals was no longer able to decrease social exploration in rats and mice (*Laye* et al., 1995; *Bluthe* et al., 1994). In the same manner, vagotomy blocked the depressing effects of LPS on food-motivated behaviour in mice (*Bret-Dibat* et al., 1995).

VAGAL STIMULATION AS AN ANTI-DEPRESSANT

While vagal activation by cytokines has been associated with sickness and depressive type behaviour it is also emerging that stimulating the vagus can lead to a reduction in anxiety and depression associated behaviours. In one study, rats were administered 30 minutes per day of continuous vagus nerve stimulation for 4 days, and were then subjected to the forced swim test, a well validated assessment of antidepressant activity. Vagus nerve stimulation significantly reduced immobility time compared to unstimulated controls, reflective of antidepressant effects (*Krahl* et al., 2004). Interestingly, vagal nerve stimulation-induced decreases in immobility were associated with increased swimming behaviour, which has been linked to a predominantly serotonergic mechanism of action *Cryan* et al., 2005). In a subsequent controlled trial, rats received desipramine or vagal nerve stimulation for 2 h at three time points over a 24 hour period, prior to undergoing the

forced swim test and both treatments resulted in reduced immobility compared to saline control (*Cunningham* et al., 2008). However, chronic vagal nerve stimulation for 1 month failed to show any behavioural alterations in rats subjected to the forced swim test or the elevated plus maze test, in contrast to treatment with another classical antidepressant, imipramine (*Biggio* et al., 2009). Vagal stimulation is an FDA accepted alternative treatment for intractable depression and has also been used successfully in the treatment of refractory epilepsy, demonstrating clear behavioural effects of modulating vagal afferent signals (*Walsh* and *Kling*, 2004). While this treatment is controversial, largely due to a lack of positive sham treatment controlled clinical trials, there have been reports that vagal nerve stimulation is beneficial in at least some patients with depression and may be particularly effective with chronic treatment (*Rizvi* et al., 2011; *Martin* and *Martin-Sanchez*, 2012).

VAGAL INFLAMMATORY REFLEX

The vagus innervates tissues known to participate in immune functions and/or contain important immune elements, such as thymus, lung, liver, and gastrointestinal tract. Furthermore, trunks or branches of the vagus are often associated with lymph nodes that drain regions in which immune activation occurs. It has recently been identified that the vagus plays a critical role in a neural circuit that controls the inflammatory response in a reflex-like manner. The vagus nerve senses inflammation sending afferent signals to the brain that then activates an efferent response, releasing mediators including acetylcholine that, through an interaction with immune cells, attenuates inflammation.

Tracey and colleagues first highlighted the anti-inflammatory role of the vagus demonstrating that direct electrical stimulation of the peripheral vagus nerve in vivo during lethal endotoxaemia in rats prevented the development of shock through the inhibition of TNF synthesis (*Borovikova* et al., 2000). The vagus nerve also plays a counter-inflammatory role in the experimental colitis (*Ghia* et al., 2006). Macrophages have been identified as the major source of TNF during endotoxaemia, and are suggested to be the main target of the anti-inflammatory function of the vagus nerve in a murine model of inflammatory bowel disease (*Ghia* et al., 2006).

In addition to suppressive effects on macrophages the vagus nerve also acts to regulate T cell function. *O'Mahony* et al. (2009) demonstrated that transfer of $CD4^+$ T cells from vagotomised donors into non-vagotomised with DSS induced colitis reduced the number of splenic $Foxp3^+$ regulatory T cells in recipient animals, and was associated with aggravated disease symptoms mimicking the effects of vagotomy on colitis. Subdiaphragmatic vagotomy leads to a dramatic increase in T cell proliferation and production of inflammatory cytokines when compared to cells from sham-operated animals (*Karimi* et al., 2010). The effect of vagotomy is not limited to the spleen as lymphocytes isolated from the mesenteric lymph nodes also demonstrated a significant increase in inflammatory cytokine production. Overall this data suggests that $CD4^+$ T cell are also under tonic inhibitory control from the vagus. This anti-inflammatory efferent response may, in certain circumstances play a

role in mediating the anti-depressive effects of vagal nerve stimulation.

Immune system dysfunction has also been linked to depression (*Connor* and *Leonard*, 1998; *Miller* et al., 2009; *Li* et al., 2011). Approximately one-third of people with depression, without co-morbid disease, have higher levels of inflammatory markers compared with the normal, non-depressed population. Furthermore, inflammatory illnesses are associated with greater rates of major depression while patients treated with cytokines for various illnesses are at increased risk of developing major depressive illness. Conversely, successful treatment with an antidepressant decreases levels of pro-inflammatory cytokines such as IL-6 and TNF (*Capuron* and *Dantzer*, 2003; *O'Brien* et al., 2007; *Hernandez* et al., 2008). While it is as yet unclear whether neurostimulation therapies for depression affect immune function, there is evidence in vagal nerve stimulation treated epilepsy patients that pro-inflammatory cytokine levels were reduced with successful treatment (*De Heerdt* et al., 2009; *Majoie* et al., 2011).

VAGUS AND THE GUT

Vagal primary afferents innervate the muscular and mucosal layers of the gut. They are 30,000 to 80,000 vagal afferent nerves (and as many spinal afferents) that supply the intestine with the ratio of afferent to efferent fibres in peripheral nerve bundles being 9:1 (*Mei*, 1983; *Blackshaw* et al., 2007). Vagal afferents innervate all of gut with the coeliac branch supplying the intestine from proximal duodenum to the distal part of the descending colon (*Wang* and *Powley*, 2007). Vagal innervation is densest proximally but still substantial for the colon. Histological and electrophysiological data reveal that visceral afferent chemosensitive endings are free endings (*Mei*, 1983) that express a large mixture of chemical and mechanosensitive receptors (*Blackshaw* et al., 2007).

Chemosensitive receptors are the targets of gut hormones and regulatory peptides such as ghrelin, CCK, GLP-1 and PYY(3–36) that activate vagal afferent neurons, whose terminals lie in the mucosa that can powerfully influence the control of food intake and regulation of energy balance (*Blackshaw* et al., 2007). Labelling experiments have identified vagal afferent fibres in the lamina propria of duodenal and jejunal villi and crypts of Lieberkühn, but they do not cross the basal membrane to innervate the epithelial layer (*Wang* and *Powley*, 2007). Thus, vagal afferents are not in a position to sense luminal nutrients directly, but are in close anatomical apposition to the basal membrane of entero-endocrine cells (*Li*, 2007).

Intraganglionic laminar vagal afferent endings (IGLEs) are located in the connective tissue capsule of myenteric plexus ganglia, between the longitudinal (outer) and circular (inner) muscle layers. These fibres respond to muscle tension generated by both passive stretch and active contraction of the muscle layers (*Berthoud* et al., 2001). This type of vagal afferent ending is found in large numbers throughout the oesophagus and gastrointestinal tract and is thought to be important for generating vagal afferent tone for balanced interoceptive awareness and emotional well-being.

Thus, given the key role of the vagus in communicating visceral signals to brain and particularly to neural circuitry associated with mood and anxiety it is perhaps not surprising that many investigations of communication between gut bacteria and the CNS have examined the role of the vagus. There is now strong evidence from animal studies that gut microorganisims can activate the vagus nerve and that such activation plays a critical role in mediating effects on the brain and subsequently, behaviour.

Such evidence came early from the study of animals infected with pathogens. Subdiaphragmatic vagotomy attenuated c-fos expression in the PVN of rats inoculated with *Salmonella typhimurium* (*Wang* et al., 2002). Although *S. typhimurium* infection was accompanied by intestinal inflammation subsequent studies have indicated that microorganisms in the gastrointestinal tract can directly activate neural pathways even in the absence of an identified immune response (*Goehler* et al., 2005). The anxiogenic effect of orally administered subclinical doses of *Campylobacter jejuni*, in mice was associated with a significant increase in c-Fos expression in neurons bilaterally in the vagal ganglia and activated visceral sensory nuclei in the brainstem. The areas of brainstem activation, the NTS and lateral parabrachial nucleus, participate in neural information processing that ultimately leads to autonomic neuroendocrine and behavioural responses (*Goehler* et al., 2005).

Non-pathogenic bacteria also activate vagal signalling from gut to brain. *Tanida* et al. (2005) demonstrated that intraduodenal injection of the bacterial strain *Lactobacillus johnsonii* La1 reduced renal sympathetic nerve activity and blood pressure while enhancing gastric vagal nerve activity. All of these effects could be abolished by pre-treatment with a histaminergic H3-receptor antagonist. Similarly the effects were absent in animals that had bilateral lesions of the hypothalamic suprachiasmatic nucleus, a major regulator of circadian rhythm. These findings suggest that the influence of the bacteria on autonomic neurotransmission and subsequently blood pressure is mediated centrally, likely through histaminergic nerves and the suprachiasmatic nucleus (*Tanida* et al., 2005).

Consequently, infradiaphragmatic denervation of vagal nerve fibres surrounding the oesophagus eliminated the ability of *L. johnsonii* La1 to reduce renal sympathetic nerve activity and blood pressure indicating that at least some of the effects of this bacterium on autonomic nerve responses were elicited by interaction with afferent vagal nerve fibres (*Tanida* et al., 2005).

Recently it was demonstrated that oral administration of a *L. rhamnosus* strain (JB1) could alter the normal behaviour of adult balb/c mice (*Bravo* et al., 2011). Chronic treatment with the bacteria reduced anxiety-like behaviour as assessed in an elevated plus maze and decreased the time spent immobile in a forced swim test. In addition, stress-induced plasma corticosterone levels were lower in treated mice a similar effect to subchronic or chronic treatment with antidepressants that can prevent forced swim stress-induced increases in plasma corticosterone in both mice and rats. Overall, changes induced with *L. rhamnousus* were indicative of reduced anxiety, and decreased depression-like behaviour Assessment of neural correlates to behavioural changes determined that mice receiving *L. rhamno-*

sus had alterations in central GABA receptor subunit mRNA expression. *L. rhamnosus* administration decreased expression of GABA type B (GABAB) subunit 1 isoform b (GABAB1b) mRNA in the amygdala and hippocampus, while increasing expression in cortical areas. Expression of GABAAα2 receptor mRNA was reduced in the amygdala and cortical areas, whereas levels were increased in the hippocampus (*Bravo* et al., 2011). It is difficult to attribute a causal relationship between behavioural effects observed and neural correlates. However, reduced expression of GABAB1b mRNA, in the amygdala, hippocampus, and locus ceruleus is consistent with the antidepressant-like effect of GABAB receptor antagonists (*Cryan* and *Slattery*, 2010) and with studies of GABAB1b-deficient animals, indicating an important role for this subunit in the development of cognitive processes, including those relevant to fear (*Jacobson* et al., 2007a, 23007b). It is also interesting to note that in a recent study of transcriptomes from the mucosa of the proximal small intestines of healthy human subjects following treatment with different lactobacillus species, there was a strong correspondence between *in vivo* transcriptional networks altered after consumption of one of the strains, *Lactobacillus casei*, and the response of human cells to the anxiolytic GABA A receptor modulator, Tracazolate (*van Baarlen* et al., 2011).

Subdiaphragmatic vagotomy blocked the anxiolytic and antidepressant effects of chronic *L. rhamnosus* ingestion in normal adult Balb/c mice while also preventing the associated alterations in GABAAα2 mRNA expression in the amygdala (*Bravo* et al., 2011). Similarly, the ability of *B. longum* to attenuate DSS colitis induced anxiety was abolished by vagotomy (*Bercik* et al., 2011a).

CONCLUSION AND FUTURE DIRECTIONS

Overall, studies indicate that vagal pathways mediate signals that can induce both anxiogenic and anxiolytic effects depending on the nature of the stimulus and, interestingly, the vagus appears to differentiate between non-pathogenic and potentially pathogenic bacteria even in the absence of overt inflammation.

It is therefore clear that the involvement of the vagus in microbiota-gut-brain communication is not straightforward or simply dependent on "activation". Assuming that an increase in c-fos expression always uniquely reflects an increase in neuronal firing rates, a dubious assumption, existing anatomical data cannot answer the question why in some cases vagal activation causes depression and in others, for example, electrical stimulation of the vagus, eases depression. What is currently lacking is relevant data on the electrophysiology of the system. In spite of our obvious lack of understanding of how probiotics encode vagal neural discharge, very little work has been published on a probiotic neural code. *Tanida* et al. (2005) showed that injecting *L. johnsonii* into rat duodenum increased gastric vagal multiunit firing rate by about 10% within 15 minutes, and this slowly grew to a 90% increase over the baseline 1 h after the injection was delivered. Clearly, much more work of this sort needs to be done and should compared with vagal responses to anxiogenic and anxiolytic peripheral stimuli.

Electrophysiology may also be utilized to determine the nature of the peripheral signal acting to stimulate the

vagus nerve in the gut following exposure to specific bacteria. Single chemosensitive vagal afferent units supplying the gut are normally silent or have a low resting discharge of 0-3 Hz (*Blackshaw* and *Grundy*, 1993). They respond to most luminal molecules by increasing their firing rate. Response latencies vary according to the chemical nature of the stimulus. The short chain fatty acid butyrate had a response onset latency of 2-3 ms (*Lal* et al., 2001), the long chain fatty acid sodium oleate had a latency of 15 ms (*Lal* et al., 2001), amino acids evoked responses within about 9 ms (*Mei*, 1983) the response to casein acid hydrolysate has a latency and of 19 ms (*Eastwood* et al., 1998), and glucose takes 20 ms (*Hardcastle* et al., 1978). *Salmonella typhimurium* lipopolysaccharide evoked an increase in the mesenteric nerve discharge with 30 min (*Liu* et al., 2009) while LPS from a commensal *E. coli* had no effect (*Liu* et al., 2009). Mesenteric vagal afferents are mainly unmyelinated fibres with conductions speeds of 0.7 m/s (*Cervero* and *Sharkey*, 1988), so the conduction from mucosa to the mesenteric recording electrode (less than 10 mm distance) would be $\leq$ 7 ms. Thus most of the response times could be attributed to mucosal endocrine cells sensing and transduction, transmission to adjacent vagal endings and generation of a suprathreshold neurite receptor potential.

Certainly, important advances in our understanding of the microbiome-gut-brain axis will come from studies of how distinct microbial stimuli activate the vagus and the nature of the signals transmitted to the brain that lead to differential changes in the neurochemistry of the brain and behaviour. However, while it appears that the vagus in critical to mediating gut-brain communication by specific bacteria in some model systems, it is by no means the only potential signalling method. Indeed, largely due to technical difficulties, few studies have investigated the role of spinal afferents in mediating bacteria induced changes in behaviour and brain chemistry. It is certainly possible that the observed changes in brain chemistry behaviour induced by gut bacteria require parallel input from both the vagal and spinal afferents. Furthermore, behavioural changes induced through disruption of the microbiota by antibiotic treatment have been demonstrated to be independent of vagal signalling (*Bercik* et al., 2011b) with some additional evidence that neither sympathetic afferents nor immune modulation is required. This clearly suggests that the bacteria in the gut can communicate to the brain through multiple pathways. A potential means of communication, that has been somewhat neglected in existing studies, involves direct hormonal signalling to the brain. Nevertheless understanding the induction and transmission of anxiolytic signals in the vagus nerve may have important implications for the development of microbial-based therapeutic strategies for mood disorders.

LITERATURE

Arborelius, L., Owens, M.J., Plotsky, P.M., and Nemeroff, C.B.: The role of corticotropin-releasing factor in depression and anxiety disorders. J. Endocrinol. 160, 1-12 (1999).

Aston-Jones, G., Ennis, M., Pieribone, V.A., Nickell, W.T., and Shipley, M.T.: The brain nucleus locus coeruleus: Restricted afferent control of a broad efferent network. Science 234, 734-737 (1986).

Aston-Jones, G., Rajkowski, J., Kubiak, P., Valentino, R.J., and Shipley, M.T.: Role of the locus coeruleus in emotional activa-

tion. Prog. Brain Res. 107, 379-402 (1996).

Bercik, P., Park, A.J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P.A., Fahnestock, M., Moine D., Berger, B., Huizinga, J.D., Kunze, W., McLean, P.G., Bergonzelli, G.E., Collins, S.M., and Verdu, E.F.: The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. Neurogastroenterol. Motil. 23, 1132-1139 (2011a).

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., and Collins, S.M.: The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141, 599-609 (2011b).

Berthoud, H.R., Lynn, P.A., and Blackshaw, L.A.: Vagal and spinal mechanosensors in the rat stomach and colon have multiple receptive fields. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280, R1371-R1381 (2001).

Biggio, F., Gorini, G., Utzeri, C., Olla, P., Marrosu, F., Mocchetti, I., and Follesa, P.: Chronic vagus nerve stimulation induces neuronal plasticity in the rat hippocampus. Int. J. Neuropsychopharmacol. 12, 1209-1221 (2009).

Blackshaw, L.A. and Grundy, D.: Effects of 5-hydroxytryptamine (5-HT) on the discharge of vagal mechanoreceptors and motility in the upper gastrointestinal tract of the ferret. J. Auton. Nerv. Syst. 45, 51-59 (1993).

Blackshaw, L.A., Brookes, S.J., Grundy, D., and Schemann, M.: Sensory transmission in the gastrointestinal tract. Neurogastroenterol. Motil. 19, 1-19 (2007).

Bluthe, R.M., Walter, V., Parnet, P., Laye, S., Lestage, J., Verrier, D., Poole, S., Stenning, B.E., Kelley, K.W., and Dantzer, R.: Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. C. R. Acad. Sci. III 317, 499-503 1994,.

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., and Tracey, K.J.: Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405, 458-462 (2000).

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA 108, 16050-16055 (2011).

Bret-Dibat, J.L., Bluthe, R.M., Kent, S., Kelley, K.W., and Dantzer, R.: Lipopolysaccharide and interleukin-1 depress food-motivated behavior in mice by a vagal-mediated mechanism. Brain Behav. Immun. 9, 242-246 (1995).

Browning, K.N. and Mendelowitz, D.: Musings on the wanderer: What's new in our understanding of vago-vagal reflexes? II. Integration of afferent signaling from the viscera by the nodose ganglia. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G8-G14 (2003).

Capuron, L. and Dantzer, R.: Cytokines and depression: The need for a new paradigm. Brain Behav. Immun. 17, Suppl. 1, S119-S124 (2003).

Cervero, F. and Sharkey, K.A.: An electrophysiological and anatomical study of intestinal afferent fibres in the rat. J. Physiol. 401, 381-397 (1988).

Connor, T.J. and Leonard, B.E.: Depression, stress and immunological activation: the role of cytokines in depressive disorders. Life Sci. 62, 583-606 (1998).

Crucian, G.P., Hughes, J.D., Barrett, A.M., Williamson, D.J., Bauer, R.M., Bowers, D., and Heilman, K.M.: Emotional and physiological responses to false feedback. Cortex 36, 623-647 (2000).

Cryan, J.F., Valentino, R.J., and Lucki, I.: Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci. Biobehav. Rev. 29, 547-569 (2005).

Cryan, J.F. and Slattery, D.A.: GABAB receptors and depression. Current status. Adv. Pharmacol. 58, 427-451 (2010).

Cunningham, J.T., Mifflin, S.W., Gould, G.G., and Frazer, A.: Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. Neuropsychopharmacology 33, 1884-1895 (2008).

Dantzer, R., Konsman, J.P., Bluthe, R.M., and Kelley, K.W.: Neural and humoral pathways of communication from the immune system to the brain: Parallel or convergent? Auton. Neurosci. 85, 60-65 2000.

De Herdt, V., Bogaert, S., Bracke, K.R., Raedt, R., De Vos, M., Vonck, K., and Boon, P.: Effects of vagus nerve stimulation on pro- and anti-inflammatory cytokine induction in patients with refractory epilepsy. J. Neuroimmunol. 214, 104-108 (2009).

Eastwood, C., Maubach, K., Kirkup, A.J., and Grundy, D.: The role of endogenous cholecystokinin in the sensory transduction of luminal nutrient signals in the rat jejunum. Neurosci. Lett. 254, 145-148 (1998).

Ghia, J.E., Blennerhassett, P., Kumar-Ondiveeran, H., Verdu, E.F., and Collins, S.M.: The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. Gastroenterology 131, 1122-1130 (2006).

Goehler, L.E., Gaykema, R.P., Opitz, N., Reddaway, R., Badr, N., and Lyte, M.: Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with *Campylobacter jejuni*. Brain Behav. Immun. 19, 334-344 (2005).

Hardcastle, J., Hardcastle, P.T., and Sanford, P.A.: Effect of actively transported hexoses on afferent nerve discharge from rat small intestine. J. Physiol. 285, 71-84 (1978).

Hernandez, M.E., Mendieta, D., Martinez-Fong, D., Loria, F., Moreno, J., Estrada, I., Bojalil, R., and Pavon, L.: Variations in circulating cytokine levels during 52 week course of treatment with SSRI for major depressive disorder. Eur. Neuropsychopharmacol. 18, 917-924 (2008).

Jacobson, L.H., Bettler, B., Kaupmann, K., and Cryan, J,F.: Behavioral evaluation of mice deficient in GABA(B(1)) receptor isoforms in tests of unconditioned anxiety. Psychopharmacology (Berl.) 190, 541-553 (2007a).

Jacobson, L.H., Kelly, P.H., Bettler, B., Kaupmann, K., and Cryan, J.F.: Specific roles of GABA(B(1)) receptor isoforms in cognition. Behav. Brain Res. 181, 158-162 (2007b).

Karimi, K., Bienenstock, J., Wang, L., and Forsythe, P.: The vagus nerve modulates CD4+ T cell activity. Brain Behav. Immun. 24, 316-323 (2010).

Konsman, J.P., Luheshi, G.N., Bluthe, R.M., and Dantzer, R.: The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. Eur. J. Neurosci. 12, 4434-4446 (2000).

Krahl, S.E., Senanayake, S.S., Pekary, A.E., and Sattin, A.: Vagus nerve stimulation (VNS) is effective in a rat model of antidepressant action. J. Psychiatr. Res. 38, 237-240 (2004).

Lal, S., Kirkup, A.J., Brunsden, A.M., Thompson, D.G., and Grundy, D.: Vagal afferent responses to fatty acids of different chain length in the rat. Am. J. Physiol. Gastrointest. Liver Physiol. 281, G907-G915 (2001).

Laye, S., Bluthe, R.M., Kent, S., Combe, C., Medina, C., Parnet, P., Kelley, K., and Dantzer, R.: Subdiaphragmatic vagotomy blocks induction of IL-1 beta mRNA in mice brain in response to peripheral LPS. Am. J. Physiol. 268, R1327-R1331 (1995).

Li, Y.: Sensory signal transduction in the vagal primary afferent neurons. Curr. Med. Chem. 14, 2554-2563 (2007).

Li, M., Soczynska, J.K., and Kennedy, S,H.: Inflammatory biomarkers in depression: An opportunity for novel therapeutic interventions. Curr. Psychiatry Rep. 13, 316-320 (2011).

Liu, C.Y., Mueller, M.H., Rogler, G., Grundy, D., and Kreis, M.E.: Differential afferent sensitivity to mucosal lipopolysaccharide from *Salmonella typhimurium* and *Esche-*

richia coli in the rat jejunum. Neurogastroenterol. Motil. 21, 1335-e129 (2009).

Luheshi, G.N., Bluthe, R.M., Rushforth, D., Mulcahy, N., Konsman, J.P., Goldbach, M., and Dantzer, R.: Vagotomy attenuates the behavioural but not the pyrogenic effects of interleukin-1 in rats. Auton. Neurosci. 85, 127-132 (2000).

Majoie, H.J., Rijkers, K., Berfelo, M.W., Hulsman, J.A., Myint, A., Schwarz, M., and Vles, J.S: Vagus nerve stimulation in refractory epilepsy: Effects on pro- and anti-inflammatory cytokines in peripheral blood. Neuroimmunomodulation 18, 52-56 (2011).

Martin, J.L., and Martin-Sanchez, E.: Systematic review and meta-analysis of vagus nerve stimulation in the treatment of depression: Variable results based on study designs. Eur. Psychiatry 27, 147-155 (2012).

Mei, N.: Recent studies on intestinal vagal afferent innervation. Functional implications. J. Auton. Nerv. Syst. 9, 199-206 (1983).

Miller, A.H., Maletic, V., and Raison, C,L.: Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732-741 (2009).

O'Brien, S.M., Scully, P., Fitzgerald, P., Scott, L.V., and Dinan, T,G.: Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. J. Psychiatr. Res. 41, 326-331 (2007).

O'Mahony, C., van der Kleij, H., Bienenstock, J., Shanahan, F., and O'Mahony, L.: Loss of vagal anti-inflammatory effect: In vivo visualization and adoptive transfer. Am. J. Physiol. Regul. Integr. Comp. Physiol. 297, R1118-R1126 (2009).

Rizvi, S.J., Donovan, M., Giacobbe, P., Placenza, F., Rotzinger, S., and Kennedy, S.H.: Neurostimulation therapies for treatment resistant depression: A focus on vagus nerve stimulation and deep brain stimulation. Int. Rev. Psychiatry 23, 424-436 (2011).

Tanida, M., Yamano, T., Maeda, K., Okumura, N., Fukushima, Y., and Nagai, K.: Effects of intraduodenal injection of *Lactobacillus johnsonii* La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. Neurosci. Lett. 389, 109-114 (2005).

van Baarlen, P., Troost, F., van der Meer, C., Hooiveld, G., Boekschoten, M., Brummer, R.J., and Kleerebezem, M.: Human mucosal *in vivo* transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. Proc. Natl. Acad. Sci. USA 108, Suppl. 1, 4562-4569 (2011).

Walsh, S.P. and Kling, M.A.: VNS and depression: Current status and future directions. Expert. Rev. Med. Devices 1, 155-160 (2004).

Wang, X., Wang, B.R., Zhang, X.J., Xu, Z., Ding, Y.Q., and Ju, G.: Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. World J. Gastroenterol. 8, 540-545 (2002).

Wang, F.B. and Powley, T.L.: Vagal innervation of intestines: Afferent pathways mapped with new en bloc horseradish peroxidase adaptation. Cell Tissue Res. 329, 221-230 (2007).

Zagon, A.: Does the vagus nerve mediate the sixth sense? Trends Neurosci. 24, 671-673 (2001).

Ziegler, D.R., Cass, W.A., and Herman, J.P.: Excitatory influence of the locus coeruleus in hypothalamic-pituitary-adrenocortical axis responses to stress. J. Neuroendocrinol. 11, 361-369 (1999).

MAPPING THE CONSEQUENCES OF METABOLIC INTERACTIONS BETWEEN HOST AND MICROBIOME ON THE BRAIN

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INTRODUCTION

The gut microbiome, defined by Joshua Lederberg as the sum of the bacterial genomes and their environmental interactions (*Lederberg,* 2001), acts as an extra organ in the mammalian host with a vast capacity for metabolism of chemicals deriving from endogenous mammalian metabolism, nutrients and other xenobiotics. This surrogate organ not only functions to shape the immune system and harvest energy, but is also capable of direct chemical communication and contributes to a wide range of signalling pathways that connect various organs and tissues throughout the body (*Cryan* and *O'Mahony,* 2011; *Nicholson* et al., 2012). The communication between the gut and the brain, the so called 'gut-brain axis' is a critical factor in many physiological and pathological processes. In addition to direct interaction between the brain and enteric nervous system in the gut via the vagus nerve, a great deal of chemical communication occurs and many of the chemicals produced by the gut microbiota are potentially neuroactive including γ-amino butyric acid (GABA), 5-hydroxytryptophan and indoxyl compounds (*Wikoff* et al., 2009; *Barrett* et al., 2012). Conversely the brain can modulate the behaviour of the microbiota via mechanisms such as stimulation of the endochromaffin cells. This review addresses the influence of the gut microbiota on the brain and central nervous system (CNS) and explores their consequent impact on metabolism under various physiological and pathological conditions.

METABOLIC SIGNATURE OF AUTISM: CONTRIBUTIONS FROM THE MICROBIOME

Autism spectrum disorder (ASD) represents a group of developmental conditions characterised by dysfunction in verbal and non-verbal communication and social interactions and is associated with repetitive behaviour and difficulty in imaginative play in children. First described by Kanner in 1943, autism typically manifests during the first three years of life and is part of the Pervasive Developmental Disorders (PDD) family. It is diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and there are several clinical scoring scales used for establishing the severity and type of autism (*Faras* et al., 2010). A recent review reports that whilst the conservative estimate of autistic spectrum disorder prevalence is 27.5 per 10,000 individuals, the real prevalence estimate based on newer surveys is 60 per 10,000 individuals, with a 4:1 male-to-female ratio (*Faras* et al., 2010), thus autism is a growing clinical, social and financial concern.

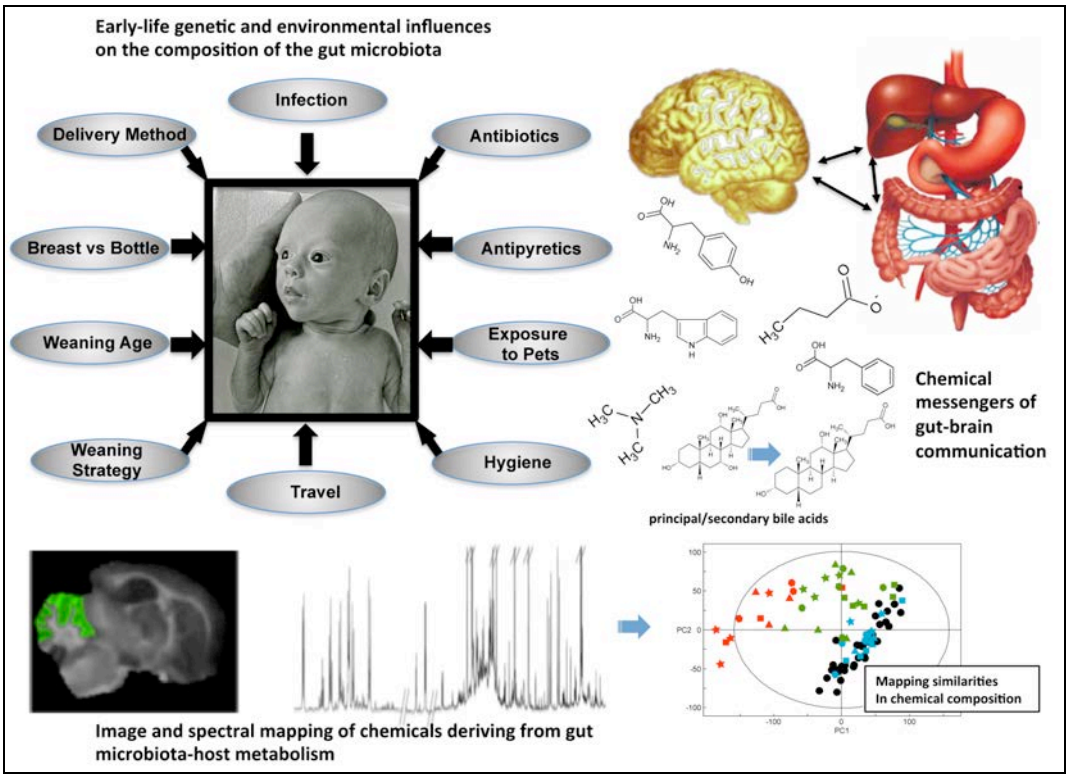

Figure 1: The early life environment influences the composition of the gut microbiota with consequent risk or protection for later-life disease. One of the ways in which the brain and gut are inextricably linked is through chemical dialogue between the gut microbiota and the brain in the form of small molecule messengers such as bile acids, biogenic amines, products of choline degradation and short chain fatty acids. These molecules can be detected in the NMR and MS profiles of urine, plasma, cerebrospinal fluid and other biofluids. The distribution of these chemicals can also be mapped in the brain in order to ascertain their topographical distribution. Computational modelling can be used to efficiently extract and visualise information relating to gut-brain communication and to unravel the profound dependence of humans on their microbiota.

Clinicians, scientists and parents of autistic children have proposed multiple theories regarding the causes of autism. However, only limited clinical data are available and in some cases the data are contradictory and/or inconclusive. Numerous aetiological hypotheses have been postulated including: *In utero* exposure to chemicals such as pesticides, genetic causes (particular association with the X chromosome), oxidative stress, measles, mumps and rubella (MMR) vaccination or its thiomersal carrier, advanced maternal age, planned caesarean section, low Apgar scores, hyperbilirubinaemia and birth defects (*Duchan* et al., 2012; *Guinchat* et al., 2012). Inflammation has also been associated with autism and could be mediated by a defective placenta, immature blood-brain barrier, the immune response of the mother to infection while pregnant, a premature birth, encephalitis in the child after birth, or a toxic environment either *in utero* or in the first few months of life (*Faras* et al., 2010). Although some of the theories on the causes of autism, such as the MMR vaccine, have been largely discredited, it is evident that ASD repre-

sents a complex set of interrelated conditions and is likely to originate from a highly multifactorial aetiology. This is further supported by the vast array of co-morbidities that typically accompany autism, in particular gastrointestinal dysfunction. Understanding the role of the gut microbiota in autism would undoubtedly offer new therapeutic avenues, regardless of whether the intestinal dysfunction is causal or merely coincidental.

The compositional landscape and functionality of the gut microbiota are determined by a sequence of factors beginning at or before birth. The first large-scale exposure to bacteria occurs during the birth process and continues to be modulated by subsequent exposure to the external environment, feeding method and early life infections. Whilst the early microbiotal composition is dynamic, this plasticity diminishes over time to form a relatively stable microbiome that persists throughout adulthood. However, it is thought that these early influences on the microbiota can persist and influence disease risk later in life. For example preterm infants have a higher risk of cardiovascular disease, pulmonary disease, metabolic diseases and several psychological and neurodevelopmental conditions including autism (*Rogers* and *Velten*, 2011). Metabolic profiling of biofluids and tissues can detect changes in microbial activity via profiling or imaging of microbial products such as cresols, biogenic amines, short chain fatty acids etc. (Figure 1), and represents one means of understanding the relationship between the gut and the brain.

Gastrointestinal (GI) dysfunction is reported in a high percentage of children diagnosed with autism with reported symptoms ranging from diarrhoea to constipation and abdominal bloating and pain. In one study, intestinal permeability was found to be higher in individuals with ASD (36.7%) in comparison with healthy controls (4.8%), whereas ASD participants on a casein free diet did not show this increase in intestinal permeability (*de Magistris* et al., 2012). Interestingly, family members of autistic children also showed increased intestinal permeability. Given the prevalence of GI dysfunction in children with ASD, there has been growing interest in the role of the gut microbiota, specifically bacterial populations such as the Clostridia, which are known to produce toxins and often flourish in a perturbed gut ecosystem. However, as yet the literature contains few examples of studies in which the microbiome of autistic individuals has been systematically characterised and those that have been reported suffer from a low sample size and poor comparison between autistic children with and without GI dysfunction (*Critchfield* et al., 2011). Williams and colleagues showed in a study of the mucosal biopsies of 22 children with autism that disaccharidases and hexose transporters were deficient and that this deficiency correlated with the intestinal transcription factor caudal type homeobox 2 (CDX2) that regulates transporters of glucose and other sugars (*Williams* et al., 2011). This change in transcription factors was correlated with the bacterial profiles of the mucosal samples that showed an increase in the *Firmicute:Bacteroidete* ratio and increased numbers of Betaproteobacteria.

A series of relatively small scale studies have demonstrated that autistic individuals harbour a distinctive faecal microflora compared with that of healthy children (*Parracho* et al., 2005; *Finegold* et al., 2010), with autistic children carrying greater numbers of bacteria of the *Clostridium histolyticum* group (*Parracho* et al., 2005) and *Desulfovibrio* (*Finegold* et al., 2010)

and manifesting a shift at the phylum level towards an increased *Bacteroidetes:Firmicute* ratio (*Finegold* et al., 2010). Other bacteria found in high concentrations in the epithelial mucosa of children with ASD associated GI symptoms are the *Sutterella* genus, predominantly *Sutterella wadsworthensis* and *Sutterella stercoricanis* (*Williams* et al., 2012).

Metabolic profiling studies have also generated indirect evidence of a shift in clostridial species. Urinary excretion of 4-cresyl sulphate is increased in autistic children, and to a lesser extent their siblings (*Yap* et al., 2010). 4-Cresol is a gut microbial metabolite that can be synthesized by several bacteria including *Clostridium difficile* and *Clostridium scatalogens*, and which subsequently undergoes phase II metabolism to the sulphate conjugate with minor amounts of the glucuronide conjugate formed. Children with ASD have also been found to have higher urinary levels of another clostridial metabolite, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), which is thought to be a metabolite of the tyrosine analogue 3-hydroxyphenylalanine (*Shaw,* 2010). This metabolite was also found in high levels in the urine of an adult that had experienced repeated *C. difficile* infections (*Shaw,* 2010).

Other metabolites that characterize the urine of autistic children include bacterial products of choline degradation such as methylamine and dimethylamine, hippurate, phenylacetylglutamine, reinforcing the possible involvement of the microbiota in autism. In contrast, Lis and colleagues. reported lower urinary levels of hippurate and 4-hydroxyhippurate in autistic children compared to age matched controls, as measured by ion exchange chromatography (*Lis* et al., 1976). Thus the relationship between autism and the gut microbiota is not straightforward, like most other aspects of ASD.

As with the role of the gut microbiota, the involvement of oxidative stress in the aetiology of ASD is similarly ambiguous. Both oxidized glutathione and the ratio of oxidized to reduced glutathione are increased in autism (*Ghanizadeh* et al., 2012; *Frustaci* et al., 2012), whilst blood levels of reduced glutathione, glutathione peroxidase and sulphur-containing amino acids cysteine and methionine were found to be reduced in a meta analysis of ASD (*Frustaci* et al., 2012). There is some evidence that dietary supplementation with glutathione may improve the levels of trans-sulphuration metabolites (*Kern* et al., 2011).

Low levels of reduced plasma glutathione and sulphur-containing amino acids are consistent with the observation that autistic children are deficient in sulphation capacity (*Alberti* et al., 1999), thus any intervention that challenges the already impaired capacity for sulphation has the potential to compound the expression of ASD. Acetaminophen use in children has been suggested as a trigger for autism via activation of the endocannabinoid system, which is known to be capable of modulating brain function during development (*Schultz*, 2010). The primary route of acetaminophen excretion in children is sulphation. In autistic children that are already sulphur deficient, acetaminophen could further deplete already low sulphur pools with subsequent impact on the glutathione pathway. Interestingly the metabolic fate of acetaminophen can be predicted from pre-dose urinary profiles and the most predictive metabolite for high acetaminophen sulphate excretion is 4-cresyl sulphate (*Clayton* et al., 2009), the synthesis of which has been largely attributed to *Clostridium difficile* and *C. scatalogens*. Thus it is possible that the gut microbiota contribute to the deple-

tion of sulphate pools in autistic children by generating phenols and other chemicals that undergo sulphation prior to excretion.

In conclusion, the role of the gut microbiota in ASD is far from clear. Various studies show differential microbial profiles between autistic and healthy children, and the presence of various GI-related co-morbidities points to an association between the microbiome and ASD. The fact that children with ASD tend towards food sensitivity and are typically on numerous therapeutic regimes including controlled diets, chelation agents and a cocktail of drugs such as ariprazole, altrexone, buspirone, divalproex sodium, lamotrigine, levetiracetam, memantine, mirtazapine, riluzole, pioglitazone, and topiramate (*Doyle and McDougle*, 2012) confounds the picture as these agents may in themselves induce a change in the gut microbiota. This underscores the requirement for high quality, systematic and controlled studies to be carried out in order to ascertain the true relationship between the gut microbiota and ASD.

INFECTION-INDUCED DISRUPTION OF THE GUT MICROBIOTA-HOST METABOLIC INTERFACE

Both chronic and acute infections have been shown to have profound impact on the gut microbiome. Reduced cognitive function in children in developing countries has been correlated with repeated incidences of infection causing diarrhoea (*Oria* et al., 2009). Evidence of both direct and indirect influence of the microbiota on the central nervous system (CNS) can be found in the literature. For example, increased anxiety, brought about through viscerosensory signalling from the gastrointestinal tract, can be induced in mice by experimental infection with *Campylobacter jejuni* (*Goehler* et al., 2007).

The hygiene hypothesis has lead to the association of certain autoimmune diseases with infection and bacteria. The onset of these diseases has been linked to bacterially driven immune responses. In an animal model of multiple sclerosis, it has been shown that commensal gut microbiota are necessary for initiating immune responses that result in myelin-specific CD4(+) T cells attacking the CNS (*Berer* et al., 2011).

Examples in both the insect and in the human world provide evidence that parasitic infection can alter behaviour. Parasitic hairworms manipulate their cricket host by inducing suicide behaviour, causing the insect to leap into water, where the parasite continues its life cycle (*Ponton* et al., 2011). Epidemiological studies have found associations between certain helminths, including *Schistosoma mansoni, Ascaris lumbracoides* and *Trichuris trichiura,* and cognitive impairment in school age children (*Jardim-Botelho* et al., 2008; *Shang* and *Tang*, 2010) but the mechanism is unknown. Direct contact of a parasite with the CNS can result in overt histological and biochemical changes. Most prominent examples are cerebral manifestations of malaria, due to malaria toxin excretion, excess cytokine secretion and billharziosis-induced granuloma formation caused by sequestration of Schistosome erythrocytes. Serendipitous ectopic worm manifestations in the brain by cestodes, trematodes and protozoa have also been reported to cause lesions and haemorrhages (*Walker* et al., 2005). However, for most host-parasite interactions involving neurological damage or altered behaviour, the molecular mechanisms

are not well understood.

There is some suggestion that certain changes in brain biochemistry following helminth infection may be associated with dysregulation of the microbiota. The parasitology literature contains several examples of infection-induced disturbances of the microbiota (*Wang* et al., 2010; *Li* et al., 2012). Many parasites ranging from *Giardia* to hookworm are associated with gastrointestinal disturbances. It is unsurprising that parasites that reside in the gut should impact upon the microbiota, since they share the same physical environment. However, studies in animal models have shown that several parasites, some of which reside in the blood or in non-gastrointestinal tissues, can modulate the excretion of gut microbial metabolites.

Parasite infections have been shown to perturb several metabolites of the gut microbiota in various animal models and in humans (*Wang* et al., 2004, 2010). Metabolic profiling studies in humans and animal models have shown modulation of urinary gut microbial metabolites including hippurate, 4-cresyl sulphate and glucuronide, phenylacetyl glycine / glutamine, 4-hydroxyphenylacetic acid and 4-hydroxy-3-methy-phenylpropionate acid (*Wang* et al, 2004, *Wang* et al, 2010; *Balog* et al, 2011). In a study investigating the metabolic effects of a foodborne trematode, *Fasciola hepatica*, in a rat model, clear evidence of altered gut microbial metabolism was found in both the urine (decreased hippurate levels and modified bacterial products of choline degradation) and faecal metabolite profiles and, more interestingly, these changes correlated with altered brain biochemistry following infection. Significant perturbations of the nucleotide balance in the brain suggested a shift toward modulation of immune reactions, which could serve to prolong the camouflage of the parasite within the host via minimization of inflammatory damage (*Saric* et al., 2010).

Thus, it seems that there is a complex tripartite interaction between host, parasite and microbiota mediated by both immunoregulatory mechanisms and direct chemical communication between microbe and host. Moreover, this three-way relationship has system-wide impact, including neurological consequences and warrants deeper interrogation.

METABOLIC EFFECTS OF THE GUT MICROBIOTA IN RELATION TO IMPACT ON MOOD AND BEHAVIOUR

There is a growing body of evidence associating depression and other psychiatric disorders with perturbed gut microbiota. The cytokine hypothesis of depression suggests that increased translocation of LPS from Gram-negative bacteria and inflammation are causally associated with clinical depression and neurodegeneration (*Qin* et al., 2007; *Maes*, 2008). In another stress-induced rat model of depression associated with leaky gut, the administration of *Lactobacillus farciminis* was shown to reverse the stress-induced hyperpermeability and behavioural changes (*Ait-Belgnaoui* et al., 2012) In addition to producing LPS and other neurotoxic chemicals, the gut bacteria can synthesize metabolites such as pyrogallol and urolithins from dietary polyphenols that are neuroprotective and can counteract the effects of diabetes-induced neurodegeneration (*Verzelloni* et al., 2011).

Gut microbial metabolites found in urine and other biofluids in animal and

human studies of depression also point to the involvement of the gut bacteria in behaviour. An LC-MS profiling study of a rat model of depression found elevated urinary levels of kynurenic acid, hippurate, phenylacetylglycine and xanthurenic acid with lower levels of tryptophan, indoxyl sulphate, indole-3-acetate and tricarboxylic acid cycle intermediates in rats challenged by chronic unpredictable mild stress suggesting that gut microbial metabolism and modulation of the TCA cycle were key components of this model of depression (*Zheng* et al., 2009). Interferon-α administration has been associated with depressive disorders in cancer or hepatitis-C patients treated with interferon-α (*Raison* et al., 2006). Interferon-α induced activation of the tryptophan degrading enzyme indoleamine 2,3-dioxygenase results in the generation of quinolinic acid and other neuroactive metabolites, which are thought to relate to depression. In a bacterially-induced animal model of depression, induced by inoculation with Bacillus Calmette-Guérin (BCG), administration of indoleamine 2,3-dioxygenase resulted in increased plasma concentrations of the kynurenine:tryptophan ratio (*O'Connor* et al., 2009).

Examples of the effect of microbiota on cognition are scattered throughout the literature. As previously mentioned, repeated diarrhoeal infections have been associated with detrimental effects on cognition in children. Similarly cirrhotic patients with encephalopathy show poor cognition, which is correlated with *Veillonellaceae* in faecal material (*Bajaj* et al., 2012). Higher faecal levels of *Enterobacteriaceae, Alcaligeneceae* and *Fusobacteriaceae* with lower levels of *Ruminococcaceae* and *Lachnospiraceae* were also found in cirrhotic patients.

There is increasing acceptance of the fact that surgery *per se* can impose short-term or long-term consequences on mood, behaviour and cognition. Several analyses of patients undergoing coronary artery bypass graft surgery (CABG) have indicated that neurological complications can include stroke, depression or mild cognitive decline reflected by short-term memory loss or psychomotor slowing (*Hawkes* et al., 2006). The cause of these neurological effects is not well understood but surgical trauma, genetic susceptibility, interoperative or post-operative ischaemia and body temperature during surgery have all been proposed as causal factors (*Hawkes* et al., 2006). Probiotics and synbiotics (a combination of pre- and probiotics) have been used to reduce the incidence of post-operative sepsis after elective surgery and to counteract the effect of antibiotics, typically administered as part of surgical procedures (*Kinross* et al., 2012). Prebiotics have also been shown to influence anxiety and mood. For example, Silk and colleagues showed that administration of a trans-galacto-oligosaccharide enhanced faecal bifidobacteria and improved anxiety and depression symptoms in patients with irritable bowel syndrome (*Silk* et al., 2009). Similarly probiotics such as lactobacilli have been reported to regulate emotional behaviour in mice through vagus nerve stimulated modulation of GABA receptor expression (*Bravo* et al., 2011).

Obesity has been associated with poor cognitive function, particularly in executive function (*Lokken* et al., 2010). There is controversy regarding the effect of weight loss surgery (bariatric surgery) on cognition and memory with some suggestion that bariatric surgery can cause memory impairment and depression. An animal model of gastric restriction was able to show structural alterations in the hippocampus (*Sonoda* et al., 2011). The mecha-

nisms of bariatric surgery-induced weight loss are known to involve alteration of gastrointestinal hormones, which are important in appetite regulation. Several gastrointestinal hormones can contribute to obesity by modulating the activity of the gut-brain axis. Recently we have characterized the metabolic response of rats undergoing RYGB surgery using high resolution spectroscopic profiling and integrated the response with the post surgical change in the microbiome and shown a dramatic shift towards the gammaproteobacteria. The surgery also resulted in a modulation of gut microbial metabolites, some of which were neuroactive. Increased urinary cresols, phenylacetylglycine and indoxylsulfate occurred post surgery together with increased production of γ-aminobutyric acid (GABA) (*Li* et al., 2011).

CONCLUDING REMARKS

The manner in which the gut bacteria influence the CNS largely remains elusive and merits systematic study. The gut and brain use several communication axes involving nerve stimulation, immunological correspondence and direct metabolic interactions in order to maintain homeostasis. The gut microbiota contribute to the communication highway between the gut and the brain and there is enough evidence to implicate the microbiota in various neurodegenerative diseases, behavioural disorders, neurodevelopmental conditions and cognition. The partnership between culture independent sequencing of the microbiome, the human genome and metabolic profiling of the microbial components of biofluids should help to elucidate the complex interactions of host and microbe and to place this interaction in terms of regulation/ dysregulation of the CNS. Understanding this partnership will open new avenues for therapeutic intervention and opportunities for improving disease risk by modulation of the microbiota early in life or even prenatally.

LITERATURE

Alberti, A., Pirrone, P., Elia, M., Waring, R.H., and Romano, C.: Sulphation deficit in "low-functioning" autistic children: A pilot study. Biol. Psychiatry 46, 420-424 (1999).

Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., Houdeau, E., Fioramonti, J., Bueno, L., and Theodorou, V.: Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 37, 1885-1895 (2012).

Bajaj, J.S., Ridlon, J.M., Hylemon, P.B., Thacker, L.R., Heuman, D.M., Smith, S., Sikaroodi, M., and Gillevet, P.M.: Linkage of gut microbiome with cognition in hepatic encephalopathy. Am. J. Physiol. Gastrointest. Liver Physiol. 302, G168-G175 (2012).

Balog, C.I., Meissner, A., Goraler, S., Bladergroen, M.R., Vennervald, B.J., Mayboroda, O.A., and Deelder, A.M.: Metabonomic investigation of human *Schistosoma mansoni* infection. Mol. Biosyst. 7, 1473-1480 (2011).

Barrett, E., Ross, R.P., O'Toole, P.W., Fitzgerald, G.F., and Stanton, C.: Gamma-aminobutyric acid production by culturable bacteria from the human intestine. J. Appl. Microbiol. 113, 411-417 (2012).

Berer, K., Mues, M., Koutrolos, M., Rasbi,

Z.A., Boziki, M., Johner, C., Wekerle, H., and Krishnamoorthy, G.: Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature 479, 538-541 (2011).

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J. and Cryan, J.F.: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA 108, 16050-16055 (2011).

Clayton, T.A., Baker, D., Lindon, J.C., Everett, J.R., and Nicholson, J.K.: Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. Proc. Natl. Acad. Sci. USA 106, 14728-14733 (2009).

Critchfield, J.W., van Hemert, S., Ash, M., Mulder, L., and Ashwood, P.: The potential role of probiotics in the management of childhood autism spectrum disorders. Gastroenterol. Res. Pract. 2011:161358, Epub (2011).

Cryan, J.F. and O'Mahony, S.M.: The microbiome-gut-brain axis: From bowel to behavior. Neurogastroenterol. Motil. 23, 187-192 (2011).

da Mota, F.F., Marinho, L.P., Moreira, C.J., Lima, M.M., Mello, C.B., Garcia, E.S., Carels, N., and Azambuja, P.: Cultivation-independent methods reveal differences among bacterial gut microbiota in triatomine vectors of Chagas disease. PLoS Negl. Trop. Dis. 6, e1631 (2012).

de Magistris, L., Familiari, V., Pascotto, A., Sapone, A., Frolli, A., Iardino, P., Carteni, M., De Rosa, M., Francavilla, R., Riegler, G., Militerni, R., and Bravaccio, C.: Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J. Pediatr. Gastroenterol. Nutr. 51, 418-424 (2010).

Doyle, C.A. and McDougle, C.J.: Pharmacotherapy to control behavioral symptoms in children with autism. Expert. Opin. Pharmacother. 13, 1615-1629 (2012).

Duchan, E. and Patel, D.R.: Epidemiology of autism spectrum disorders. Pediatr. Clin. North Am. 59, 27-43 (2012).

Faras, H., Al Ateeqi, N., and Tidmarsh, L.: Autism spectrum disorders. Ann. Saudi Med. 30, 295-300 (2010).

Finegold, S.M., Dowd, S.E., Gontcharova, V., Liu, C., Henley, K.E., Wolcott, R.D., Youn, E., Summanen, P.H., Granpeesheh, D., Dixon, D., Liu, M., Molitoris, D.R., and Green, J.A. 3rd.: Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16, 444–453 (2010).

Frustaci, A., Neri, M., Cesario, A., Adams, J.B., Domenici, E., Dalla Bernardina, B., and Bonassi, S.: Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. Free Radic. Biol. Med. 52, 2128-2141 (2012).

Ghanizadeh, A., Akhondzadeh, S., Hormozi, M.A, Abotorabi, M., and Firoozabadi, A.: Glutathione-related factors and oxidative stress in autism, a review. Curr. Med. Chem. 19, 4000-4005 (2012).

Goehler, L.E., Lyte, M., and Gaykema, R.P.: Infection-induced viscerosensory signals from the gut enhance anxiety: implications for psychoneuroimmunology. Brain Behav. Immun. 21, 721-726 (2007).

Guinchat, V., Thorsen, P., Laurent, C., Cans, C., Bodeau, N., and Cohen, D.: Pre-, peri- and neonatal risk factors for autism. Acta Obstet. Gynecol. Scand. 91, 287-300 (2012).

Hawkes, A.L., Nowak, M., Bidstrup, B., and Speare, R.: Outcomes of coronary artery bypass graft surgery. Vasc. Health Risk Manag. 2, 477-484 (2006).

Jardim-Botelho, A., Raff, S., Rodrigues, R.A, Hoffman, H.J., Diemert, D.J., Correa-Oliveira, R., Bethony, J.M., and Gazzinelli, M.F.: Hookworm, *Ascaris lumbricoides* infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. Trop. Med. Int. Health 13, 994-1004 (2008).

Kern, J.K., Geier, D.A., Adams, J.B., Garver, C.R., Audhya, T., and Geier, M.R.: A clinical trial of glutathione supplementation in

autism spectrum disorders. Med. Sci. Monit. 17, CR677-682 (2011).

Kinross, J.M., Marakar, S., Karthikesalingam, A., Chow, A., Penney, N., Silk, D., and Darzi, A.: A meta-analysis of probiotic and synbiotic use in elective surgery: Does nutrition modulation of the gut microbiome improve clinical outcome? JPEN J. Parenter. Enteral Nutr. 2012 Jul 10, Epub ahead of print (2012).

Lederberg, J. and McCray, A.T.: 'Ome Sweet 'Omics—a genealogical treasury of words. The Scientist 17, No. 7 (2001).

Li, J.V., Ashrafian, H., Bueter, M., Kinross, J., Sands, C., le Roux, C.W., Bloom, S.R., Darzi, A., Athanasiou, T., Marchesi, J.R., Nicholson, J.K., and Holmes, E.: Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. Gut 60, 1214-1223 (2011).

Li, R.W., Wu, S., Li, W., Navarro, K., Couch, R.D., Hill, D., and Urban, J.F. Jr.: Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. Infect. Immun. 80, 2150-2157 (2012).

Lis, A.W., Mclaughlin, I., Mclaughlin, R.K., Lis, E.W., and Stubbs, E.G.: Profiles of ultraviolet-absorbing components of urine from autistic children, as obtained by high-resolution ion-exchange chromatography. Clin. Chem. 22, 1528-1532 (1976).

Lokken, K.L., Boeka, A.G., Yellumahanthi, K., Wesley, M., and Clements, R.H.: Cognitive performance of morbidly obese patients seeking bariatric surgery. Am. Surg. 76, 55-59 (2010).

Maes, M.: The cytokine hypothesis of depression: Inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro Endocrinol. Lett. 29, 287-291 (2008).

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. Host-gut microbiota metabolic interactions. Science 336,1262-1267 (2012).

O'Connor, J.C., Lawson, M.A., Andre, C., Briley, E.M., Szegedi, S.S., Lestage, J., Castanon, N., Herkenham, M., Dantzer, R., and Kelley, K.W.: Induction of IDO by bacille Calmette-Guérin is responsible for development of murine depressive-like behavior. J. Immunol. 182, 3202-3212 (2009).

Oria, R.B., Costa, C.M., Lima, A.A., Patrick, P.D., and Guerrant, R.L.: Semantic fluency: A sensitive marker for cognitive impairment in children with heavy diarrhea burdens? Med. Hypotheses 73, 682-686 (2009).

Parracho, H.M.R.T., Bingham, M.O., Gibson, G.R., and McCartney, A.L.: Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J. Med. Microbiol. 54, 987-991 (2005).

Ponton, F., Otalora-Luna, F., Lefevre, T., Guerin, P.M., Lebarbenchon, C., Duneau, D., Biron, D.G., and Thomas, F.: Water-seeking behavior in worm-infected crickets and reversibility of parasitic manipulation. Behav. Ecol. 22, 392-400 (2011).

Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., and Crews, F.T.: Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55, 453-462 (2007).

Rogers, L.K. and Velten, M.: Maternal inflammation, growth retardation, and preterm birth: Insights into adult cardiovascular disease. Life Sci. 89, 417-421 (2011).

Saric, J., Li, J.V., Utzinger, J., Wang, Y., Keiser, J., Dirnhofer, S., Beckonert, O., Sharabiani, M.T., Fonville, J.M., Nicholson, J.K., and Holmes, E.: Systems parasitology: Effects of *Fasciola hepatica* on the neurochemical profile in the rat brain. Mol. Syst. Biol. 6, 396 (2010).

Shang, Y. and Tang, L.H.: Intelligence level and characteristics of cognitive structure in school-age children infected with soil-transmitted helminthes. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 28, 423-426 (2010).

Shaw, W.: Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine

metabolite of Clostridia spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. Nutr. Neurosci. 13, 135-143 (2010).

Schultz, S.T.: Can autism be triggered by acetaminophen activation of the endocannabinoid system? Acta Neurobiol. Exp. (Wars) 70, 227-231 (2010).

Silk, D.B., Davis, A., Vulevic, J., Tzortzis, G., and Gibson, G.R.: Clinical trial: The effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. Aliment. Pharmacol. Ther. 29, 508-518 (2009).

Sonoda, E.Y., Gomes da Silva, S., Arida, R.M., Giglio, P.N., Margarido, N.F., Martinez, C.A., Pansani, A.P., Maciel Rde, S., Cavalheiro, E.A., and Scorza, F.A.: Hippocampal plasticity in rats submitted to a gastric restrictive procedure. Nutr. Neurosci. 14, 181-185 (2011).

Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S., Calani, L., Costa, L.G., Brighenti, F., Borges, G., Crozier, A., Conte, A., and Del Rio, D.: Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. Mol. Nutr. Food Res. 55, Suppl. 1, S35-S43 (2011).

Walker, M.D. and Zunt J.R.: Neuroparasitic Infections: Cestodes, Trematodes, and Protozoans. Semin. Neurol. 25, 262-277 (2005).

Wang, Y., Holmes, E., Nicholson, J.K., Cloarec, O., Chollet, J., Tanner, M., Singer, B.H., and Utzinger, J.: Metabonomic investigations in mice infected with *Schistosoma mansoni*: An approach for biomarker identification Proc. Natl. Acad. Sci. USA 101, 12676-12681 (2004).

Wang, Y., Li, J.V., Saric, J., Keiser, J., Wu, J., Utzinger, J., and Holmes, E.: Advances in metabolic profiling of experimental nematode and trematode infections. Adv. Parasitol. 73, 373-404 (2010).

Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C., and Siuzdak, G.: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. USA 106, 3698-3703 (2009).

Williams, B.L., Hornig, M., Buie, T., Bauman, M.L., Cho Paik, M., Wick, I., Bennett, A., Jabado, O., Hirschberg, D.L., and Lipkin, W.I.: Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One. 6, e24585 (2011).

Williams, B.L., Hornig, M., Parekh, T., and Lipkin, W.I.: Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio. 3, e00261-11 (2012).

Yap, I.K., Angley, M., Veselkov, K.A., Holmes, E., Lindon, J.C., and Nicholson, J.K.: Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. J. Proteome Res. 9, 2996-3004 (2010).

Zheng, S., Yu, M., Lu, X., Huo, T., Ge, L., Yang, J., Wu, C., Li, F.: Urinary metabonomic study on biochemical changes in chronic unpredictable mild stress model of depression. Clin. Chim. Acta 411, 204-209 (2010).

MICROBIAL "OLD FRIENDS", IMMUNOREGULATION AND PSYCHIATRIC DISORDERS

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SUMMARY

Many diseases are increasing in prevalence in urban communities in developed countries. Immigrants are also at increased disease risk, particularly if they move from a poor to a wealthy country during infancy. Moreover, the prevalence of many of these conditions increases further in second-generation immigrants, suggesting that exposure (or lack of exposure) to critical influences during pregnancy and infancy may play an important role in conferring risk for many chronic diseases common in the modern world. The diversity of diseases involved is remarkable. They include chronic inflammatory disorders (autoimmunity, allergy and inflammatory bowel disease), many cancers, (such as prostate, colorectal and various childhood cancers) and a range of psychiatric disorders including depression, disorders precipitated by psychosocial stressors or low socioeconomic status, and disorders with a developmental component such as schizophrenia and autism. Here we explore important parallels among the increases in these diverse types of disease. We merge the immunological explanation (hygiene, or "Old Friends" hypothesis) with psychosocial explanations, and suggest that there are underlying mechanisms, many involving the gut microbiota, that are relevant to *all* these disorders and that transcend the boundaries between traditional medical disciplines.

INTRODUCTION

Many diseases are increasing in incidence in developed countries. These increases tend to be greatest in urban communities. Immigrants are also especially vulnerable to these diseases, particularly if they move from a poor to a wealthy country during infancy, suggesting that infancy provides a critical window for the influence of factors that are protective in poor countries, or detrimental in rich ones. Remarkably, the incidence of many of these conditions increases further in second-generation immigrants, suggesting that exposure (or lack of exposure) to critical influences during pregnancy may play an important role in conferring risk for many diseases common in the modern world. The most remarkable aspect of this problem is the diversity of diseases

involved. These include chronic inflammatory disorders (such as autoimmunity, allergy and inflammatory bowel disease), many cancers, (such as prostate, colorectal and various childhood cancers [Hodgkin's lymphoma, acute lymphatic leukaemia]) and, still more unexpectedly, a range of psychiatric disorders including depression and disorders with a developmental component such as schizophrenia and autism. Medicine tends to be strictly compartmentalized, so the hypotheses put forward to explain the increases in, for example, psychiatric disorders do not usually overlap with the hypotheses put forward to explain parallel increases in cancer or inflammatory diseases, despite the similar epidemiology and the very obvious tendency for all three types of disorder to occur in the same individuals. An immigrant from a developing country arriving in a modern developed city is confronted with psychosocial changes and stressors, a new diet, and a totally different microbiological environment. Psychiatrists and psychologists interpret increased incidences of psychiatric diseases in terms of the changed psychosocial environment, while immunologists naturally try to interpret the increases in the diseases that fall within their domain in terms of the changing microbial environment, and immunological inputs. The aim of this paper is to explore the parallels between the increases in these diverse types of disease in developed countries and to suggest that there are underlying mechanisms, relevant to them *all* that transcend the boundaries between traditional medical disciplines. The keys to this discussion are the profound effects that psychosocial stressors exert on the immune system and vice versa, so that the two types of influence end up operating through similar pathways, often involving the intestinal microbiota.

INFLAMMATION AND PSYCHIATRIC DISORDERS

It is increasingly apparent that inflammation is involved not only in chronic inflammatory disorders such as allergies, autoimmunity and inflammatory bowel disease (IBD) but also in many psychiatric disorders. We have pointed out elsewhere that inflammation and immunoregulation are important in depression (*Raison* et al., 2010; *Rook* and *Lowry*, 2008). A large subset of depressed individuals has persistently raised levels of pro-inflammatory cytokines and other downstream inflammatory markers (*Maes* et al., 1992; *Miller* et al., 2009a), together with a relative deficit in anti-inflammatory mediators and regulatory T cells (fully referenced in *Raison* et al., 2010). When cytokines such as interferon-alpha (IFN-α) are used therapeutically (to treat viral hepatitis or some cancers) they can cause depression-like symptoms that can be treated with standard anti-depressants, such as selective serotonin reuptake inhibitors (SSRI) (*Miller* et al., 2009a; *Musselman* et al., 2001).

It is also clear that inflammatory events during pregnancy, often triggered by infections, can lead to the central nervous system (CNS) developmental abnormalities that underlie the subsequent appearance of autism and schizophrenia (extensively reviewed and referenced in *Meyer* et al., 2011) (*Crespi* and *Thiselton*, 2011; *Schwarz* et al., 2011; *Zerbo* et al., 2012). Indeed autism is very clearly associated with a family history of other chronic inflammatory disorders

such as autoimmunity and allergies, and it is accompanied by abnormal microbiota (*Finegold* et al., 2010) (of which the immunoregulatory role is discussed more below and in other chapters in this volume) and by incontrovertible evidence of background inflammatory activity (*Becker*, 2007; *Onore* et al., 2012). This concept is powerfully supported by animal models (rodents and monkeys) showing that inflammation in the mother during pregnancy induced by injecting lipopolysaccharide (LPS) or poly I:C, or by direct injection of IL-6, will cause changes in the grey and white matter of the foetuses and behavioural changes that are reminiscent of autism and schizophrenia (*Brown* and *Derkits*, 2010; *Smith* et al., 2007; *Willette* et al., 2011). Thus immunregulatory deficits during pregnancy or adult life could play a role in these conditions.

Finally, as discussed at greater length later, inflammation is also involved in the detrimental health consequences of low socio-economic status (SES), both in humans (*Hemingway* et al., 2003; *Miller* et al., 2009b) and in captive colonies of rhesus macaques (*Tung* et al., 2012).

EPIDEMIOLOGICAL PARALLELS

The parallels between the epidemiology of chronic inflammatory disorders and the epidemiology of psychiatric disorders highlight factors that expose the individual simultaneously to a changing psychosocial environment, and to changing immunological input. In the text that follows we use as examples the psychiatric disorders outlined in the previous section, and several particularly prevalent chronic inflammatory disorders; allergies, IBD and two autoimmune disorders (multiple sclerosis [MS] and Type 1 diabetes [T1D]).

Urban versus rural

A feature shared by most of the disorders discussed here is a higher prevalence in urban communities, compared to rural ones. For example a meta-analysis of high quality studies performed in high-income countries since 1985 found that the prevalence of depression in urban areas was 39% higher than in rural areas. Similarly, the prevalence of anxiety disorders was 21% higher in urban than in rural areas (*Peen* et al., 2010), though a small minority of studies fails to find this urban-rural difference (*Kovess-Masfety* et al., 2005). Peen and colleagues also noted an increased urban prevalence of psychiatric disorders in general (38% more in urban communities) (*Peen* et al., 2010). This agrees well with another large meta-analysis which found a significantly raised prevalence of schizophrenia in urban communities (*McGrath* et al., 2004). Similarly, a study of all children born in Denmark between 1 January 1984 and 31 December 1998 found that the degree of urbanisation of place of birth was very significantly correlated to risk of autism ($p<0.0001$) (*Lauritsen* et al., 2005).

It has been suggested that mentally healthy people move away from socially deprived inner cities while vulnerable mentally ill people tend to gravitate towards these areas where deviant behaviour might be more easily tolerated (*Freeman* and *Alpert*, 1986; *Moorin* et al., 2006). However available data suggest that it is the *urban upbringing* rather than a selective migration into cities that lies behind the repeated associations of exposure to the

urban environment and an increased risk of psychiatric disturbance (*Blazer* et al., 1985; *Verheij*, 1996). This view is strongly supported by the other epidemiological parallels discussed below.

The urban-rural phenomenon is also well established for chronic inflammatory disorders, where the aetiology is known to involve dysregulation of the immune system. This has been explored in some detail in the allergic disorders. Contact with the farming environment, whether postnatal (*Riedler* et al., 2001) or prenatal (*Ege* et al., 2008; *Schaub* et al., 2009) protects against allergic disorders, whereas the prevalence of these conditions increases with increasing urbanization (*Nicolaou* et al., 2005). The same is true for inflammatory bowel diseases (*Hou* et al., 2009), and for autoimmune diseases such as multiple sclerosis (MS) (*Antonovsky* et al., 1965; *Beebe* et al., 1967; discussed in *Lowis*, 1990). Interestingly, Type 1 diabetes (T1D, caused by autoimmune destruction of the pancreatic β cells) is more common in urban than in rural areas in some countries (Greece, southern Italy, Lithuania) (*Cherubini* et al., 1999; *Dacou-Voutetakis* et al., 1995; *Pundziute-Lycka* et al., 2003), but not in others (Finland, New Zealand or the UK) (*Miller* et al., 2011). This pattern of findings might imply that the effect is seen when the comparison is made in poorly developed countries where rural life is "traditional", with multiple exposures to animals, farm buildings, and soil.

The urban-rural comparison therefore suggests either that something beneficial is absent from the urban environment, or that something detrimental is present, and these possibilities are as relevant for chronic inflammatory disorders as they are for psychiatric conditions. However, these findings do not allow us to determine the relative importance of psychosocial factors versus immunological inputs for either group of diseases, despite the copious literature implicating the former for the psychiatric diseases, and the latter for the chronic inflammatory ones.

Immigrants

Another striking parallel between chronic inflammatory diseases and psychiatric disorders concerns the effects of immigration on these conditions. All the diseases discussed here, whether chronic inflammatory (*Ahlgren* et al., 2011; *Hou* et al., 2009; *Rottem* et al., 2005; *Söderström* et al., 2012) or psychiatric (*Breslau* et al., 2011; *Dealberto*, 2010; *Keen* et al., 2010), tend to be more common in immigrants than in the birth population from which the immigrant was derived, at least when the migration is from a developing to a developed country. Other relevant variables include the age of the individual at the time of immigration, and whether the prevalence increases in second generation immigrants, born in the adopted country. A study of these parameters provides some insight into whether the relevant influences, be they psychosocial or immunological, need to occur before birth, or in early childhood, or whether they can still exert their effects on adults.

Age at immigration, 2nd generation immigrants and psychiatric disorders

Depression is particularly interesting in this respect (*Breslau* et al., 2009; *Vega* et al., 2004). Mexicans, Cubans and African/Caribbean peoples have a 2-3-fold increase in the prevalence of depression if immigration to the USA occurred when the individual was less than 13 years old, or was born in the USA, compared to the prevalence in those who migrated after the age of 13

(*Breslau* et al., 2009). But this is not likely due to psychosocial stress related to skin colour, because white Eastern European immigrants show the same effect. In sharp contrast, the effect is not seen in immigrants from Western Europe, or from Puerto Rico, which is closely associated with the USA. (These last two populations already have a high prevalence of depression that is not increased by immigrating to, or being born in, the USA) (*Breslau* et al., 2009). These findings imply that influences important for depression occur perinatally, or in the early years of life.

The same is true for psychotic disorders (*Coid* et al., 2008). A large Danish study noted that immigration into Denmark when less than 4 years old was associated with a strikingly increased risk for psychotic disorders, whereas the increased risk gradually decreased with older age at migration and disappeared in those immigrating when more than 29 years old (*Veling* et al., 2011). Similarly a large meta-analysis confirmed that schizophrenia was increased amongst 1st generation immigrants, and further increased amongst 2nd generation immigrants, particularly when the country of origin was a developing one (*Cantor-Graae* and *Selten*, 2005). Again, early events seem crucial.

Age at immigration is irrelevant to an early onset condition such as autism, but autism is strikingly (as much as 10-fold) increased in 2nd generation Caribbean or African immigrants born in the UK, compared to children of white UK-born mothers, as discussed below (*Keen* et al., 2010).

These findings implicate crucial early events in the perinatal period or early childhood as risk factors for depression, schizophrenia and autism. Do these more specific findings, usually attributed to the psychosocial challenges of immigrant status, differentiate these psychiatric disorders from the chronic inflammatory disorders? The answer to this question is again no, as explained in the next section.

Age at immigration, 2nd generation immigrants and chronic inflammatory disorders

Migration has clear effects on the prevalence of MS, and the crucial events that confer increased risk for the disease occur very early in life, as is true for the psychiatric disorders (reviewed and referenced in *Gale* and *Martyn*, 1995; *Milo* and *Kahana*, 2010). Iranians who migrate to Sweden have twice the prevalence of MS seen in their birth country (*Ahlgren* et al., 2011). Interestingly, if the 2nd (or later) generation immigrants return to their developing country of origin, they retain their increased susceptibility to MS, which remains higher than in the local population that was not born abroad (*Cabre*, 2009). A similar phenomenon was seen when people born in the United Kingdom (UK: a high MS country) migrated to South Africa (SA: a low MS country). Migration from the UK to SA was protective when the migrant was a child, whereas adult migrants retained their high UK prevalence of MS (*Dean*, 1967). Analysis of this and other studies suggests that the environmental factors that protect from or predispose to MS act during the first two decades of life (*Gale* and *Martyn*, 1995; *Milo* and *Kahana*, 2010). The same is true for T1D. Here the crucial factor is to have been *born* in the receiving developed country, again suggesting that relevant environmental factors act very early, or even in the prenatal period (*Söderström* et al., 2012).

The role of migration in conferring risk for allergic disorders has been intensively examined. A study of chil-

dren adopted into Sweden from developing countries showed that the prevalences of asthma, hay fever and eczema were highest in those adopted when less than 2 yrs. old (*Hjern* et al., 1999). Similarly, for Mexican immigrants to the USA, the prevalence of asthma was highest for those born in the USA, while in those not born in the USA, the prevalence of asthma decreased as the age at immigration increased (*Eldeirawi* et al., 2009). This effect of age at the time of childhood immigration was also seen in immigrants to Israel from the former Soviet Union or Ethiopia who were assessed when 17 years old (*Pereg* et al., 2008). These observations suggest the importance of early environmental influences for allergy/asthma risk, a conclusion that is powerfully supported by evidence that prenatal exposure (i.e. of the pregnant mother) to the farming environment protects the infant against some allergic manifestations (*Ege* et al., 2008; *Schaub* et al., 2009). This is discussed later in another context.

Finally, a definitive study of all first- and second-generation immigrants in Sweden between January 1, 1964, and December 31, 2007 showed that some 1st generation immigrants remain partially protected from both ulcerative colitis (UC) and Crohn's disease (CD), presumably by environmental factors encountered in their countries of origin, but the diseases increased in prevalence in 2nd generation immigrants, relative to 1st generation immigrants (*Li* et al., 2011). Similarly, the prevalence of UC in South Asian immigrants to Leicester in the UK was higher in 2nd than in 1st generation immigrants (*Carr* and *Mayberry*, 1999). This again implicates perinatal factors as potentially causative of this migration effect.

Thus the influence of immigration, acting via factors that occur perinatally or very early in life, is equally consistent and highly apparent for both psychiatric and chronic inflammatory disorders.

Birth order

The study of disease prevalence in relation to birth order focuses rather specifically on perinatal factors. It was the observation that having multiple older siblings, especially male ones, provided some protection against hay fever that led to the first use of the term "Hygiene Hypothesis", highlighting the notion that grubby older brothers provided an expanded and protective microbiological environment (*Strachan*, 1989). However, subsequent studies of the effects of birth order on other inflammatory conditions have painted a less consistent picture. For example, where IBD is concerned birth order effects are sometimes significant, but the direction of the association is inconsistent (*Hampe* et al., 2003; *Van Kruiningen* et al., 2007). Results are also often contradictory in MS (*Zilber* et al., 1988). Similarly variation was seen in a meta-analysis of data from studies of childhood onset T1D, but overall, there does appear to be increased prevalence in firstborn children (*Cardwell* et al., 2011).

What about the psychiatric disorders? Again the picture is rather variable. For depression an association with birth order is sometimes reported, but the relationship is inconsistent (*Bergeron* et al., 2007; *Schmidt* and *Tolle*, 1977; *Wells* et al., 1985). The effect may be more pronounced for autism and schizophrenia. A comprehensive Finnish study of families with at least two children, one of whom was schizophrenic, found that being the first-born was a significant risk factor for schizophrenia, but the protective effect of older siblings was complex and depended on how much older they

were (*Haukka* et al., 2004). The relevance of birth order to autism has been reviewed in detail elsewhere (*Becker*, 2007). Briefly, the risk of autism has been shown to fall as the number of older siblings rises in studies in the United States, Western Australia and England, though not every study shows this (*Becker*, 2007). In view of the significant epidemiological association with familial allergic disorder, where the birth order effect is clear, as mentioned earlier, this is of great interest (discussed in *Meyer* et al., 2011; *Onore* et al., 2012).

THE OLD FRIENDS HYPOTHESIS AND IMMUNOREGULATION

These epidemiological findings, when applied to the chronic inflammatory disorders, are usually explained by the Hygiene Hypothesis or by the variant of that hypothesis that we prefer, the "Old Friends" hypothesis (plus a few additional recent factors discussed later). The Old Friends hypothesis states that mammals co-evolved with an array of organisms and conditions that, because they needed to be tolerated, took on a role as inducers of immunoregulatory circuits (*Rook*, 2010; *von Hertzen* et al., 2011a). Such organisms and conditions include various microbiotas and commensals (gut, skin, lung etc.), chronic infections picked up at birth, helminths that persist for life, and environmental organisms from animals, mud and untreated water with which we were in daily contact. For example, helminthic parasites need to be tolerated because although not always harmless, once they are established in the host any effort by the immune system to eliminate them is futile, and merely causes tissue damage (*Babu* et al., 2006). Contact with the "Old Friends" rapidly diminishes when industrialization occurs, and we start to inhabit a plastic and concrete environment, to consume washed food and chlorine-treated water, and to minimize our contact with mud, animals and faeces. This withdrawal of the organisms that drive immunoregulatory circuits results in defective immunoregulation that, depending on the genetic background of any given individual, can manifest as a variety of chronic inflammatory disorders, including allergies, IBD and autoimmunity. In contradistinction to early articulations of the hygiene hypothesis that focused more exclusively on allergic conditions, we now know that a failure of immunoregulatory mechanisms really can lead to simultaneous increases in diverse types of pathology. For example, genetic defects of the gene encoding the transcription factor Foxp3 lead to the X-linked autoimmunity–allergic dysregulation syndrome (XLAAD) that includes aspects of allergy, autoimmunity and enteropathy (*Wildin* et al., 2002).

The crucial underlying points are these. First, the chronic inflammatory disorders all show evidence of failed immunoregulation (*Rook*, 2009). Secondly, "Old Friends" (such as helminths, non-pathogenic environmental bacteria [pseudo-commensals] or certain gut commensals, probiotics) can be shown to drive immunoregulation, and to block or treat models of *all* of these chronic inflammatory conditions (*Karimi* et al., 2009; *Osada* and *Kanazawa*, 2010; *Round* and *Mazmanian*, 2009). Thirdly, some Old Friends, or molecules that they secrete, can be shown to specifically expand Treg populations (*Atarashi* et al., 2011; *Grainger* et al., 2010; *Karimi* et al.,

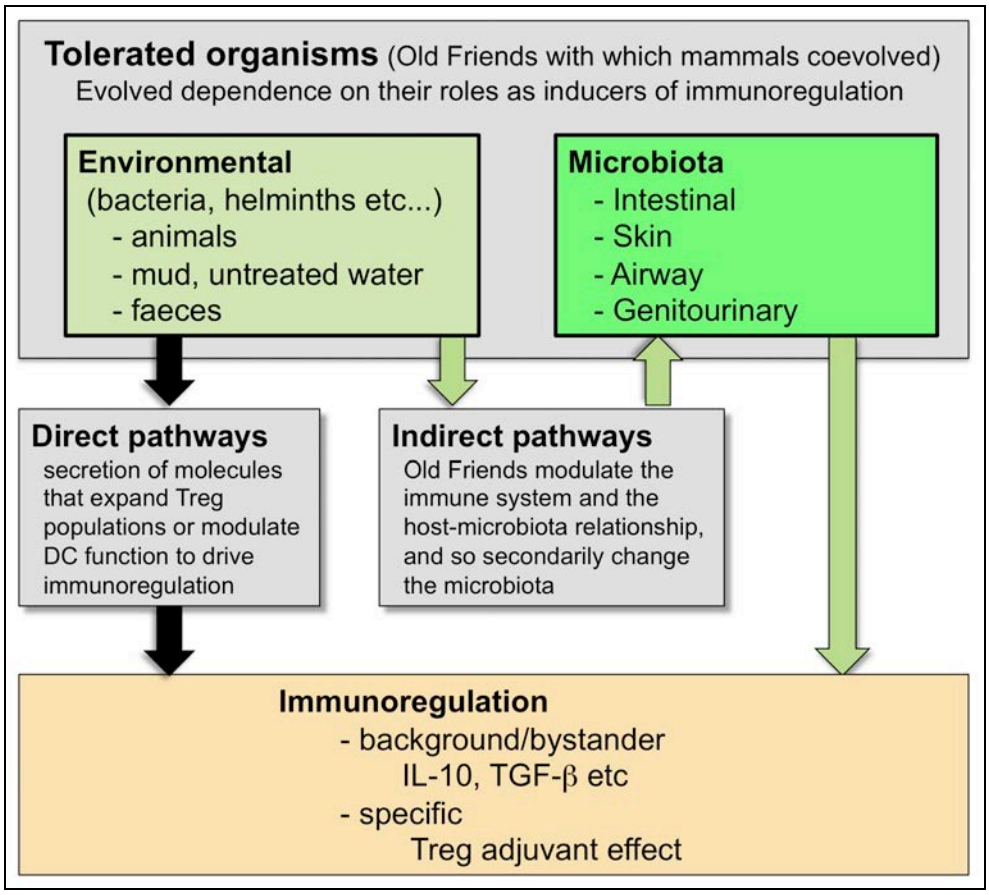

Figure 1: Microorganisms with which mammals co-evolved ("Old Friends"), and that had to be tolerated, have been entrusted with roles in the induction of immunoregulation. These include direct effects on the immune system (for example, driving expansion of Treg, and of regulatory DC), and indirect effects secondary to changes in the host-microbiota relationship. The latter causes changes in the composition of the microbiota that then secondarily modify immune function.

2009; *Round* et al., 2011), or to cause dendritic cells (DC) to switch to regulatory DC that preferentially drive immunoregulation (*Smits* et al., 2005). Finally, when MS patients become infected with helminths, the disease stops progressing, and circulating myelin-recognising regulatory T cells (Treg) appear in the peripheral blood (*Correale* and *Farez*, 2007; *Correale* and *Farez*, 2011), indicating that the helminths act as Treg adjuvants. This is an exciting observation that has led to formal clinical trials (*Fleming* et al., 2011).

Old Friends Hypothesis and the gut Microbiota

We emphasise that many "Old Friends" are (or were, until changed or depleted) gut microbiota, or gut parasites (*Atarashi* et al., 2011; *Grainger* et al., 2010; *Round* et al., 2011). Others were environmental saprophytes in mud and untreated water that inevitably passed though the gut in large numbers every day (*Le Bert* et al., 2011). Moreover new data show that other microbiota such as those of the skin or oral mucosa can also be relevant to immunoregulation (*Friberg* et al., 2010;

Hanski et al., 2012; *Singhal* et al., 2011). But one of the most important discoveries in recent years is the fact that manipulations of the immune system may act *indirectly* via changes in the gut microbiota. For example, Wen and colleagues showed that specific pathogen free (SPF) non-obese diabetic (NOD) mice that spontaneously develop a condition resembling T1D, are protected from the disease following knockout of the gene encoding MyD88 (an adaptor for multiple Toll-like receptors) (*Wen* et al., 2008). However this did not mean that MyD88 was directly involved in the autoimmune response to β cells in the pancreas. Rather it emerged that the modification of the immune system resulting from knocking out MyD88 caused profound changes in the interactions between the immune system and the microbiota, leading to changes in the composition of the latter. It was these changes in the composition of the microbiota that were responsible for the immunoregulatory effect that blocked the autoimmune process. Thus changes in the microbiota, which is profoundly different in Europeans than in people living in a traditional rural African village (*De Filippo* et al., 2010), must be regarded as part of the Old Friends hypothesis, whether these changes are attributable to diet (*Cani* and *Delzenne*, 2011) or to diminished exposures. In either case altered exposure to "Old Friends" will simultaneously exert direct effects on the immune system, and indirect effects via secondarily induced changes in the microbiota (Figure 1).

The Old Friends hypothesis and genetics

In parts of the world where there was a heavy load of organisms causing immunoregulation (such as helminths), there has been selection for single nucleotide polymorphisms (SNP) or other variants to partially compensate for this immunoregulation, or to combat new infections such as malaria that spread from gorilla to man about 10,000 years ago (*Liu* et al., 2010; *Sotgiu* et al., 2008). This is seen for several pro-inflammatory cytokines (*Fumagalli* et al., 2009), IgE (*Barnes* et al., 2005) and STAT6, a transcription factor involved in Th2 responses (*Moller* et al., 2007). There is also an increased frequency of a truncated form of the serotonin transporter promoter that also has a marked pro-inflammatory effect (*Fredericks* et al., 2010). The problem here is clear (Figure 2). As soon as the immunoregulation-inducing organisms are withdrawn by the modern lifestyle, these genetic variants lead to excessive inflammation, and become risk factors for chronic inflammatory disorders (*Barnes* et al., 2005; *Fredericks* et al., 2010; *Fumagalli* et al., 2009; *Moller* et al., 2007). This constitutes a second layer of evolved dependence on the continuing presence of the "Old Friends" (Figure 2).

This is important because work that identifies proximate "causes" for diseases that were rare or non-existent before the Second Epidemiological Transition may merely be unravelling a problem that would be irrelevant if the microbial status of the modern world could be returned to that seen in the paleolithic. For instance, the recent claim to have discovered that the "cause" of Crohn's disease is a genetically determined defect in the homing of neutrophils (*Smith* et al., 2009) is difficult to reconcile with the fact that 100 years ago the disease barely existed. But recent environmental changes could conceivably have caused this phenotype to become a risk factor (Figure 2).

It is clear how the Old Friends hypothesis can provide an explanation for

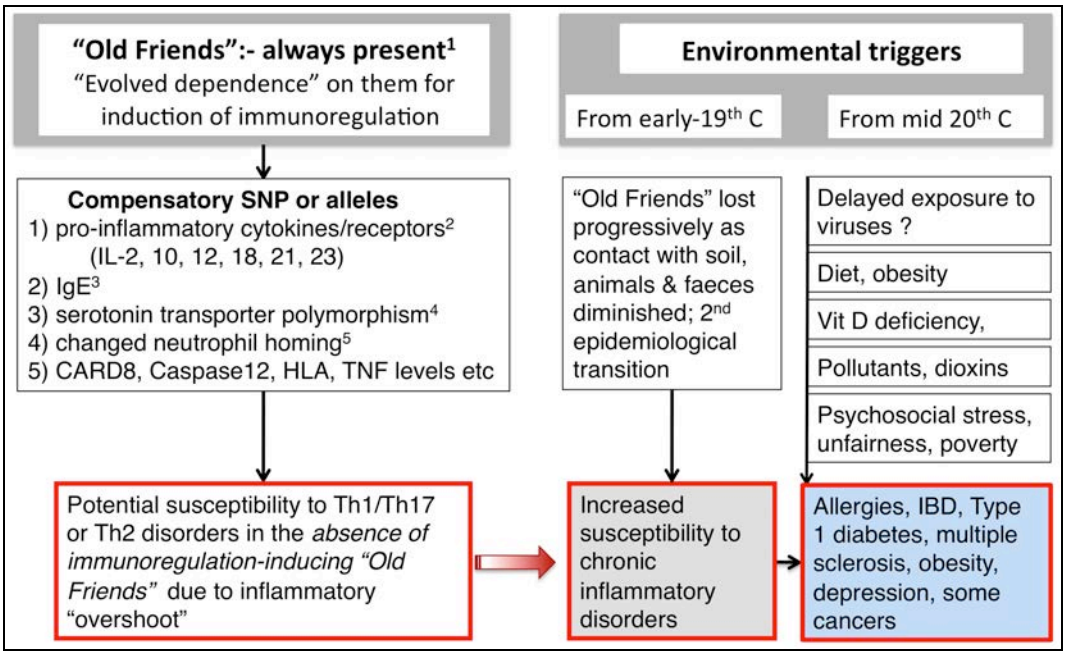

Figure 2: Interaction of genetics and loss of the "Old Friends". The Old Friends had to be tolerated and so co-evolved roles as triggers of immunoregulatory pathways. In areas with very high loads of these and other organisms, particularly helminths, compensatory genetic variants accumulated, to partially restore inflammatory responses. In the absence of the Old Friends, not only is immunoregulation inadequately primed, but also these genetic variants cause excessive inflammation and become risk factors for chronic inflammatory disorders. Genetic variants that were advantageous, and did not cause disease in the past, start to do so when the Old Friends are lost after the 2nd epidemiological transition (referenced in main text). More recently several aspects of modern life are exacerbating the effects of the lack of "Old Friends" at the level of immunoregulation. Obesity is associated with altered gut microbiota and excessive release of pro-inflammatory cytokines. Stress also alters gut microbiota, and drives corticotropin-releasing hormone (CRH) that increases permeability of the gut mucosa. Increased absorption of lipopolysaccharide (LPS) and other microbial components drives further release of pro-inflammatory cytokines. Lack of vitamin D exacerbates immunodysregulation, as does the triggering of Th17 cells by dioxins. Meanwhile the changes in the gut are also likely to impact on Th17 development. Viruses that used to be encountered harmlessly in early infancy (under cover perhaps of maternal antibody) can trigger autoimmunity if encountered for the first time later in life. Psychosocial stressors exacerbate these problems, as outlined in the main text.

rural-urban differences in the prevalence of chronic inflammatory diseases, and for the consequences of immigration from poor to rich countries. The rural environment and above all the farming environment, provide a microbiological input that is closer to that with which we evolved, so more likely to prime appropriate immunoregulation. Similarly, the developing country lifestyle exposes us to more diverse and numerous Old Friends than does the rich Western lifestyle.

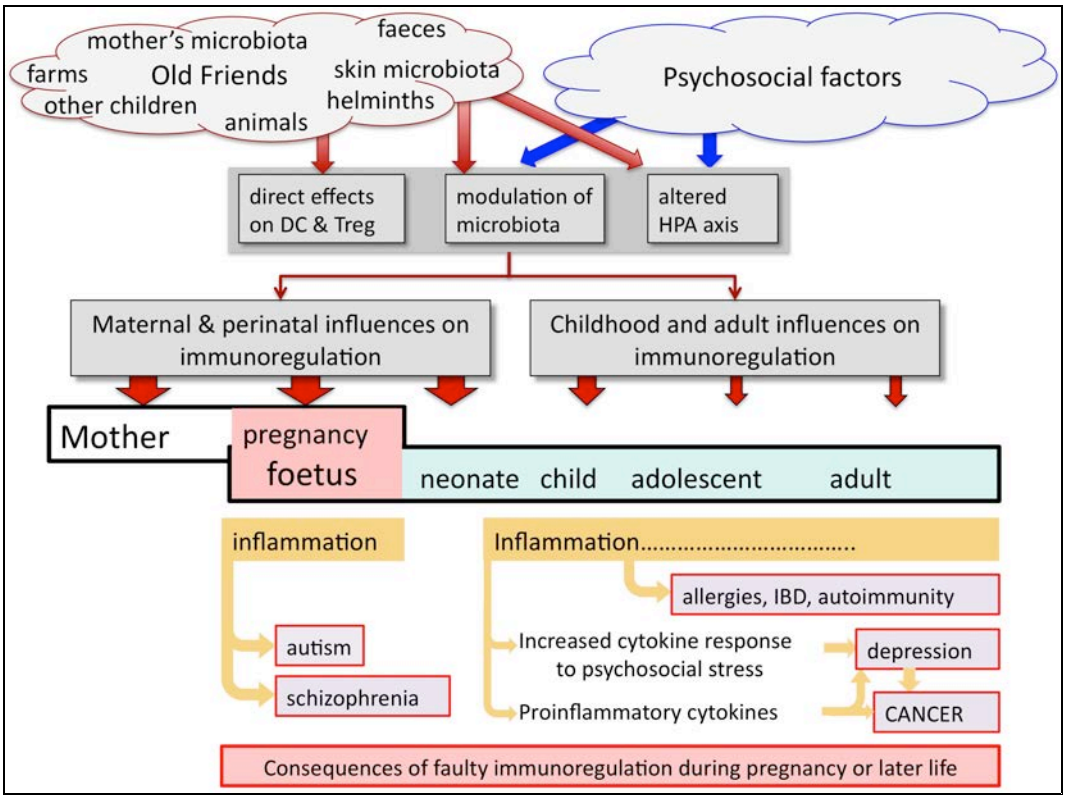

Figure 3: Immunoregulatory influences throughout life, and the consequences of their failure. The immunoregulatory Old Friends and psychosocial factors influence immunoregulation, and converge strikingly on the microbiota and HPA axis in the perinatal period. Withdrawal of Old Friends, and/or perinatal psychosocial stressors predispose to inflammatory disorders. During pregnancy this can predispose to neurodevelopmental problems such as autism and schizophrenia. In adolescence or adult life poor immunoregulation can predispose to chronic inflammatory disorders or to depression or to both together. A late consequence, exacerbated by depression, is inflammation-associated oncogenesis, and promotion of cancer growth and spread.

OLD FRIENDS AND PSYCHOSOCIAL STRESSORS IN THE PERINATAL PERIOD

The previous sections have used three psychiatric disorders to point out that aspects of their epidemiology that are usually interpreted as indicating a crucial role for exposure to psychosocial stressors, are not significantly different from the findings that emerge from the epidemiology of chronic inflammatory disorders such as allergies, IBD and autoimmunity. In the context of the latter disorders these findings are usually explained by the "Old Friends" hypothesis. Does this mean that we should we be changing our view of the role of the psychosocial stressors in psychiatric disease, or should be blaming psychosocial stressors for these chronic inflammatory disorders? The answer, we suggest, is both. Psychosocial stressors and inflammation are closely linked, as discussed below.

Perinatal psychosocial stress and immunoregulation

Although immigrants certainly meet a changed microbial environment, it is equally certain that they face a barrage of psychosocial stressors. Indeed we know that prenatal psychosocial stress (i.e. experienced by the pregnant mother) or early postnatal stress can cause long-term changes in neurogenesis (reviewed in *Korosi* et al., 2012), in cognition, memory (*Entringer* et al., 2009a) and in hypothalamic-pituitary-adrenal (HPA) axis function (*Entringer* et al., 2009b). These dilemmas are partly resolved when one considers the close links between psychosocial stressors and the immune system. We suggest below that psychosocial stressors will exacerbate the immunoregulatory dysfunction caused by the lack of "Old Friends", while the lack of "Old Friends" will exacerbate the inflammatory effects of psychosocial stressors (Figure 3). There are clear synergistic pathways to the simultaneous development of a mixture of psychiatric and chronic inflammatory problems. The crucial point is that perinatal stress also has long-term modulatory effects on immune system function. This issue is the topic of the following sections.

Perinatal stress and long-term changes to immune function

Many studies in animals and humans have shown that a variety of stressors, including psychosocial ones, during pregnancy activate inflammation (*Haroon* et al., 2012; *Howerton* and *Bale*, 2012). For example, prenatal maternal stress during otherwise normal human pregnancies was associated with raised circulating levels of the pro-inflammatory cytokines IL-6 and tumour necrosis factor-alpha (TNF-α), raised C-reactive protein (CRP) and low levels of the anti-inflammatory cytokine IL-10 (*Coussons-Read* et al., 2005). Similarly overall stress levels during pregnancy correlated with increased release of IL-1β and IL-6 by lymphocytes stimulated *in vitro* during the 3rd trimester (*Coussons-Read* et al., 2007).

However, the important point here is that perinatal stress results in adults who themselves show exaggerated inflammatory responses to stress (*Carpenter* et al., 2010; *Danese* et al., 2007, 2008). For example, peripheral blood mononuclear cells from healthy young women whose mothers had experienced major negative life events during pregnancy showed altered responses to phytohaemagglutinin compared to cells from a control group. There was a bias towards production of T-helper 2 (Th2) cytokines and both IL-6 and IL-10 were also significantly elevated (*Entringer* et al., 2008). Similarly maltreated children develop higher levels of IL-6 in response to a standardized social stressor (the Trier Social Stress Test; TSST) when tested as adults in comparison to a non-maltreated control group (*Carpenter* et al., 2010; *Pace* et al., 2006), and maltreated children tend to have higher levels of CRP 20 years later (*Danese* et al., 2007). Low early life social class (socio-economic status; SES) is similarly associated in adult life (aged 25-40) with increased production of IL-6 in cultures of peripheral blood leukocytes stimulated with ligands for toll-like receptor 3 (TLR3) or TLR5 (*Miller* et al., 2009b). These findings all imply that perinatal stress itself leads to long-lasting problems with immunoregulation (*Carpenter* et al., 2010; *Danese* et al., 2007, 2008). Interestingly, negative life events during the first years of life, whether they affect the child directly, or indirectly via traumatic experiences of the mother, predispose to the autoimmune disease T1D (reviewed in *Peng* and *Hagopian*, 2006; *Sepa* et al.,

2005; *Vlajinac* et al., 2006). It is likely that this reflects an influence of perinatal negative life events on subsequent immunoregulation.

Many mechanisms are involved in the relationship between perinatal stress and immune activation (*Haroon* et al., 2012; *Howerton* and *Bale*, 2012). We consider two particularly relevant and important mechanisms below; the HPA axis and the microbiota.

Perinatal stress and long-term changes to the HPA axis

In monkeys, exposure to high levels of maternal stress hormones (whether induced by stressing the mother, or by injecting dexamethasone or adrenocorticotropic hormone [ACTH] during pregnancy) causes prolonged changes in the reactivity of the infant's lymphocytes *in vitro* (*Coe* et al., 1996, 1999). Numerous animal models have demonstrated associations between prenatal stress and long-term alterations in HPA axis function (*Kapoor* et al., 2006; *Weinstock*, 2005).

Healthy young human adults who had been exposed to "prenatal stress", because their mothers had experienced severe negative life events such as the death of someone close during pregnancy, responded differently to a standardized social stressor (TSST) when compared to an age-matched comparison group of healthy young women who had not been exposed to prenatal stress. The prenatal stress group had lower cortisol levels ($p=0.007$) before the TSST, and a larger increase in response to the TSST ($p=0.03$) (*Entringer* et al., 2009b). Similar changes have been associated with severe stress in early childhood (*Heim* et al., 2000). Moreover adults with PTSD symptoms who were abused as children show increased NFκB and decreased glucocorticoid sensitivity and these two findings are highly correlated (*Pace* et al., 2012). This is consistent with the idea that HPA axis changes as a result of early abuse or neglect contribute to increased inflammatory drive.

Interestingly, a transcriptional profiling of adults whose childhood background had been of low or high socioeconomic status (SES) revealed that those from a low childhood SES background had up-regulation of genes bearing response elements for the cAMP response element binding (CREB)/activating transcription factors (ATF) family of transcription factors involved in signalling to leukocytes, heightened expression of transcripts bearing response elements for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and down-regulation of genes with response elements for the glucocorticoid receptor (GR) involved in anti-inflammatory function (*Miller* et al., 2009b). A similar pro-inflammatory bias in gene expression emerged from a comparison of asthmatic children from low or high SES backgrounds (*Chen* et al., 2009). In conclusion, perinatal and early neonatal stressors are likely to induce long-term changes in HPA axis function (Figure 3), with obvious consequences for immune function.

Although perhaps beyond the scope of this discussion document, it is worth noting briefly that the persistence into adult life of HPA axis effects triggered in the perinatal period might be explained by epigenetic changes, such as altered DNA methylation patterns (*Guo* et al., 2011; *Miller* et al., 2008; *Weaver* et al., 2004), or by shortened telomere length, and reduced telomerase activity (*Choi* et al., 2008; *Entringer* et al., 2011; *Jacobs* et al., 2011; *Ornish* et al., 2008). Reduced telomere length is associated with inflammation and autoimmunity as well as with premature immunosenescence (*Carrero* et al., 2008; *Fitzpatrick* et al., 2007; *Hohen-*

sinner et al., 2011), though whether as cause or consequence is not certain.

Perinatal stress and long-term changes to the intestinal microbiota

It has been known for some time that stress alters the microbiota of experimental animals (*Kiliaan* et al., 1998), and the same is true of the microbiota of severely stressed critically ill humans, where the changes are rapid and prolonged (*Hayakawa* et al., 2011). Crucially important in the current context is the observation that the stress of maternal separation for 3 hours per day from post-natal days 2-12 has long-term effects on the subsequent 16S rRNA diversity of the microbiota that is still apparent when the pups become adult rats, compared to control adults that had not been exposed to maternal separation as pups (*O'Mahony* et al., 2009). Similarly, *prenatal* stressors have been shown to alter the microbiome in rhesus monkeys by reducing the overall numbers of bifidobacteria and lactobacilli during adulthood (*Bailey* et al., 2004).

This might be an important mechanism because the nature of the microbiota during the first weeks of life has a profound effect on development of the CNS and the HPA axis. For example, germ-free mice have increased motor activity, reduced anxiety, altered gene expression in several brain areas, and increased turnover of noradrenaline, dopamine and 5-HT in the striatum (*Heijtz* et al., 2011). These abnormalities persist into adulthood, and cannot be corrected by reconstitution of the microbiota of adult animals (*Heijtz* et al., 2011). Moreover, the nature of the microbiota is crucial. In another study it was noted that germ-free mice had abnormal responses to restraint stress, specifically increased ACTH and corticosterone responses, together with reduced expression of brain-derived neurotrophic factor (BDNF) in cortex and hippocampus, reduced glucocorticoid receptors (GR) in the cortex, and raised corticotrophin-releasing hormone (CRH) in the hypothalamus (*Sudo* et al., 2004). Oral reconstitution with a normal microbiota normalised the HPA axis function if done at 6 weeks, but not if done later. Early mono-association with *Bifidobacterium infantis* also normalized HPA axis function, but mono-association with enteropathogenic *E. coli* made the abnormalities more severe. Thus not only is the microbiota modified by stress, but it is also involved in development of the CNS, so given these observations it is possible that perinatal stress might exert physiological effects on the brain in adulthood at least in part via its impact on the microbiota

Consequences of changes to the intestinal microbiota

The nature of the microbiota is likely to modulate both the subsequent response to psychosocial stressors and the functions of the immune system. When mice are stressed by being housed with an aggressive dominant male, they develop altered microbiota, and raised circulating levels of IL-6, TNF and IFN-γ. However, if the microbiota is depleted by an antibiotic cocktail, the same social stressor fails to cause increased pro-inflammatory cytokine levels (*Bailey* et al., 2011). It seems that increased permeability in the gut caused by stress, results in increased intake of LPS and other pro-inflammatory molecules, which exert a positive feedback on the stress-induced inflammatory response. Stress-induced increases in gut permeability might be partly due to release of CRH by T cells, and by cells within the submucosa and muscle layers of the gut, myenteric neurons, serotonin-containing enterochromaffin cells, and lamina propria

cells of the mucosa in stomach and colon (*Stengel* and *Tache*, 2009). CRH is not only a regulator of intestinal permeability (*Gareau* et al., 2008; *Teitelbaum* et al., 2008), but also a potent pro-inflammatory cytokine in its own right in the periphery of the body (*Calcagni* and *Elenkov*, 2006). These changes may help explain the finding that depressed patients have raised levels of antibody to a range of intestinal bacteria (*Maes* et al., 2008).

Alterations in the microbiota will also impact immunoregulation (Figure 3). The fundamental role of the microbiota in immunoregulation has been reviewed extensively elsewhere (*Round* and *Mazmanian*, 2009). The organisms that comprise the microbiota can be thought of as one component of the "Old Friends". Recent observations suggested that, as in animals, in humans fluctuations in the microbiota early after surgery (bone marrow transplantation) may lead to an increased risk of immunoregulatory failure, manifested in this study as graft-versus-host disease (*Jenq* et al., 2012).

SYNERGY BETWEEN THE OLD FRIENDS MECHANISM AND STRESS

The previous section makes clear that perinatal stress can cause long-term dysregulation of the immune response, both via changes to the HPA axis and changes to the microbiota. (No doubt there are other changes too, e.g., changes to the sympathetic and parasympathetic systems, but these are beyond the scope of this paper). However we have also pointed out that in the modern urban environment a second mechanism is also reducing the efficiency of immunoregulatory mechanisms: depletion of immunoregulation-inducing "Old Friends". In this section we explore the hypothesis that these two mechanisms interact to modulate those psychiatric disorders where inflammation is known to play a role. As outlined in the section immediately after the introduction, inflammation is implicated in depression (*Raison* et al., 2010), and in driving the developmental abnormalities that underlie many cases of schizophrenia and autism (*Meyer* et al., 2011) and in the health consequences of low socio-economic status (*Hemingway* et al., 2003; *Miller* et al., 2009b; *Tung* et al., 2012). There are several ways in which the psychosocial and microbial pathways might work together via immunoregulation to alter patterns of psychiatric disease.

Urban versus rural upbringing, and response to an experimental stressor

A recent functional magnetic resonance imaging (fMRI) study compared the effects of an experimental social stressor on individuals brought up in urban or rural environments. Urban versus rural upbringing correlated with significant differences in activation of the perigenual anterior cingulate cortex, a region involved in regulation of negative affect and the physiological stress response (*Lederbogen* et al., 2011). The authors attributed their findings to putatively different levels of social stressors in individuals with an urban versus rural upbringing. But would social stressors in children differ significantly in the two environments in a wealthy European country (Germany)? It is equally likely that the findings were due to the "Old Friends" mechanism, leading to diminished regulation of pro-inflammatory mediators in those subjects who had an urban upbringing. Indeed the protective effects of the German farming environment against allergies and early onset in-

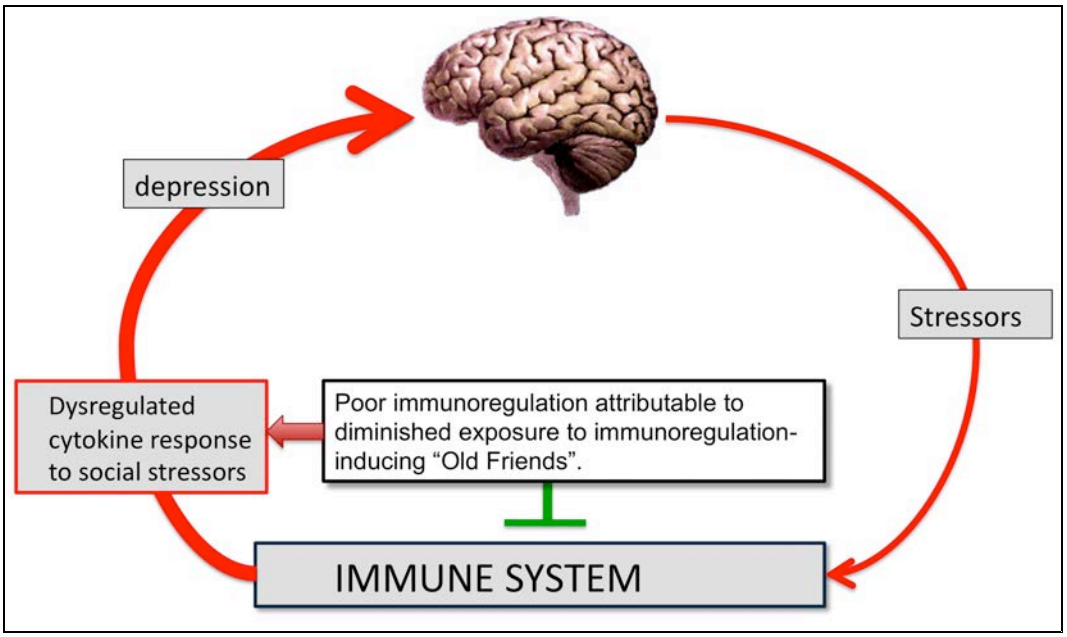

Figure 4: Exaggerated cytokine release in response to a psychosocial stressor leading to depression. Populations that have undergone the 2nd epidemiological transition have minimal exposure to immunoregulation-inducing "Old Friends" such as helminths. The consequent diminished efficiency of immunoregulation is manifested as rising prevalences of chronic inflammatory disorders such as allergies, autoimmunity and inflammatory bowel disease (IBD). Poor immunoregulation will also leave the individual susceptible to excessive and prolonged cytokine release in response to psychosocial stressors, which may result in inappropriate triggering of depressive episodes.

flammatory bowel disease are well documented and require that a child be exposed to the farming environment during the first 2.5 years of life…a rural upbringing (*Radon* et al., 2007; *Riedler* et al., 2001). The authors of the fMRI study did not measure the stress-induced levels of circulating pro-inflammatory cytokines in the two populations. The "Old Friends" view of the data would postulate higher levels in the subjects who had urban upbringings.

Depression

Exposure to psychosocial stressors in later life would be expected to cause greater increases in circulating pro-inflammatory cytokines in individuals with poor immunoregulation as a consequence of lack of exposure to Old Friends. Thus people living in rich developed countries should have a greater likelihood of becoming depressed when confronted with a given level of psychosocial stress, compared to the citizens of a developing country environment rich in immunoregulation-inducing "Old Friends" (Figure 4). We know that depressed individuals with histories of early life trauma or neglect release more IL-6 in response to the TSST (*Pace* et al., 2006). To put it another way, it might be that depression is increasing in the USA (*Compton* et al., 2006) not because our lives are becoming more stressful, but rather because in developed countries our immune systems release more depression-inducing pro-inflammatory cytokines in response to any given level of psychosocial stressor. This would imply

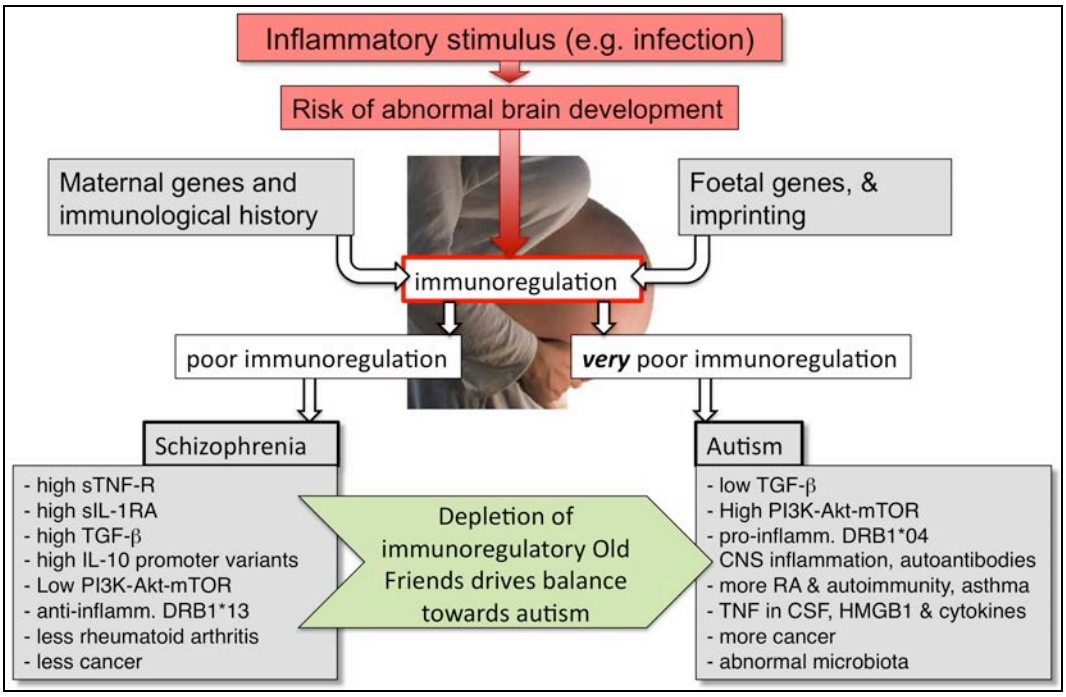

Figure 5: Reduced efficiency of immunoregulation during pregnancy, due to loss of the immunoregulation-inducing Old Friends could explain the reduction in schizophrenia and the increase in autism that are suggested in the recent literature (see main text). Both disorders are associated with inflammatory episodes during pregnancy that lead to neurodevelopmental abnormalities. But the severity of the failure of immunoregulation is much greater in autism, and persists into adulthood. The immunological points listed in the boxes at lower left and right are taken from, and fully explained within, the references discussed in the main text.

that the prevalence of depression should be greater in developed countries than in developing ones. Comparative studies are difficult to do but this is indeed what is indicated by data gathered by the World Health Organization (*Ustun* et al., 2004).

Schizophrenia and autism

In developed countries pregnant women will themselves have poor immunoregulation as a result of reduced contact with immunoregulation-inducing Old Friends. This will mean that they have reduced ability to shut off or modulate inflammatory episodes during pregnancy (Figure 5), such as those involved in driving the developmental abnormalities that lead to many cases of autism or schizophrenia (*Meyer* et al., 2011), (see Figure 3). Because the inflammatory component of autism is much greater than that seen in schizophrenia, one likely consequence of failing immunoregulation would be a reduction in the prevalence of schizophrenia, and an increase in autism. There is evidence that autism is becoming more common (*Williams* et al., 2006) while schizophrenia may be showing the opposite trend (*Der* et al., 1990; *Woogh*, 2001). It is of particular interest to note that genetic studies have revealed that some maternal genes, such as HLA DR4, are involved in modulating the risk of these disorders even when not inherited by the foetus (*Johnson* et al., 2009). It will be

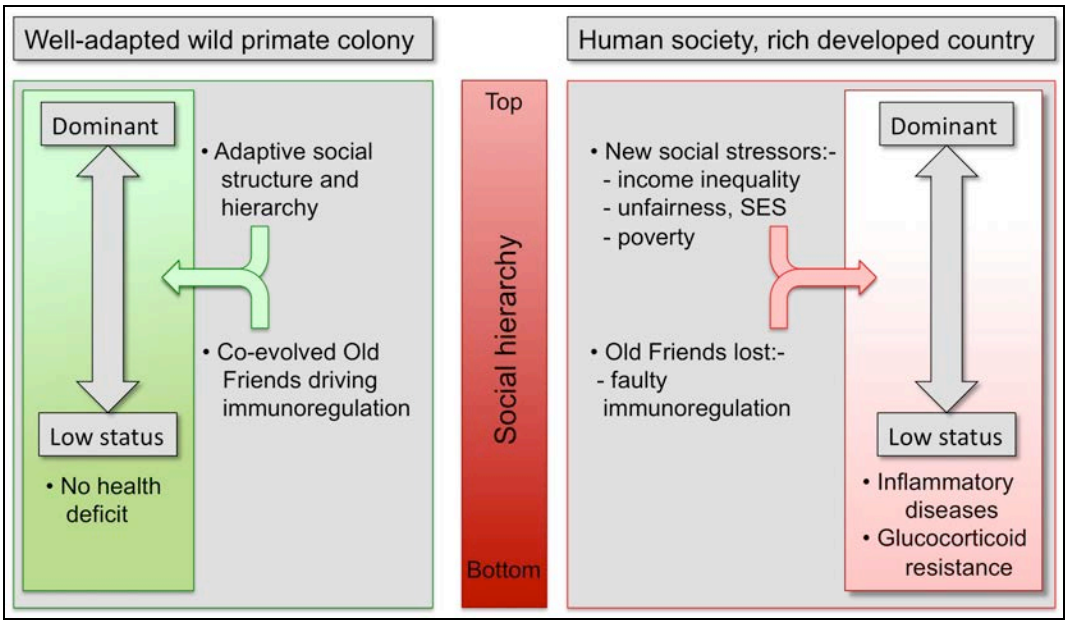

Figure 6: The consequences for long-term health of socioeconomic status and dominance hierarchies. It is often assumed that low position in a dominance hierarchy automatically leads to health deficits, particularly chronic inflammatory problems and cardiovascular disease. This seems maladaptive in a Darwinian sense because low status individuals may later become leaders and breeders. Some recent studies have revealed that in monkeys and primates low status can be compatible with health (i.e. no evidence of stress or raised glucocorticoids). This is likely to be the evolutionarily adaptive state, prevalent in the wild, when habitat is undisturbed and unrestricted, and human observers are absent. We suggest that the health penalty of low socioeconomic status (SES) in modern human communities is attributable to failing immunoregulation (loss of Old Friends) superimposed on the social stress of poverty and perceived "unfairness" (income inequality etc.).

interesting to ask whether many of these genes, like HLA DR4 (*Johnson* et al., 2009), have a role in the control of inflammation.

Health deficits of low socioeconomic status

In the Whitehall study of UK civil servants, circulating levels of CRP and IL-6 were inversely correlated with employment grade, implying an inverse relationship between socioeconomic status (SES) and background inflammation (*Hemingway* et al., 2003). This and other studies show that this gradient is associated with a health deficit that is measurable at every rank below the top of the gradient, despite the fact that at these upper levels diet and nutrition and healthcare access are not significantly different (*Marmot*, 2006; *Sapolsky*, 2004). A similar phenomenon has been seen in rhesus macaques. In this species there is a linear dominance hierarchy. It has emerged that the lower the position of a given animal within this hierarchy in a captive colony, the higher the expression in the peripheral blood of genes involved in inflammatory responses (*Tung* et al., 2012). There are also links with chronic inflammatory disorders (*Chen* et al., 2009), and low SES in early life leads to decreased glucocorticoid sensitivity and increased pro-inflammatory signalling in adulthood when cells are

stimulated with various ligands *in vitro* (*Miller* et al., 2009b).

While these findings are often thought to indicate that psychosocial stress increases as one's position in a hierarchy decreases, it may well be that disruptions in our relationship with Old Friend organisms may contribute to the association between status and health and inflammation. This possibility is highlighted by the fact that the Old Friends mechanism also impacts serum CRP levels (*McDade* et al., 2010). CRP is lower in adults who experienced greater microbial exposures in childhood (*McDade* et al., 2010). Moreover when CRP levels were followed in the same individuals over several months it emerged that in a developing country the baseline CRP is very low (i.e. fully shut off), with intermittent peaks when inflammation is needed to cope with infection. Thus the prevalence of ''high risk'' CRP (>3 mg/l) is greater in a remote Amazonian population with high rates of infectious disease than in the USA (*Gurven* et al., 2008), but these are *transient peaks* triggered by infection (*McDade* et al., 2012). In contrast, in the USA CRP is often not fully turned off despite lack of a valid requirement for inflammation (*McDade* et al., 2012). There is chronic persistent low-level inflammation indicating poor immunoregulation (*McDade* et al., 2012), and it is this uncontrolled chronic inflammation that can predispose to chronic inflammatory disorders, including the psychiatric effects outlined above.

A simple hypothesis therefore would be that the health deficit driven by low SES is exaggerated in individuals deprived of immunoregulation-inducing Old Friends (Figure 6). If we could compare the health gradients of SES hierarchies in developing and rich countries, an Old Friends perspective would predict that we would find less health deficit at the bottom end of the social gradient in developing countries (assuming that we could eliminate confounders such as unequal access to health care, etc.) than in a developed ones.

But there is also the possibility of a much stronger version of this hypothesis. Perhaps the health deficit of low SES does not occur at all unless immunoregulation is compromised. The notion that any position below the top of a dominance or SES hierarchy is associated with long-term inflammation-mediated damage to health is anti-intuitive and incompatible with Darwinian medicine. Subordinate individuals may later become dominant and play crucial roles as the main leaders and breeders (reviewed in *Sapolsky*, 2004). It is maladaptive for such future breeding stock to receive permanent damage (for instance to the cardiovascular system) earlier in life. Most observations of stress and/or inflammation in subdominant animals have been made in populations that were captive (so partly depleted of Old Friends), and/or undergoing social disruption as a result of human observers and interventions and perimeter fencing. For example in many troops of macaques or baboons subordinate animals have high basal glucocorticoid levels, but in other troops of the same species this effect is not seen (reviewed in *Sapolsky*, 2004). The latter, more difficult to observe and record, is likely to be the adaptive situation, and the norm in thriving undisturbed communities. We tentatively suggest that in human communities, when confounders such as access to nutrition and health care have been eliminated, it will be found that the SES-linked health deficit is a Western artefact of modern social stressors (poverty, unfairness, income disparity) driving inflammation in the context of a dysregulated immune system (Figure 6).

FINAL REMARKS

The purpose of this paper is to explore the relationship between the Hygiene or "Old Friends" hypothesis and the psychosocial stressor hypothesis. The "bottom line" is found in Figures 4, 5 and 6 where we illustrate diagrammatically the ways in which we suggest that these two mechanisms, both equally valid and proven, interact and contribute to the changing patterns of disease in the modern world. These consequences are relevant both to psychiatric disorders and to chronic inflammatory disorders.

This paper is not intended to be a comprehensive review. We did not include the role of immunoregulation and inflammation in cancer apart from a brief comment in the introduction, because this topic was extensively reviewed elsewhere (*Rook* and *Dalgleish*, 2011; *von Hertzen* et al., 2011b). Similarly, we do not include discussion of all the factors known to be relevant to associations between environmental conditions, immune function and physical and mental health (though some of these are listed in Figure 2). For example diet is a major factor that has been omitted. Diet has profound effects on the microbiota, and therefore indirectly on immunoregulation (*Maslowski* and *Mackay*, 2011). Another important factor is delayed exposure to viruses caused by hygienic modern living conditions. Many viruses are harmless when met by neonates, perhaps because of the presence of maternal antibodies, but when encountered later such viruses may trigger inflammatory disorders such as allergies and autoimmunity (*Filippi* and *von Herrath*, 2008; *Harrison* et al., 2008; *Serreze* et al., 2000). Lack of vitamin D is also a feature of modern western lifestyles that has a major impact on immunoregulation and has been implicated in schizophrenia (*McGrath* et al., 2010) as well as in several chronic inflammatory disorders (*Hewison*, 2010; *Poon* et al., 2004; *VanAmerongen* et al., 2004), and exposure to modern pollutants such as dioxins might drive pro-inflammatory Th17 cells via the aryl hydrocarbon receptor (*Veldhoen* et al., 2008).

Similarly, had space permitted there are other psychiatric conditions that could have been included in the discussion because of evidence for inflammatory components (attention deficit hyperactivity disorder [ADHD], and post-traumatic stress disorder [PTSD]) (*Oades*, 2011; *Sommershof* et al., 2009).

In conclusion, we suggest here that the pathways controlling brain development, stress responses and mood are so closely related to those controlling immunoregulation that they all need to be considered together. Breaking down the interdisciplinary barriers might focus more attention on the relevance of psychosocial stressors in inflammatory disorders, and more attention on the potential for anti-inflammatory and immunomodulatory treatments for psychiatric ones.

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LITERATURE

Ahlgren, C., Oden, A., and Lycke, J.: A nationwide survey of the prevalence of multiple sclerosis in immigrant populations of Sweden. Mult. Scler. 18, 1099-1107 (2011).

Antonovsky, A., Leibowitz, U., Smith, H.A., Medalie, J.M., Balogh, M., Kats, R., Halpern, L., and Alter, M.: Epidemiologic study of multiple sclerosis in Israel. I. An overall review of methods and findings. Arch. Neurol. 13, 183-193 (1965).

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, II, Umesaki, Y., Itoh, K., and Honda, K.: Induction of colonic regulatory T cells by indigenous *Clostridium* species. Science 331, 337-341 (2011).

Babu, S., Blauvelt, C.P., Kumaraswami, V., and Nutman, T.B.: Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: Implications for parasite persistence. J. Immunol. 176, 3248-3256 (2006).

Bailey, M.T., Lubach, G.R., and Coe, C.L.: Prenatal stress alters bacterial colonization of the gut in infant monkeys. J. Pediatr. Gastroenterol. Nutr. 38, 414-421 (2004).

Bailey, M.T., Dowd, S.E., Galley, J.D., Hufnagle, A.R., Allen, R.G., and Lyte, M.: Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. Brain Behav. Immun. 25, 397-407 (2011).

Barnes, K.C., Grant, A.V., and Gao, P.: A review of the genetic epidemiology of resistance to parasitic disease and atopic asthma: Common variants for common phenotypes? Curr. Opin. Allergy Clin. Immunol. 5, 379-385 (2005).

Becker, K.G.: Autism, asthma, inflammation, and the hygiene hypothesis. Med. Hypotheses 69, 731-740 (2007).

Beebe, G.W., Kurtzke, J.F., Kurland, L.T., Auth, T.L., and Nagler, B.: Studies on the natural history of multiple sclerosis. 3. Epidemiologic analysis of the army experience in World War II. Neurology 17, 1-17 (1967).

Bergeron, L., Valla, J.P., Smolla, N., Piche, G., Berthiaume, C., and St-Georges, M.: Correlates of depressive disorders in the Quebec general population 6 to 14 years of age. J. Abnorm. Child Psychol. 35, 459-474 (2007).

Blazer, D., George, L.K., Landerman, R., Pennybacker, M., Melville, M.L., Woodbury, M., Manton, K.G., Jordan, K., and Locke, B.: Psychiatric disorders. A rural/urban comparison. Arch. Gen. Psychiatry 42, 651-656 (1985).

Breslau, J., Borges, G., Hagar, Y., Tancredi, D., and Gilman, S.: Immigration to the USA and risk for mood and anxiety disorders: Variation by origin and age at immigration. Psychol. Med. 39, 1117-1127 (2009).

Breslau, J., Borges, G., Tancredi, D., Saito, N., Kravitz, R., Hinton, L., Vega, W., Medina-Mora, M.E., and Aguilar-Gaxiola, S.: Migration from Mexico to the United States and subsequent risk for depressive and anxiety disorders: A cross-national study. Arch. Gen. Psychiatry 68, 428-433 (2011).

Brown, A.S. and Derkits, E.J.: Prenatal infection and schizophrenia: A review of epidemiologic and translational studies. Am. J. Psychiatry 167, 261-280 (2010).

Cabre, P.: Environmental changes and epidemiology of multiple sclerosis in the French West Indies. J. Neurol. Sci. 286, 58-61 (2009).

Calcagni, E. and Elenkov, I.: Stress system activity, innate and T helper cytokines, and susceptibility to immune-related diseases. Ann. NY Acad. Sci. 1069, 62-76 (2006).

Cani, P.D. and Delzenne, N.M.: The gut microbiome as therapeutic target. Pharmacol. Ther. 130, 202-212 (2011).

Cantor-Graae, E. and Selten, J.P.: Schizophrenia and migration: A meta-analysis and review. Am. J. Psychiatry 162, 12-24 (2005).

Cardwell, C.R., Stene, L.C., Joner, G., Bulsara, M.K., Cinek, O., Rosenbauer, J., Ludvigsson, J., Svensson, J., Goldacre, M.J., Waldhoer, T., Jarosz-Chobot, P., Gimeno, S.G., Chuang, L.M., Roberts, C.L., Parslow, R.C., Wadsworth, E.J., Chetwynd, A., Brigis, G., Urbonaite, B., Sipetic, S., Schober, E., Devoti, G., Ionescu-Tirgoviste, C., de Beaufort, C.E., Stoyanov, D., Buschard, K., Radon, K., Glatthaar, C., and Patterson, C.C.: Birth order and childhood type 1 diabetes risk: A pooled analysis of 31 observational studies. Int. J. Epidemiol. 40, 363-374 (2011).

Carpenter, L.L., Gawuga, C.E., Tyrka, A.R., Lee, J.K., Anderson, G.M., and Price, L.H.: Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. Neuropsychopharmacology 35, 2617-2623 (2010).

Carr, I. and Mayberry, J.F.: The effects of migration on ulcerative colitis: A three-year prospective study among Europeans and first- and second- generation South Asians in Leicester (1991-1994). Am. J. Gastroenterol. 94, 2918-2922 (1999).

Carrero, J.J., Stenvinkel, P., Fellstrom, B., Qureshi, A.R., Lamb, K., Heimburger, O., Barany, P., Radhakrishnan, K., Lindholm, B., Soveri, I., Nordfors, L., and Shiels, P.G.: Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients. J. Intern. Med. 263, 302-312 (2008).

Chen, E., Miller, G.E., Walker, H.A., Arevalo, J.M., Sung, C.Y., and Cole, S.W.: Genome-wide transcriptional profiling linked to social class in asthma. Thorax 64, 38-43 (2009).

Cherubini, V., Carle, F., Gesuita, R., Iannilli, A., Tuomilehto, J., Prisco, F., Iafusco, D., Altobelli, E., Chiarelli, F., De Giorgi, G., and Falorni, A.: Large incidence variation of Type I diabetes in central-southern Italy 1990-1995: Lower risk in rural areas. Diabetologia 42, 789-792 (1999).

Choi, J., Fauce, S.R., and Effros, R.B.: Reduced telomerase activity in human T lymphocytes exposed to cortisol. Brain Behav. Immun. 22, 600-605 (2008).

Coe, C.L., Lubach, G.R., Karaszewski, J.W., and Ershler, W.B.: Prenatal endocrine activation alters postnatal cellular immunity in infant monkeys. Brain Behav. Immun. 10, 221-234 (1996).

Coe, C.L., Lubach, G.R., and Karaszewski, J.W.: Prenatal stress and immune recognition of self and nonself in the primate neonate. Biol. Neonate 76, 301-310 (1999).

Coid, J.W., Kirkbride, J.B., Barker, D., Cowden, F., Stamps, R., Yang, M., and Jones, P.B.: Raised incidence rates of all psychoses among migrant groups: Findings from the East London first episode psychosis study. Arch. Gen. Psychiatry 65, 1250-1258 (2008).

Compton, W.M., Conway, K.P., Stinson, F.S., and Grant, B.F.: Changes in the prevalence of major depression and comorbid substance use disorders in the United States between 1991-1992 and 2001-2002. Am. J. Psychiatry 163, 2141-2147 (2006).

Correale, J. and Farez, M.: Association between parasite infection and immune responses in multiple sclerosis. Ann. Neurol. 61, 97-108 (2007).

Correale, J. and Farez, M.F.: The impact of parasite infections on the course of multiple sclerosis. J. Neuroimmunol. (2011).

Coussons-Read, M.E., Okun, M.L., Schmitt, M.P., and Giese, S.: Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. Psychosom. Med. 67, 625-631 (2005).

Coussons-Read, M.E., Okun, M.L., and Nettles, C.D.: Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav. Immun. 21, 343-350 (2007).

Crespi, B.J., and Thiselton, D.L.: Comparative immunogenetics of autism and schizophrenia. Genes Brain Behav. 10, 689-701 (2011).

Dacou-Voutetakis, C., Karavanaki, K., and Tsoka-Gennatas, H.: National data on the epidemiology of IDDM in Greece. Cases diagnosed in 1992. Hellenic Epidemiology Study Group. Diabetes Care 18, 552-554

(1995).

Danese, A., Pariante, C.M., Caspi, A., Taylor, A., and Poulton, R.: Childhood maltreatment predicts adult inflammation in a life-course study. Proc. Natl. Acad. Sci. USA 104, 1319-1324 (2007).

Danese, A., Moffitt, T.E., Pariante, C.M., Ambler, A., Poulton, R., and Caspi, A.: Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch. Gen. Psychiatry 65, 409-415 (2008).

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G., and Lionetti, P.: Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci. USA 107, 14691-14696 (2010).

Dealberto, M.J.: Ethnic origin and increased risk for schizophrenia in immigrants to countries of recent and longstanding immigration. Acta Psychiatr. Scand. 121, 325-339 (2010).

Dean, G.: Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African-born and in white immigrants to South Africa. Br. Med. J. 2, 724-730 (1967).

Der, G., Gupta, S., and Murray, R.M.: Is schizophrenia disappearing? Lancet 335, 513-516 (1990).

Ege, M.J., Herzum, I., Buchele, G., Krauss-Etschmann, S., Lauener, R.P., Roponen, M., Hyvarinen, A., Vuitton, D.A., Riedler, J., Brunekreef, B., Dalphin, J.C., Braun-Fahrlander, C., Pekkanen, J., Renz, H., and von Mutius, E.: Prenatal exposure to a farm environment modifies atopic sensitization at birth. J. Allergy Clin. Immunol. 122, 407-412, 412.e411-414 (2008).

Eldeirawi, K., McConnell, R., Furner, S., Freels, S., Stayner, L., Hernandez, E., Amoruso, L., Torres, S., and Persky, V.W.: Associations of doctor-diagnosed asthma with immigration status, age at immigration, and length of residence in the United States in a sample of Mexican American School Children in Chicago. J. Asthma 46, 796-802 (2009).

Entringer, S., Kumsta, R., Nelson, E.L., Hellhammer, D.H., Wadhwa, P.D., and Wust, S.: Influence of prenatal psychosocial stress on cytokine production in adult women. Dev. Psychobiol. 50, 579-587 (2008).

Entringer, S., Buss, C., Kumsta, R., Hellhammer, D.H., Wadhwa, P.D., and Wust, S.: Prenatal psychosocial stress exposure is associated with subsequent working memory performance in young women. Behav. Neurosci. 123, 886-893 (2009a).

Entringer, S., Kumsta, R., Hellhammer, D.H., Wadhwa, P.D., and Wust, S.: Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. Horm. Behav. 55, 292-298 (2009b).

Entringer, S., Epel, E.S., Kumsta, R., Lin, J., Hellhammer, D.H., Blackburn, E.H., Wust, S., and Wadhwa, P.D.: Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proc. Natl. Acad. Sci. USA 108, E513-518 (2011).

Filippi, C.M. and von Herrath, M.G.: Viral trigger for type 1 diabetes: Pros and cons. Diabetes 57, 2863-2871 (2008).

Finegold, S.M., Dowd, S.E., Gontcharova, V., Liu, C., Henley, K.E., Wolcott, R.D., Youn, E., Summanen, P.H., Granpeesheh, D., Dixon, D., Liu, M., Molitoris, D.R., and Green, J.A., 3rd: Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16, 444-453 (2010).

Fitzpatrick, A.L., Kronmal, R.A., Gardner, J.P., Psaty, B.M., Jenny, N.S., Tracy, R.P., Walston, J., Kimura, M., and Aviv, A.: Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am. J. Epidemiol. 165, 14-21 (2007).

Fleming, J., Isaak, A., Lee, J., Luzzio, C., Carrithers, M., Cook, T., Field, A., Boland, J., and Fabry, Z.: Probiotic helminth administration in relapsing-remitting multiple sclerosis: A phase 1 study. Mult. Scler. 17,

743-754 (2011).
Fredericks, C.A., Drabant, E.M., Edge, M.D., Tillie, J.M., Hallmayer, J., Ramel, W., Kuo, J.R., Mackey, S., Gross, J.J., and Dhabhar, F.S.: Healthy young women with serotonin transporter SS polymorphism show a pro-inflammatory bias under resting and stress conditions. Brain Behav. Immun. 24, 350-357 (2010).
Freeman, H. and Alpert, M.: Prevalence of schizophrenia in an urban population. Br. J. Psychiatry 149, 603-611 (1986).
Friberg, I.M., Bradley, J.E., and Jackson, J.A.: Macroparasites, innate immunity and immunoregulation: Developing natural models. Trends Parasitol. 26, 540-549 (2010).
Fumagalli, M., Pozzoli, U., Cagliani, R., Comi, G.P., Riva, S., Clerici, M., Bresolin, N., and Sironi, M.: Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. J. Exp. Med. 206, 1395-1408 (2009).
Gale, C.R. and Martyn, C.N.: Migrant studies in multiple sclerosis. Prog. Neurobiol. 47, 425-448 (1995).
Gareau, M.G., Silva, M.A., and Perdue, M.H.: Pathophysiological mechanisms of stress-induced intestinal damage. Curr. Mol. Med. 8, 274-281 (2008).
Grainger, J.R., Smith, K.A., Hewitson, J.P., McSorley, H.J., Harcus, Y., Filbey, K.J., Finney, C.A.M., Greenwood, E.J.D., Knox, D.P., Wilson, M.S., Belkaid, Y., Rudensky, A.Y., and Maizels, R.M.: Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. J. Exp. Med. 207, 2331-2341 (2010).
Guo, J.U., Ma, D.K., Mo, H., Ball, M.P., Jang, M.H., Bonaguidi, M.A., Balazer, J.A., Eaves, H.L., Xie, B., Ford, E., Zhang, K., Ming, G.L., Gao, Y., and Song, H.: Neuronal activity modifies the DNA methylation landscape in the adult brain. Nat. Neurosci. 14, 1345-1351 (2011).
Gurven, M., Kaplan, H., Winking, J., Finch, C., and Crimmins, E.M.: Aging and inflammation in two epidemiological worlds. J. Gerontol. A Biol. Sci. Med. Sci. 63, 196-199 (2008).
Hampe, J., Heymann, K., Krawczak, M., and Schreiber, S.: Association of inflammatory bowel disease with indicators for childhood antigen and infection exposure. Int. J. Colorectal Dis. 18, 413-417 (2003).
Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Makela, M.J., Vartiainen, E., Kosunen, T.U., Alenius, H., and Haahtela, T.: Environmental biodiversity, human microbiota, and allergy are interrelated. Proc. Natl. Acad. Sci. USA 109, 8334-8339 (2012).
Haroon, E., Raison, C.L., and Miller, A.H.: Psychoneuroimmunology meets neuropsychopharmacology: Translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 37, 137-162 (2012).
Harrison, L.C., Honeyman, M.C., Morahan, G., Wentworth, J.M., Elkassaby, S., Colman, P.G., and Fourlanos, S.: Type 1 diabetes: Lessons for other autoimmune diseases? J. Autoimmun. 31, 306-310 (2008).
Haukka, J.K., Suvisaari, J., and Lonnqvist, J.: Family structure and risk factors for schizophrenia: Case-sibling study. BMC Psychiatry 4, 41 (2004).
Hayakawa, M., Asahara, T., Henzan, N., Murakami, H., Yamamoto, H., Mukai, N., Minami, Y., Sugano, M., Kubota, N., Uegaki, S., Kamoshida, H., Sawamura, A., Nomoto, K., and Gando, S.: Dramatic changes of the gut flora immediately after severe and sudden insults. Dig. Dis. Sci. 56, 2361-2365 (2011).
Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., and Pettersson, S.: Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. USA 108, 3047-3052 (2011).
Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., Bonsall, R., Miller, A.H., and Nemeroff, C.B.: Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in

childhood. JAMA 284, 592-597 (2000).
Hemingway, H., Shipley, M., Mullen, M.J., Kumari, M., Brunner, E., Taylor, M., Donald, A.E., Deanfield, J.E., and Marmot, M.: Social and psychosocial influences on inflammatory markers and vascular function in civil servants (the Whitehall II study). Am. J. Cardiol. 92, 984-987 (2003).
Hewison, M.: Vitamin D and the immune system: New perspectives on an old theme. Endocrinol. Metab. Clin. North Am. 39, 365-379 (2010).
Hjern, A., Rasmussen, F., and Hedlin, G.: Age at adoption, ethnicity and atopic disorder: A study of internationally adopted young men in Sweden. Pediatr. Allergy Immunol. 10, 101-106 (1999).
Hohensinner, P.J., Goronzy, J.J., and Weyand, C.M.: Telomere dysfunction, autoimmunity and aging. Aging Dis. 2, 524-537 (2011).
Hou, J.K., El-Serag, H., and Thirumurthi, S.: Distribution and manifestations of inflammatory bowel disease in Asians, Hispanics, and African Americans: A systematic review. Am. J. Gastroenterol. 104, 2100-2109 (2009).
Howerton, C.L., and Bale, T.L.: Prenatal programing: At the intersection of maternal stress and immune activation. Horm. Behav. 62, 237-242 (2012).
Jacobs, T.L., Epel, E.S., Lin, J., Blackburn, E.H., Wolkowitz, O.M., Bridwell, D.A., Zanesco, A.P., Aichele, S.R., Sahdra, B.K., MacLean, K.A., King, B.G., Shaver, P.R., Rosenberg, E.L., Ferrer, E., Wallace, B.A., and Saron, C.D.: Intensive meditation training, immune cell telomerase activity, and psychological mediators. Psychoneuroendocrinology 36, 664-681 (2011).
Jenq, R.R., Ubeda, C., Taur, Y., Menezes, C.C., Khanin, R., Dudakov, J.A., Liu, C., West, M.L., Singer, N.V., Equinda, M.J., Gobourne, A., Lipuma, L., Young, L.F., Smith, O.M., Ghosh, A., Hanash, A.M., Goldberg, J.D., Aoyama, K., Blazar, B.R., Pamer, E.G., and van den Brink, M.R.: Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. J. Exp. Med. 209, 903-911 (2012).
Johnson, W.G., Buyske, S., Mars, A.E., Sreenath, M., Stenroos, E.S., Williams, T.A., Stein, R., and Lambert, G.H.: HLA-DR4 as a risk allele for autism acting in mothers of probands possibly during pregnancy. Arch. Pediatr. Adolesc. Med. 163, 542-546 (2009).
Kapoor, A., Dunn, E., Kostaki, A., Andrews, M.H., and Matthews, S.G.: Fetal programming of hypothalamo-pituitary-adrenal function: Prenatal stress and glucocorticoids. J. Physiol. 572, 31-44 (2006).
Karimi, K., Inman, M.D., Bienenstock, J., and Forsythe, P.: *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. Am. J. Respir. Crit. Care Med. 179, 186-193 (2009).
Keen, D.V., Reid, F.D., and Arnone, D.: Autism, ethnicity and maternal immigration. Br. J. Psychiatry 196, 274-281 (2010).
Kiliaan, A.J., Saunders, P.R., Bijlsma, P.B., Berin, M.C., Taminiau, J.A., Groot, J.A., and Perdue, M.H.: Stress stimulates transepithelial macromolecular uptake in rat jejunum. Am. J. Physiol. 275, G1037-1044 (1998).
Korosi, A., Naninck, E.F., Oomen, C.A., Schouten, M., Krugers, H., Fitzsimons, C., and Lucassen, P.J.: Early-life stress mediated modulation of adult neurogenesis and behavior. Behav. Brain Res. 227, 400-409 (2012).
Kovess-Masfety, V., Lecoutour, X., and Delavelle, S.: Mood disorders and urban/rural settings: comparisons between two French regions. Soc. Psychiatry Psychiatr. Epidemiol. 40, 613-618 (2005).
Lauritsen, M.B., Pedersen, C.B., and Mortensen, P.B.: Effects of familial risk factors and place of birth on the risk of autism: A nationwide register-based study. J. Child. Psychol. Psychiatry 46, 963-971 (2005).
Le Bert, N., Chain, B.M., Rook, G., and Noursadeghi, M.: DC priming by *M. vaccae* inhibits Th2 responses in contrast to specific TLR2 priming and is associated with se-

lective activation of the CREB pathway. PLoS One 6, e18346 (2011).

Lederbogen, F., Kirsch, P., Haddad, L., Streit, F., Tost, H., Schuch, P., Wust, S., Pruessner, J.C., Rietschel, M., Deuschle, M., and Meyer-Lindenberg, A.: City living and urban upbringing affect neural social stress processing in humans. Nature 474, 498-501 (2011).

Li, X., Sundquist, J., Hemminki, K., and Sundquist, K.: Risk of inflammatory bowel disease in first- and second-generation immigrants in Sweden: A nationwide follow-up study. Inflamm. Bowel Dis. 17, 1784-1791 (2011).

Liu, W., Li, Y., Learn, G.H., Rudicell, R.S., Robertson, J.D., Keele, B.F., Ndjango, J.B., Sanz, C.M., Morgan, D.B., Locatelli, S., Gonder, M.K., Kranzusch, P.J., Walsh, P.D., Delaporte, E., Mpoudi-Ngole, E., Georgiev, A.V., Muller, M.N., Shaw, G.M., Peeters, M., Sharp, P.M., Rayner, J.C., and Hahn, B.H.: Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. Nature 467, 420-425 (2010).

Lowis, G.W.: The social epidemiology of multiple sclerosis. Sci. Total Environ. 90, 163-190 (1990).

Maes, M., Scharpe, S., Van Grootel, L., Uyttenbroeck, W., Cooreman, W., Cosyns, P., and Suy, E.: Higher alpha 1-antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression: Further evidence for the existence of an inflammatory response during that illness. J. Affect. Disord. 24, 183-192 (1992).

Maes, M., Kubera, M., and Leunis, J.C.: The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuro Endocrinol. Lett. 29, 117-124 (2008).

Marmot, M.G.: Status syndrome: A challenge to medicine. JAMA 295, 1304-1307 (2006).

Maslowski, K.M. and Mackay, C.R.: Diet, gut microbiota and immune responses. Nat. Immunol. 12, 5-9 (2011).

McDade, T.W., Rutherford, J., Adair, L., and Kuzawa, C.W.: Early origins of inflammation: Microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. Proc. Biol. Sci. 277, 1129-1137 (2010).

McDade, T.W., Tallman, P.S., Madimenos, F.C., Liebert, M.A., Cepon, T.J., Sugiyama, L.S., and Snodgrass, J.J.: Analysis of variability of high sensitivity C-reactive protein in lowland ecuador reveals no evidence of chronic low-grade inflammation. Am. J. Hum. Biol. 24, 675-681 (2012).

McGrath, J., Saha, S., Welham, J., El Saadi, O., MacCauley, C., and Chant, D.: A systematic review of the incidence of schizophrenia: The distribution of rates and the influence of sex, urbanicity, migrant status and methodology. BMC Med. 2, 13 (2004).

McGrath, J.J., Burne, T.H., Feron, F., Mackay-Sim, A., and Eyles, D.W.: Developmental vitamin D deficiency and risk of schizophrenia: A 10-year update. Schizophr. Bull. 36, 1073-1078 (2010).

Meyer, U., Feldon, J., and Dammann, O.: Schizophrenia and autism: Both shared and disorder-specific pathogenesis via perinatal inflammation? Pediatr. Res. 69, 26R-33R (2011).

Miller, C.A., Campbell, S.L., and Sweatt, J.D.: DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol. Learn. Mem. 89, 599-603 (2008).

Miller, A.H., Maletic, V., and Raison, C.L.: Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732-741 (2009a).

Miller, G.E., Chen, E., Fok, A.K., Walker, H., Lim, A., Nicholls, E.F., Cole, S., and Kobor, M.S.: Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. Proc. Natl. Acad. Sci. USA 106, 14716-14721 (2009b).

Miller, L.J., Willis, J.A., Pearce, J., Barnett, R., Darlow, B.A., and Scott, R.S.: Urban-rural variation in childhood type 1 diabetes incidence in Canterbury, New Zealand, 1980-2004. Health Place 17, 248-256 (2011).

Milo, R. and Kahana, E.: Multiple sclerosis: Geoepidemiology, genetics and the environment. Autoimmun. Rev. 9, A387-394 (2010).

Moller, M., Gravenor, M.B., Roberts, S.E., Sun, D., Gao, P., and Hopkin, J.M.: Genetic haplotypes of Th-2 immune signalling link allergy to enhanced protection to parasitic worms. Hum. Mol. Genet. 16, 1828-1836 (2007).

Moorin, R.E., Holman, C.D., Garfield, C., and Brameld, K.J.: Health related migration: Evidence of reduced "urban-drift". Health Place 12, 131-140 (2006).

Musselman, D.L., Lawson, D.H., Gumnick, J.F., Manatunga, A.K., Penna, S., Goodkin, R.S., Greiner, K., Nemeroff, C.B., and Miller, A.H.: Paroxetine for the prevention of depression induced by high-dose interferon alfa. N. Engl. J. Med. 344, 961-966 (2001).

Nicolaou, N., Siddique, N., and Custovic, A.: Allergic disease in urban and rural populations: Increasing prevalence with increasing urbanization. Allergy 60, 1357-1360 (2005).

O'Mahony, S.M., Marchesi, J.R., Scully, P., Codling, C., Ceolho, A.M., Quigley, E.M., Cryan, J.F., and Dinan, T.G.: Early life stress alters behavior, immunity, and microbiota in rats: Implications for irritable bowel syndrome and psychiatric illnesses. Biol. Psychiatry 65, 263-267 (2009).

Oades, R.D.: An exploration of the associations of pregnancy and perinatal features with cytokines and tryptophan/kynurenine metabolism in children with attention-deficit hyperactivity disorder (ADHD). Atten. Defic. Hyperact. Disord. 3, 301-318 (2011).

Onore, C., Careaga, M., and Ashwood, P.: The role of immune dysfunction in the pathophysiology of autism. Brain Behav. Immun. 26, 383-392 (2012).

Ornish, D., Lin, J., Daubenmier, J., Weidner, G., Epel, E., Kemp, C., Magbanua, M.J., Marlin, R., Yglecias, L., Carroll, P.R., and Blackburn, E.H.: Increased telomerase activity and comprehensive lifestyle changes: A pilot study. Lancet Oncol. 9, 1048-1057 (2008).

Osada, Y. and Kanazawa, T.: Parasitic helminths: New weapons against immunological disorders. J. Biomed. Biotechnol. 2010, 743-758 (2010).

Pace, T.W., Mletzko, T.C., Alagbe, O., Musselman, D.L., Nemeroff, C.B., Miller, A.H., and Heim, C.M.: Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am. J. Psychiatry 163, 1630-1633 (2006).

Pace, T.W., Wingenfeld, K., Schmidt, I., Meinlschmidt, G., Hellhammer, D.H., and Heim, C.M.: Increased peripheral NF-kappaB pathway activity in women with childhood abuse-related posttraumatic stress disorder. Brain Behav. Immun. 26, 13-17 (2012).

Peen, J., Schoevers, R.A., Beekman, A.T., and Dekker, J.: The current status of urban-rural differences in psychiatric disorders. Acta Psychiatr. Scand. 121, 84-93 (2010).

Peng, H. and Hagopian, W.: Environmental factors in the development of Type 1 diabetes. Rev. Endocr. Metab. Disord. 7, 149-162 (2006).

Pereg, D., Tirosh, A., Lishner, M., Goldberg, A., Shochat, T., and Confino-Cohen, R.: Prevalence of asthma in a large group of Israeli adolescents: Influence of country of birth and age at migration. Allergy 63, 1040-1045 (2008).

Poon, A.H., Laprise, C., Lemire, M., Montpetit, A., Sinnett, D., Schurr, E., and Hudson, T.J.: Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. Am. J. Respir. Crit. Care Med. 170, 967-973 (2004).

Pundziute-Lycka, A., Urbonaite, B., Ostrauskas, R., Zalinkevicius, R., and Dahlquist, G.G.: Incidence of type 1 diabetes in Lithuanians aged 0-39 years varies

by the urban-rural setting, and the time change differs for men and women during 1991-2000. Diabetes Care 26, 671-676 (2003).

Radon, K., Windstetter, D., Poluda, A.L., Mueller, B., von Mutius, E., and Koletzko, S.: Contact with farm animals in early life and juvenile inflammatory bowel disease: A case-control study. Pediatrics 120, 354-361 (2007).

Raison, C.L., Lowry, C.A., and Rook, G.A.W.: Inflammation, sanitation and consternation: Loss of contact with co-evolved, tolerogenic micro-organisms and the pathophysiology and treatment of major depression. Arch. Gen. Psychiatry 67, 1211-1224 (2010).

Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D., and von Mutius, E.: Exposure to farming in early life and development of asthma and allergy: A cross-sectional survey. Lancet 358, 1129-1133 (2001).

Rook, G.A.W. and Lowry, C.A.: The hygiene hypothesis and psychiatric disorders. Trends Immunol. 29, 150-158 (2008).

Rook, G.A.W.: The broader implications of the hygiene hypothesis. Immunology 126, 3-11 (2009).

Rook, G.A.W.: 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin. Exp. Immunol. 160, 70-79 (2010).

Rook, G.A.W. and Dalgleish, A.: Infection, immunoregulation and cancer. Immunol. Rev. 240, 141-159 (2011).

Rottem, M., Szyper-Kravitz, M., and Shoenfeld, Y.: Atopy and asthma in migrants. Int. Arch. Allergy Immunol. 136, 198-204 (2005).

Round, J.L. and Mazmanian, S.K.: The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9, 313-323 (2009).

Round, J.L., Lee, S.M., Li, J., Tran, G., Jabri, B., Chatila, T.A., and Mazmanian, S.K.: The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332, 974-977 (2011).

Sapolsky, R.M.: Social status and helath in humans and other animals. Annu. Rev. Anthropol. 33, 393-418 (2004).

Schaub, B., Liu, J., Hoppler, S., Schleich, I., Huehn, J., Olek, S., Wieczorek, G., Illi, S., and von Mutius, E.: Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J. Allergy Clin. Immunol. 123, 774-782.e775 (2009).

Schmidt, C.W. and Tolle, R.: [Family constellation in mental disorder. Results and methods of statistical investigations of family size, birth order and sib position (author's transl.)]. Fortschr. Neurol. Psychiatr. Grenzgeb. 45, 20-52 (1977).

Schwarz, E., Guest, P.C., Rahmoune, H., Harris, L.W., Wang, L., Leweke, F.M., Rothermundt, M., Bogerts, B., Koethe, D., Kranaster, L., Ohrmann, P., Suslow, T., McAllister, G., Spain, M., Barnes, A., van Beveren, N.J., Baron-Cohen, S., Steiner, J., Torrey, F.E., Yolken, R.H., and Bahn, S.: Identification of a biological signature for schizophrenia in serum. Mol. Psychiatry 17, 494-502 (2011).

Sepa, A., Frodi, A., and Ludvigsson, J.: Mothers' experiences of serious life events increase the risk of diabetes-related autoimmunity in their children. Diabetes Care 28, 2394-2399 (2005).

Serreze, D.V., Ottendorfer, E.W., Ellis, T.M., Gauntt, C.J., and Atkinson, M.A.: Acceleration of type 1 diabetes by a coxsackievirus infection requires a preexisting critical mass of autoreactive T-cells in pancreatic islets. Diabetes 49, 708-711 (2000).

Singhal, S., Dian, D., Keshavarzian, A., Fogg, L., Fields, J.Z., and Farhadi, A.: The role of oral hygiene in inflammatory bowel disease. Dig. Dis. Sci. 56, 170-175 (2011).

Smith, A.M., Rahman, F.Z., Hayee, B., Graham, S.J., Marks, D.J., Sewell, G.W., Palmer, C.D., Wilde, J., Foxwell, B.M.,

Gloger, I.S., Sweeting, T., Marsh, M., Walker, A.P., Bloom, S.L., and Segal, A.W.: Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. J. Exp. Med. 206, 1883-1897 (2009).

Smith, S.E., Li, J., Garbett, K., Mirnics, K., and Patterson, P.H.: Maternal immune activation alters fetal brain development through interleukin-6. J. Neurosci. 27, 10695-10702 (2007).

Smits, H.H., Engering, A., van der Kleij, D., de Jong, E.C., Schipper, K., van Capel, T.M., Zaat, B.A., Yazdanbakhsh, M., Wierenga, E.A., van Kooyk, Y., and Kapsenberg, M.L.: Selective probiotic bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J. Allergy Clin. Immunol. 115, 1260-1267 (2005).

Söderström, U., Aman, J., and Hjern, A.: Being born in Sweden increases the risk for type 1 diabetes - A study of migration of children to Sweden as a natural experiment. Acta Paediatr. 101, 73-77 (2012).

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.M., Elbert, T., Groettrup, M., and Kolassa, I.T.: Substantial reduction of naive and regulatory T cells following traumatic stress. Brain Behav. Immun. 23, 1117-1124 (2009).

Sotgiu, S., Angius, A., Embry, A., Rosati, G., and Musumeci, S.: Hygiene hypothesis: Innate immunity, malaria and multiple sclerosis. Med. Hypotheses 70, 819-825 (2008).

Stengel, A. and Tache, Y.: Neuroendocrine control of the gut during stress: corticotropin-releasing factor signaling pathways in the spotlight. Annu. Rev. Physiol. 71, 219-239 (2009).

Strachan, D.P.: Hay fever, hygiene, and household size. Brit. Med. J. 299, 1259-1260 (1989).

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., Kubo, C., and Koga, Y.: Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J. Physiol. 558, 263-275 (2004).

Teitelbaum, A.A., Gareau, M.G., Jury, J., Yang, P.C., and Perdue, M.H.: Chronic peripheral administration of corticotropin-releasing factor causes colonic barrier dysfunction similar to psychological stress. Am. J. Physiol. Gastrointest. Liver Physiol. 295, G452-G459 (2008).

Tung, J., Barreiro, L.B., Johnson, Z.P., Hansen, K.D., Michopoulos, V., Toufexis, D., Michelini, K., Wilson, M.E., and Gilad, Y.: Social environment is associated with gene regulatory variation in the rhesus macaque immune system. PNAS 109, 6490-6495 (2012).

Ustun, T.B., Ayuso-Mateos, J.L., Chatterji, S., Mathers, C., and Murray, C.J.: Global burden of depressive disorders in the year 2000. Br. J. Psychiatry 184, 386-392 (2004).

Van Kruiningen, H.J., Joossens, M., Vermeire, S., Joossens, S., Debeugny, S., Gower-Rousseau, C., Cortot, A., Colombel, J.F., Rutgeerts, P., and Vlietinck, R.: Familial Crohn's disease in Belgium: Pedigrees, temporal relationships among cases, and family histories. J. Clin. Gastroenterol. 41, 583-590 (2007).

VanAmerongen, B.M., Dijkstra, C.D., Lips, P., and Polman, C.H.: Multiple sclerosis and vitamin D: an update. Eur. J Clin. Nutr. 58, 1095-1109 (2004).

Vega, W.A., Sribney, W.M., Aguilar-Gaxiola, S., and Kolody, B.: 12-month prevalence of DSM-III-R psychiatric disorders among Mexican Americans: nativity, social assimilation, and age determinants. J. Nerv. Ment. Dis. 192, 532-541 (2004).

Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., Dumoutier, L., Renauld, J.C., and Stockinger, B.: The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453, 106-109 (2008).

Veling, W., Hoek, H.W., Selten, J.P., and

Susser, E.: Age at migration and future risk of psychotic disorders among immigrants in the Netherlands: A 7-year incidence study. Am. J. Psychiatry 168, 1278-1285 (2011).

Verheij, R.A.: Explaining urban-rural variations in health: A review of interactions between individual and environment. Soc. Sci. Med. 42, 923-935 (1996).

Vlajinac, H., Sipetic, S., Marinkovic, J., Bjekic, M., Kocev, N., and Sajic, S.: The Belgrade childhood diabetes study - Comparison of children with type 1 diabetes with their siblings. Paediatr. Perinat. Epidemiol. 20, 238-243 (2006).

von Hertzen, L., Hanski, I., and Haahtela, T.: Natural immunity. Biodiversity loss and inflammatory diseases are two global megatrends that might be related. EMBO Rep. 12, 1089-1093 (2011a).

von Hertzen, L.C., Joensuu, H., and Haahtela, T.: Microbial deprivation, inflammation and cancer. Cancer Metastasis Rev. 30, 211-223 (2011b).

Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., and Meaney, M.J.: Epigenetic programming by maternal behavior. Nat. Neurosci. 7, 847-854 (2004).

Weinstock, M.: The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav. Immun. 19, 296-308 (2005).

Wells, V.E., Deykin, E.Y., and Klerman, G.L.: Risk factors for depression in adolescence. Psychiatr. Dev. 3, 83-108 (1985).

Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., Hu, C., Wong, F.S., Szot, G.L., Bluestone, J.A., Gordon, J.I., and Chervonsky, A.V.: Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 455, 1109-1113 (2008).

Wildin, R.S., Smyk-Pearson, S., and Filipovich, A.H.: Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J. Med. Genet. 39, 537-545 (2002).

Willette, A.A., Lubach, G.R., Knickmeyer, R.C., Short, S.J., Styner, M., Gilmore, J.H., and Coe, C.L.: Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia. Behav. Brain Res. 219, 108-115 (2011).

Williams, J.G., Higgins, J.P., and Brayne, C.E.: Systematic review of prevalence studies of autism spectrum disorders. Arch. Dis. Child. 91, 8-15 (2006).

Woogh, C.: Is schizophrenia on the decline in Canada? Can. J. Psychiatry 46, 61-67 (2001).

Zerbo, O., Iosif, A.M., Walker, C., Ozonoff, S., Hansen, R.L., and Hertz-Picciotto, I.: Is Maternal Influenza or Fever During Pregnancy Associated with Autism or Developmental Delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) Study. J Autism Dev Disord (2012).

Zilber, N., Kutai-Berman, M., Kahana, E., and Korczyn, A.D.: Multiple sclerosis and birth order. Acta Neurol. Scand. 78, 313-317 (1988).

THE EFFECT OF INFECTION AND ANTIBIOTICS ON GUT AND BRAIN

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A growing body of evidence now supports the view that the gut and the brain can communicate and influence each other's function. There are multiple pathways involved in this bi-directional communication, including neural and humoral mechanisms (*Bercik* et al., 2012). The afferent neural pathways include the vagus nerve, spinal afferents and the enteric nervous system (ENS). The gut can influence brain's function by the release of multiple regulatory peptides and pro-inflammatory cytokines, while the brain modulates gastrointestinal function through the autonomic nervous system, hormonal and immune pathways.

Gastrointestinal infection and subsequent inflammation can markedly affect behaviour and central nervous system (CNS) function. Acute inflammation induces a complex of symptoms, termed cytokine-induced sickness behaviour, which include both a neurovegetative and a psychological component (*Dantzer* and *Kelley*, 2007). Neurovegetative symptoms include loss of appetite, sleepiness, fatigue and fever, while psychological symptoms comprise depression, anxiety and cognitive dysfunction. Chronic exposure to pro-inflammatory cytokines results in changes in behaviour and CNS function, as evidenced during experimental cytokine cancer therapy (*Denicoff* et al., 1987). Psychiatric symptoms are also a frequent side effect of interferon treatment for chronic hepatitis C (*Renault* et al., 1987; *Keefe*, 2007).

Most of the evidence that gastrointestinal infections affect brain function comes from experimental animal studies. *Lyte* et al. (1998) demonstrated that mice infected with *C. jejuni* display anxiety-like behaviour, even during the early stage of the infection, when a systemic immune response was undetectable. This suggests that non-immune factors are also involved in gut-brain communication. The same group has shown that the abnormal behaviour in infected mice was present as early as 4 hours post-infection, and this was associated with increased neural activation pattern in vagal and central autonomic pathways, suggesting that the vagus plays a crucial role in the early detection of pathogens (*Gaykema* et al., 2004; *Goehler* et al., 2005). We have shown that chronic infection with *H. pylori* induces changes in the gastric cholinergic nerve function, as well as up-regulation of substance P and CGRP within spinal afferents (*Bercik* et al., 2002). This was accompanied by increased sensitivity to gastric distension and altered gastric emptying (*Bercik* et al., 2009). The chronically infected mice also displayed an abnormal feeding pattern, eating more frequently but smaller amounts of food per feeding bout, which is similar to eating habits in patients with functional dyspepsia, whose principle complaint is early satiety and abdominal fullness. When examining the brain chemistry of these mice, we found that chronic infection altered expression of pro-opiomelanocortin (POMC) in the arcuate nucleus, one of the brain centres

involved in food control, as well increased expression of tumour necrosis factor alpha (TNF-α) in the median eminence, one of the circumventricular organs (*Bercik* et al., 2009). Interestingly, some of these abnormalities did not fully normalize even 2 months after successful *H. pylori* eradication, suggesting that chronic infection may alter permanently the neural and gut function. The infected mice also displayed a trend for anxiety-like behaviour, when assessed by the light preference test, and this abnormal behaviour became more prominent during repeated testing. On closer examination it became clear that the control mice learned and adapted their behaviour in time becoming less anxious, while the infected mice maintained the abnormal behavioural pattern. The results suggest that chronic gastrointestinal infection may affect cognition and memory.

To further explore the effect of chronic infection and inflammation on behaviour, we have used model of chronic non-invasive parasite *Trichuris muris,* which in susceptible mice leads to mild-to-moderate colitis. We have found that mice chronically infected with *T. muris* have anxiety-like phenotype compared to control healthy mice (*Bercik* et al., 2010). This abnormal behaviour was accompanied by decreased expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, which is one of the main brain centres involved in the regulation of mood and memory (*Deng* et al., 2010). BDNF is an important neurotrophin that regulates neural plasticity, and its lower levels were associated with anxiety and depression (*Duman* and *Monteggia*, 2006; *Martinowich* et al., 2007). The infected mice had modestly, but statistically significantly increased serum concentration of the pro-inflammatory cytokines TNF-α and IFN-γ. Both of these cytokines have been shown to alter tryptophan/serotonin metabolism with increasing production of kynurenine, which is able to induce anxiety/depression-like behaviour in a dose dependent fashion (*O'Connor* et al., 2009a,b). Indeed, we found that mice with *T. muris* infection had increased levels of kynurenine, which normalized together with mouse behaviour, after treatment with anti TNF-α agent etanercept (*Bercik* et al., 2010). These data suggest that chronic gut inflammation triggers alterations in the function and biochemistry of the CNS through immune mediated pathways.

It is possible that changes in gut microbiota induced by administration of antibiotics are associated with immune activation in the gut and subsequently with changes in behaviour. We have shown in a murine model, that a one-week oral treatment with non-absorbable antimicrobials induced significant alteration in gut microbiota composition, as well as mild acute inflammation (*Verdú* et al., 2006). This was associated with increased sensitivity to colorectal distension and up-regulation of substance P in the intestine. Interestingly, treatment with probiotic *L. paracasei* ameliorated visceral sensitivity and normalized expression of substance P.

In a recent study using a different mouse strain and lower concentration of the same antimicrobials, we were able to induce a significant intestinal dysbiosis, but which was not associated with over inflammation or changes in neurotransmitter content in the gut (*Bercik* et al., 2011). Interestingly, the mice treated with antimicrobials increased their exploratory behaviour and became more active. This was associated with an increase in BDNF content in the hippocampus and decreased BDNF in the amygdala. This is consistent with the role of hippocampus and amygdala in mood modulation, as

over-activation of the amygdala has been implicated in depression and anxiety (*Drevets*, 2000). Since intraperitoneal administration of antimicrobials did not alter the mouse behaviour and germ-free mice given the same antimicrobials orally did not exhibit changes in behaviour, we can conclude that the increased exploratory behaviour in orally treated mice was due to changes in gut microbiota composition. Interestingly, germ-free mice displayed marked anxiety behaviour after colonization with commensal bacteria (*Bercik* et al., 2011). Overall these data suggest that intestinal microbiota has the capacity to modulate behaviour of the host.

To investigate this concept further, we have compared the behaviour and microbiota composition of NIH Swiss and BALB/c mice. While NIH Swiss are adventurous, with high exploratory drive, BALB/c mice are shy and cautious. Interestingly, these two mouse strains also had different microbiota composition. We thus derived both mouse strains under germ-free conditions and then colonized them with their own microbiota or with microbiota from the other mouse strain. While the mice colonized with the homologous microbiota displayed identical behaviour as their conventional counterparts, germ-free NIH Swiss mice colonized with BALB/c microbiota became more shy and hesitant, and germ-free BALB/c mice colonized with NIH Swiss microbiota became more daring and active (*Bercik* et al., 2011). The changes in mouse behaviour were accompanied by alterations in hippocampal BDNF levels, but no change in immune markers or gut neurotransmitter content was observed. Thus the intestinal microbiota can affect CNS function through immune-independent pathways.

In summary, accumulating data from animal studies suggest that gut inflammation and intestinal microbiota have a profound effect on gut function, behaviour and brain biochemistry of the host. Microbiota-gut-brain communication is complex and likely involves multiple pathways, including neural, immune and metabolic mechanisms. Up to date, human data on microbiota-gut-brain axis is limited and clinical trials are needed to extend our understanding on the role of bacteria in both health and disease.

LITERATURE

Bercík, P., De Giorgio, R., Blennerhassett, P., Verdú, E.F., Barbara, G., and Collins, S.M.: Immune-mediated neural dysfunction in a murine model of chronic *Helicobacter pylori* infection. Gastroenterology 123, 1205-1215 (2002).

Bercik, P., Verdú, E.F., Foster, J.A., Lu, J., Scharringa, A., Kean, I., Wang, L., Blennerhassett, P., and Collins, S.M.: Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of *Helicobacter pylori* infection. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R587-R594 (2009).

Bercik, P., Verdu, E.F., Foster, J.A., Macri, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K.A., Lu, J., Khan, W.I., Corthesy-Theulaz, I., Cherbut, C., Bergonzelli, G.E., and Collins, S.M.: Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139, 2102-2112 (2010).

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., and Collins, S.M.: The intestinal microbi-

ota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141, 599-609 (2011).

Bercik, P., Collins, S.M., and Verdu, E.F.: Microbes and the gut-brain axis. Neurogastroenterol. Motil. 24, 405-413 (2012).

Dantzer, R. and Kelley, K.W.: Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun. 21, 153-160 (2007).

Deng, W., Aimone, J.B., and Gage, F.H.: New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? Nat. Rev. Neurosci. 11, 339-350 (2010).

Denicoff, K.D., Rubinow, D.R., Papa, M.Z., Simpson, C., Seipp, C.A., Lotze, M.T., Chang, A.E., Rosenstein, D., and Rosenberg, S.A.: The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. Ann. Intern. Med. 107, 293-300 (1987).

Drevets, W.C.: Neuroimaging studies of mood disorders. Biol. Psychiatry 48, 813-829 (2000).

Duman, R.S. and Monteggia, L.M.: A neurotrophic model for stress-related mood disorders. Biol. Psychiatry 59, 1116–1127 (2006).

Gaykema, R.P., Goehler, L.E., and Lyte, M.: Brain response to cecal infection with *Campylobacter jejuni*: Analysis with Fos immunohistochemistry. Brain Behav. Immun. 18, 238-245 (2004).

Goehler, L.E., Gaykema, R.P., Opitz, N., Reddaway, R., Badr, N., Lyte, M.: Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with *Campylobacter jejuni*. Brain Behav. Immun. 19, 334-344 (2005).

Keefe, B.: Interferon-induced depression in hepatitis C: An update. Curr. Psychiatry Rep. 9, 255-261 (2007).

Lyte, M., Varcoe, J.J., and Bailey, M.T.: Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. Physiol. Behav. 65, 63-68 (1998).

Martinowich, K., Manji, H., and Lu, B.: New insights into BDNF function in depression and anxiety. Nat. Neurosci. 10, 1089-1093 (2007).

O'Connor, J.C., André, C., Wang, Y., Lawson, M.A., Szegedi, S.S., Lestage, J., Castanon, N., Kelley, K.W., and Dantzer, R.: Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. J. Neurosci. 29, 4200-4209 (2009a).

O'Connor, J.C., Lawson, M.A., André, C., Moreau, M., Lestage, J., Castanon, N., Kelley, K.W., and Dantzer, R.: Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol. Psychiatry 14, 511-522 (2009b).

Renault, P.F., Hoofnagle, J.H., Park, Y., Mullen, K.D., Peters, M., Jones, D.B., Rustgi, V., and Jones, E.A.: Psychiatric complications of long-term interferon alfa therapy. Arch. Intern. Med. 147, 1577-1580 (1987).

Verdú, E.F., Bercik, P., Verma-Gandhu, M., Huang, X.X., Blennerhassett, P., Jackson, W., Mao, Y., Wang, L., Rochat, F., and Collins, S.M.: Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 55, 182-190 (2006).

THE EFFECT OF INFECTION AND ANTIBIOTICS ON THE GUT BRAIN AXIS

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SUMMARY

Considerable experimental evidence largely from preclinical studies supports the concept of bidirectional interactions between the gut microbiota and the central nervous system. Multiple chemical, neural and immune signalling mechanisms have been identified which can mediate the transfer of information from complex microbial communities in the gut, to various cell types in the gut wall, including enterochromaffin cells and primary afferent nerves. In rodents, the perturbation of microbial homeostasis by introduction of pathogens can influence activity within brain circuits related to emotional behaviour, even before the development of gut inflammation. The suppression or perturbation of microbial communities by oral antibiotics can also influence such behaviours, and such behavioural changes can be associated with altered brain neurochemistry. Even though it is currently unknown if similar effects occur in adult humans on antibiotic therapy, it is intriguing to speculate that the regular antibiotic consumption early in life may affect brain development via the microbiota gut brain axis.

INTRODUCTION

The importance of interactions between the brain and the digestive system in health and disease has been recognized for many centuries (reviewed in *Mayer* and *Brunnhuber*, 2012). A major scientific breakthrough in understanding these interactions occurred with the discovery of the enteric nervous system (ENS) in the middle of the 19th century (*Costa* et al., 1987; *Furness* et al., 1998; *Furness*, 2006; *Gershon* et al., 1994). The ENS has been referred to as the "second brain," based on its size, complexity and similarity in neurotransmitters and signalling molecules with the brain (*Gershon*, 1998). The topic of top-down modulation of gastrointestinal (GI) function by stress and emotions (*Cannon*, 1929), as well as bottom signalling from visceral afferents to the brain in abdominal pain syndromes, and possible emotion regulation (*James*, 1884; *Mayer*, 2011) has received increased attention during the past two decades, largely due to a series of independent, yet converging scientific discoveries from various fields of research, including enteric neuroscience (reviewed in *Furness*, 2006), neuro-imaging (reviewed in *Mayer*, 2009), intestinal microbiology and host microbial interactions (reviewed in *Artis* and *Grencis*, 2008; *Round* and *Mazmanian*, 2009), and more recently

microbial gut-brain signalling (reviewed in *Collins* and *Bercik*, 2009; *Collins* et al., 2012; *Cryan* and *Dinan*, 2012; *Forsythe* et al., 2010; *Forsythe* and *Kunze*, 2012; *Rhee* et al., 2009;), which discusses emerging pre-clinical and some clinical evidence supporting a role of infections and antibiotic treatment on bi-directional signalling between the gut and the brain.

THE GUT BRAIN AXIS IN HEALTH

There are an estimated 200-600 million neurons in the human ENS, equal to the number of neurons in the spinal cord (*Furness*, 2006). These neurons have been classified on the basis of their morphology, electrophysiological properties and chemical coding into distinct classes of functional specific neurons, including several classes of afferents (*Furness*, 2006; *Wood*, 1987). The size and complexity of the ENS is not surprising when considering some of the unique challenges posed by the interface of the organism with its luminal environment: It interfaces closely with our largest body surface (the intestinal surface area [100 m^2] is approximately 100 times larger than the surface area of the skin), with the largest population of commensal microorganisms of all body surfaces (100 trillion, 40,000 species, 100 fold greater number of genes compared to the human genome (*Kurokawa* et al., 2007), with the gut-associated immune system (containing two-thirds of the body's immune cells), and with thousands of entero-endocrine (EE) cells (containing more than 20 identified hormones). These unparalleled relationships between the GI tract and the brain, with multiple, bi-directional and often interacting interoceptive communication systems emphasize the importance of this system in the maintenance of homeostasis, and make the brain gut axis unique amongst all viscera. A rapidly growing body of evidence supports a crucial role of the gut microbial ecology in the normal functioning of the gut brain axis, with gastro-enteric infections and suppression of the normal microbial ecology by antibiotic treatment representing major threats to its homeostasis.

THE EFFECT OF INTESTINAL INFECTION ON GUT BRAIN INTERACTIONS

From a clinical perspective, profound behavioural changes, as well as changes in mood, affect, cognitive function (attention, concentration) and motivation have long been known as characteristic features of most acute gastro-enteric infections in humans. Similarly, the clinical presentation of patients with diarrhoea and cramp like abdominal pain are hallmarks of these enteric infections. However, the neurobiological mechanisms underlying the effects of gastro-enteric infections on the human gut brain axis are largely unknown.

GASTRO-ENTERIC INFECTIONS IN RODENTS WITH VARIOUS PATHOGENS

The cellular and molecular mechanisms underlying the symptoms of acute abdominal pain in enteric infections have been identified in the form of peripheral and central sensitization of visceral afferent pathways. Similarly, many of the molecular immune and neurobiological mechanisms that mediate the behavioural changes associated with acute enteric infections, including the so-called sickness syndrome (*Watkins* and *Maier*, 1999) have been identified (*Bercik* et al., 2012). More recently a series of intriguing studies from a small number of laboratories have identified behavioural, cognitive and neurobiological effects resulting from perturbations of the normal gut microbiota by different pathogens, and have described similarities and differences in the way the signals from the gut are transmitted to the brain.

Campylobacter jejuni

A pivotal study by Lyte and colleagues demonstrated that mice display altered, anxiety-like behaviour during the early phase of acute infection with *C. jejuni*, a common food-borne pathogen (*Lyte* et al., 1998). This abnormal behaviour consisted of decreased exploratory and increased non-exploratory behaviour during elevated plus-maze task on the second day after oral infection with the intestinal pathogen, compared to saline-treated animals. Interleukin-6 (IL-6) levels and peripheral blood leukocyte populations did not differ significantly between infected and control animals indicating the absence of a fully engaged immune response at the time of the behavioural tests. Based on these findings, the authors suggested that the behavioural changes were not a consequence of cytokine-induced sickness behaviour but were rather mediated by neuronal pathways transmitting information about the shift in gut microbiota to the brain. A subsequent study showed that infection with *C. jejuni* activated sensory visceral structures in the brainstem, on both the first and second day post-oral inoculation, in the nucleus tractus solitarius (NTS) and lateral parabrachial nucleus (LPBN) (*Gaykema* et al., 2004). On the second day, an increase in c-FOS mRNA expression, an indicator of neuronal activity, was observed in the hypothalamic paraventricular nucleus, a brain region involved in the stress response. Since serum levels of IL-6, IL-1ß, and tumour necrosis factor-α (TNF-α) were unchanged over both days, the data are most consistent with vagal transmission of pathogen-related signals to the CNS. Mediation by the vagal afferent pathways was confirmed by the combined result of bilateral increase of c-FOS expression in the NTS, 4-12 hours post oral inoculation with *C. jejuni*, and unchanged levels of circulating pro-inflammatory cytokines (*Goehler* et al., 2005). Other studies focusing on early (7-8 hours) responses to oral inoculation have demonstrated that infection with *C. jejuni* increased anxiety-like behaviour during the hole-board task, accompanied by activation of brain circuits related to emotional behaviour. Increased c-FOS mRNA expression was observed in central autonomic regions, including the paraventricular, basolateral nuclei of the amygdala and parts of bed nucleus striae terminalis. This data is consistent with previous studies as these brain regions are purportedly relaying the viscero-sensory stimuli from vagal afferent pathways to higher order regions mediating behavioural stress responses (*Goehler*

et al., 2008). Together with the earlier identification of vagal afferent pathways in mediating cytokine triggered sickness behaviour (*Watkins* and *Maier*, 1999) these studies illustrate an important role of vagal afferents in detecting an acute pathogen induced change in the gut and transmitting this signal to central fear circuits, even though the peripheral encoding mechanisms of such signals (e.g. inflammatory, pathogen related) may differ.

Citrobacter rodentium

Oral inoculation of mice with another pathogen, *C. rodentium*, has been found to be associated with cognitive dysfunction in non-spatial and working memory, 10 days (time of maximal inflammation) and 30 days post-inoculation (*Gareau* et al, 2011). However, these inflammation associated behavioural effects were only seen in the context of an acute water avoidance stress. On the other hand, germ-free mice, 10 days post oral pathogen inoculation had an exaggerated stress response regardless of the presence or absence of the acute stressor, presumably due to an alteration in responsiveness of the hypothalamic-pituitary-adrenal axis (*Gareau* et al., 2011). When a Lactobacillus-containing probiotic regimen was given to non-germ-free mice before and during infection, serum corticosterone responses to stress were attenuated, c-FOS expression and BDNF levels in the CA1 region of the hippocampus stabilized, and cognitive dysfunction was prevented. Based on these findings, the authors made several speculations:

1. Information about specific enteric bacterial infection reaches the brain through either enterochromaffin cell derived serotonin or corticotropin-releasing factor, priming the HPA-axis to stress.
2. At the time of the stress, abnormal behaviour is observed due to HPA axis induced alterations in hippocampal memory.
3. The primed stress response is heightened in germ free mice due to an absence of a gut microbiome.

Cognitive dysfunction and anxiety-like behaviour 7-8 hours after oral inoculation with *C. rodentium* was most likely mediated by vagal afferent pathways since vagal sensory ganglia from infected mice had higher c-FOS expression (*Lyte* et al., 2006). The data from various pathogenic infections suggests that vagal afferent transmission from gut to brain plays a crucial role in mediating the behavioural and cognitive changes, as well as the observed activation of brain circuits observed during gut infection.

Helicobacter pylori

Studies in chronic *H. pylori* infection of mice have also shown correlation with abnormal feeding behaviour consisting of frequent bouts of feeding with less food consumed per feeding than control animals. Increased levels of plasma ghrelin and postprandial CCK, higher TNF-α in the hypothalamic median eminence (ME), and lower pro-opiomelanocortin (POMC) expression in the arcuate nucleus were associated with the observed delay in gastric emptying and the development of visceral hypersensitivity in infected mice. The ME of the circumventricular organ contains a leaky area of the blood-brain barrier of the CNS, thus allowing metabolites and molecules from the systemic circulation to enter the brain directly rather than being mediated by neuronal activation (*Bercik* et al., 2009). Another study from the same group of mice chronically infected with *H. pylori* found delayed gastric emptying and visceral sensitivity associated with an up-regulation of substance P and calcitonin gene-related

peptide expression in the gut and spinal cord (*Bercik* et al., 2002). Bacterial eradication normalized gut dysfunction symptoms, but did not alter abnormal feeding behaviour and the increase in TNF-α in the brain and gastric CD3(+) T-cell counts remained elevated, suggesting different pathways monitor post-infective gut dysfunction. Interestingly these behavioural feeding changes and the biochemical changes both lasted for 2 months after the bacteria were eradicated, implying that the effects of chronic infection in GI tract can be long lasting (*Bercik* et al., 2009). When the infected animals were given probiotics, abnormal feeding behaviour was normalized and CD3(+) T-cell counts decreased, further suggesting that there are various mechanisms at play during recovery from infection that are not well understood and need further investigation (*Verdu* et al., 2008).

Trichuris muris

Chronic intestinal infestation of mice with *T. muris*, a close relative of the human parasite *Trichuris trichiura*, was accompanied by increased anxiety-like behaviour, as well as decreased expression of brain-derived neurotropic factor (BDNF) in the hippocampus, a multifunctional brain region involved in memory formation and in the inhibition of the central stress response. Intestinal inflammation, observed by the increased plasma levels of pro-inflammatory cytokines TNF-α and interferon-γ, was also accompanied by an increase in the plasma kynurenine:trytophan ratio, reflecting an alteration of tryptophan metabolism in the mice. The *T. muris* infection induced anxiety-like behaviour was not affected by a vagotomy performed prior to the infection, arguing against an important role of afferent vagal pathways in mediating the observed behavioural effects during this type of infection. In contrast, anxiety like behaviour was not observed when mice were treated with the anti-inflammatory agents etanercept (a TNF-α inhibitor) and the steroid budesonide after infection, even though hippocampal BND expression was similar to the infected-only mice. These findings suggest that in this model, the observed anxiety-like behaviour is induced by inflammatory mediators originating in the gut, and exert their effects either via circulating cytokines and inflammation-related changes in altered tryptophan metabolites, rather than mediation by vagal afferents. On the other hand, the authors reported that administration of the probiotic *Bifidobacterium longum* was able to restore normal behaviour and hippocampal BDNF expression, while plasma cytokine and kynurenine levels remained unaffected. Even though the precise mechanisms and pathways underlying the infection induced behavioural changes in this model remain to be determined, the findings suggest that multiple signalling mechanisms can be involved in the transmission of gut pathogen-related information to the brain (e.g. vagal afferents, inflammatory mediators, amino acid metabolites), and that the engagement of these mechanisms can vary depending on the pathogen and the experimental model (*Bercik* et al., 2009). Bercik and co-workeres have shown similar results in SCID mice, which had been orally administered chronic *T. muris*, which caused anxiety-like behaviour and also expressed higher levels of BDNF mRNA in the hippocampus using *in situ* hybridization. The effect of BNDF was avoided when *B. longum* was introduced, although anxiety-like behaviour remained; and the introduction of *L. rhamnosus* normalized anxiety-like behaviour but not BDNF mRNA expression. This further confirms the plethora

of data to date suggesting strongly that there are different signalling mechanisms for how information on the state of the gut's microbiota, specifically its contents work (*Bercik* et al., 2009).

In summary, considerable preclinical evidence has been reported to clearly support the ability of enteric pathogens to signal to the central nervous system and affect behaviour. This pathogen to gut to brain communication system is capable of detecting acute changes in the gut microbial ecology, and of selectively signalling the presence of a pathogen to brain circuits, which are involved in the stress response, behaviour, memory, and learning. Furthermore, there is good evidence to support the existence of multiple pathways or mechanisms mediating the transmission of pathogen related information from the GI tract to the brain. One may speculate that, while signalling via circulating immune mediators plays the dominant role during fully developed mucosal inflammation, peripheral afferent pathways, including enteric and vagal afferents may play an important role at the earliest stages of infection, prior to the full engagement of the immune response (*Bercik* et al., 2012).

THE EFFECT OF ANTIBIOTICS ON GUT BRAIN INTERACTIONS

Despite the well known side effects of orally ingested antibiotics reported in human patients, there are no reports in the literature or anecdotal reports suggesting that suppression of normal microbiota by antibiotics (dysbiosis) are associated with significant changes in mood, affect, cognition or behaviour. This is surprising, given the widespread use of antibiotics both in paediatric and adult populations, the now well established communication between the gut microbiota and the central nervous system, and the recent results from rodent studies suggesting that antibiotic induced suppression of the normal flora is associated with significant behavioural and even neurochemical changes in the brain.

Antibiotic treatment of experimental mice has become one of the most commonly used methods to induce artificial intestinal dysbiosis. Perturbation of the microbiota in adult mice by oral administration of non-absorbable antibiotics neomycin and bacitracin and the antifungal agent natamycin has been shown to increase a viscero-somatic nociceptive reflex to colorectal distension consistent with the development of visceral hypersensitivity following antibiotic induced dysbiosis (*Verdu* et al., 2006). In the same study, the antibiotic induced visceral hypersensitivity was reversed by the oral administration of the probiotic *Lactobacillus paracasei*. This study demonstrated that antibiotics can severely disrupt the central processes responsible for visceral responses by altering the neurotransmitter content of the colon, while certain probiotics may be able to re-establish intestinal symbiosis and neurotransmitter content, thereby normalizing visceral function. In another study by the same group (*Bercik* et al., 2011a), oral administration of neomycin and bacitracin along with the antifungal agent pimaricin to adult BALB/c mice, did not lead to quantitative changes in culturable bacteria but was associated with a transient change in microbial ecology: Antibiotic ingestion was associated with an increase in Actino-bacteria and Lactobacillus species and decrease in c-proteobacteria and bacteroidetes. The antibiotics also induced changes in be-

haviour, with treated animals demonstrating evidence of increased exploratory behaviour in both the step-down and light/ dark preference tests, indicating reduced levels of anxiety. As was demonstrated in comparisons between germ-free and conventional animals, behavioural changes in antibiotic-treated animals were associated with reduced BDNF levels in the amygdala, and increased levels in the hippocampus (*Bercik* et al., 2011a). The effects of antibiotic treatment on the composition of the intestinal microbiota and on behaviour were transient with treated mice resembling controls after a 2-week washout period. In these studies, a causal relationship between microbiota changes and behavioural effects is supported by the demonstration that, in contrast to oral antibiotic treatment, i.p. treatment did not influence behaviour. Furthermore, antibiotic treatment had no effect on the behaviour of germ-free animals (*Bercik* et al., 2011a). Whether the behavioural changes can be attributed to specific alterations in the microbiota, e.g., increased lactobacilli and acinto- bacteria or decreased c-proteobacteria and bacteroidetes, was not investigated. However, this is an intriguing idea especially given subsequent studies demonstrated anxiolytic effects of feeding certain lactobacilli and bifidobacterium strains (*Bercik* et al., 2011b), and as such it would be interesting to assess the effects of prebiotics, e.g. agents that promote the growth of bifidobacteria and lactobacilli, on behaviour.

SUMMARY AND CONCLUSIONS

Growing preclinical literature supports the concept of bidirectional microbiota gut brain communications providing the basis for interactions between three "super systems" within the body: The gut microbiome, the gut associated immune system and the central nervous system. However, despite the excitement about the recent findings demonstrating important influences of the gut microbiota on behaviour and brain signalling systems in rodent models, caution is in place when extrapolating the findings obtained from perturbations of the gut microbiota from rodent behaviours to complex human experiences and subjective symptoms.

In the case of gastro-enteric infections, characteristic human symptoms of nausea, fatigue, lack of motivation, anxiety, depression and abdominal pain may be the human homologues of rodent behaviours and molecular mechanisms reported in the publications discussed above. Functional brain imaging studies in human subjects may provide further evidence to support the translational significance of these observations.

In the case of antibiotics, there is currently no convincing clinical or experimental evidence in human subjects to mimic the dramatic findings observed in rodent models. The reason for this translational gap is currently unknown, but may in part be related to the fact that antibiotics are generally not given to otherwise healthy subjects, but generally in the context of on-going infections. It is conceivable that targeted evaluations of cognitive function, affect and mood in subjects undergoing oral antibiotic therapy will reveal subtle effects in these domains. In view of the widespread use of non-absorbable antibiotics for the treatment of patients with irritable bowel syndrome and symptoms of bloating and abdominal distension, it would be important to know if such treatments have effects on

the CNS. An important aspect of suppression of normal gut microbiota by antibiotics and the resulting effects on the gut brain axis may be related to brain development. Germ-free conditions, an extreme form of dysbiosis, have been found to profoundly influence brain development in rodents. Given the widespread use of antibiotics in neonatal intensive care units, and in paediatrics for common diseases as sinusitis, bronchitis and respiratory tract infections, it is conceivable that the dysbiosis resulting from these interventions may affect brain development in children. Future studies evaluating possible correlations between antibiotic use, dysbiosis and cognitive and emotional functioning and underlying brain networks in children should address this important question.

LITERATURE

Artis, D. and Grencis, R.K.: The intestinal epithelium: Sensors to effectors in nematode infection. Mucosal Immunol. 1, 252-264 (2008).

Bercik, P., Collins, S.M., and Verdu, E.F.: Microbes and the gut-brain axis. Neurogastroenterol. Motil. 24, 405-413 (2012).

Bercik, P., De Giorgio, R., Blennerhassett, P., Verdu, E.F., Barbara, G., and Collins, S.M.: Immune-mediated neural dysfunction in a murine model of chronic *Helicobacter pylori* infection. Gastroenterology 123, 1205-1215 (2002).

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., and Collins, S.M.: The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141, 599-609 (2011a).

Bercik, P., Park, A.J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P.A., Fahnestock, M., Moine, D., Berger, B., Huizinga, J.D., Kunze, W., McLean, P.G., Bergonzelli, G.E., Collins, S.M., and Verdu, E.F.: The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. Neurogastroenterol. Motil. 23, 1132-1139 (2011b).

Bercik, P., Verdu, E.F., Foster, J.A., Lu, J., Scharringa, A., Kean, I., Wang, L., Blennerhassett, P., and Collins, S.M.: Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of *Helicobacter pylori* infection. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R587-R594 (2009).

Bercik, P., Verdu, E.F., Foster, J.A., Macri, J., Potter, M., Huanf, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K.A., Lu, J., Khan, W.I., Corthesy-Theulaz, I., Cherbut, C., Bergonzelli, G.E., and Collins, S.M.: Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139, 2102-2112 (2010).

Cannon, W.B.: Organization for physiological homeostasis. Physiol. Rev. 9, 399-431 (1929).

Collins, S.M. and Bercik, P.: The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology 136, 2003-2014 (2009).

Collins, S.M., Surette, M., and Bercik, P.: The interplay between the intestinal microbiota and the brain. Nat. Rev. Microbiol. 10, 735-742 (2012).

Costa, M., Furness, J.B., and Llewellyn-Smith, I.J.: Histochemistry of the enteric nervous system. In: Physiology of the gastrointestinal tract (Ed.: Johnson, L.R.). Raven Press, New York, 1-40 (1987).

Cryan, J.F. and Dinan, T.G.: Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. Nat. Rev.

Neurosci. 13, 701-712 (2012).
Forsythe, P. and Kunze, W.A.: Voices from within: Gut microbes and the CNS. Cell. Mol. Life Sci., Epub ahead of print (2012).
Forsythe, P., Sudo, N., Dinan, T., Taylor, V.H., and Bienenstock, J.: Mood and gut feelings. Brain Behav. Immun. 24, 9-16 (2010).
Furness, J.B.: The Enteric Nervous System. Blackwell Publishing Ltd., Oxford (2006).
Furness, J.B., Kunze, W.A., Bertrand, P.P., Clerc, N., and Bornstein, J.C.: Intrinsic primary afferent neurons of the intestine. Prog. Neurobiol. 54, 1-18 (1998).
Gareau, M.G., Wine, E., Rodrigues, D.M., Cho, J.H., Whary, M.T., Philpott, D.J., Macqueen, G., and Sherman, P.M.: Bacterial infection causes stress-induced memory dysfunction in mice. Gut 60, 307-317 (2011).
Gaykema, R.P., Goehler, L.E., and Lyte, M.: Brain response to cecal infection with *Campylobacter jejuni*: Analysis with Fos immunohistochemistry. Brain Behav. Immun. 18, 238-245 (2004).
Gershon, M.D.: The second brain: The scientific basis of gut instinct and a groundbreaking new understanding of nervous disorders of the stomach and intestine. Harper Collins Publishers, New York (1998).
Gershon, M.D., Kirchgessner, A.L., and Wade, P.R.: Functional anatomy of the enteric nervous system. In: Physiology of the Gastrointestinal Tract (Eds.: Johnson, L.R., Alpers, D., Christensen, J., Jacobson, E.D., and Walsh, J.H.). Raven Press, New York, 381-422 (1994).
Goehler, L.E., Gaykema, R.P., Opitz, N., Reddaway, R., Badr, N., and Lyte, M.: Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with *Campylobacter jejuni*. Brain Behav. Immun. 19, 334-344 (2005).
Goehler, L.E., Park, S.M., Opitz, N., Lyte, M., and Gaykema, R.P.: *Campylobacter jejuni* infection increases anxiety-like behavior in the holeboard: Possible anatomical substrates for viscerosensory modulation of exploratory behavior. Brain Behav. Immun. 22, 354-366 (2008).
James, W.: What is emotion? Mind 9, 188-205 (1884).
Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K., Toh, H., Toyoda, A., Takami, H., Morita, H., Sharma, V.K., Srivastava, T.P., Taylor, T.D., Noguchi, H., Mori, H., Ogura, Y., Ehrlich, D.S., Itoh, K., Takagi, T., Sakaki, Y., Hayashi, T., and Hattori, M.: Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. DNA Res. 14, 169-181 (2007).
Lyte, M., Li, W., Opitz, N., Gaykema, R.P., and Goehler, L.E.: Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium*. Physiol. Behav. 89, 350-357 (2006).
Lyte, M., Varcoe, J.J., and Bailey, M.T.: Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. Physiol. Behav. 65, 63-68 (1998).
Mayer, E.A.: Gut feelings: the emerging biology of gut-brain communication. Nat. Rev. Neurosci. 12, 453-466 (2011).
Mayer, E.A., Aziz, Q., Coen, S., Kern, M., Labus, J.S., Lane, R., Kuo, B., Naliboff, B., and Tracey, I.: Brain imaging approaches to the study of functional GI disorders: a Rome working team report. Neurogastroenterol. Motil. 21, 579-596 (2009).
Mayer, E.A. and Brunnhuber, S.: Gastrointestinal disorders. Handb. Clin. Neurol. 106, 607-631 (2012).
Rhee, S.H., Pothoulakis, C., and Mayer, E.A.: Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat. Rev. Gastroenterol. Hepatol. 6, 306-314 (2009).
Round, J.L. and Mazmanian, S.K.: The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9, 313-323 (2009).
Verdu, E.F., Bercik, P., Huang, X.X., Lu, J.,

Al-Mutawaly, N., Sakai, H., Tompkins, T.A., Croitoru, K., Tsuchida, E., Perdue, M., and Collins, S.M.: The role of luminal factors in the recovery of gastric function and behavioral changes after chronic *Helicobacter pylori* infection. Am. J. Physiol. Gastrointest. Liver Physiol. 295, G664-G670 (2008).

Verdu, E.F., Bercik, P., Verma-Gandhu, M., Huang, X.X., Blennerhassett, P., Jackson, W., Mao, Y., Wang, L., Rochat, F., and Collins, S.M.: Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 55, 182-190 (2006).

Watkins, L.R. and Maier, S.F.: Implications of immune-to-brain communication for sickness and pain. Proc. Natl. Acad. Sci. USA 96, 7710-7713 (1999).

Wood, J.D.: Physiology of the enteric nervous system. In: Physiology of the Gastrointestinal Tract (Ed.: Johnson, L.R.). Raven Press, New York, 67-110 (1987).

IMPACT OF ENTERIC INFECTIONS ON COGNITIVE DEVELOPMENT: FIELD AND ANIMAL STUDIES OF PROTECTION BY ApoE4

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SUMMARY

Although diarrhoeal diseases in early childhood remain important causes of mortality, the long-term impact of enteric infections, with or without overt diarrhoea, may impair child growth and development. Repeated dehydrating or malnourishing infections can compound other causes of mortality and may also have lifelong consequences for those who survive. This includes a cognitive decrement that may average up to 10 IQ points by 7-9 years of age that can be attributed to diarrhoeal illnesses in the first 2 years of life alone. The cognitive function most affected is semantic, rather than phonemic, fluency, a deficit also seen in early Alzheimer's (vs. Parkinson's) dementia. These effects may be lifelong and may have selected for surprising "survival" or "thrift" genetic alleles like ApoE4 that we have shown to *protect* against the cognitive deficits seen in children with heavy diarrhoea burdens as well as providing protection against the growth and histopathologic impact of enteric infections in a murine model of malnutrition and infection.

In this overview, we review these long-term growth and cognitive effects of early childhood diarrhoea, the Alzheimer-like deficits seen and the surprising protection provided by the ApoE4 allele that increases Alzheimer risk in later life, a new example of "antagonistic pleotropy". We also show this protection in our murine model of cryptosporidiosis using targeted transgenic mice with the human E4 allele and how the molecular mechanism of this benefit can lead to novel interventions to break the vicious cycle of diarrhoea and malnutrition and their devastating consequences for child development.

BETTER COGNITION AND IQ CORRELATE WITH FEWER INFECTIONS

Over 200 million of the world's children less than 5 years of age fail to achieve their developmental potential, are stunted and live in poverty. These children do poorly in school and go on to have low incomes, high fertility and provide poor care for their children, contributing to what Dr. Grantham-McGregor has called "intergenerational transmission of poverty" (*Grantham-*

McGregor et al., 2007). Eppig and colleagues recently suggested that the "Flynn Effect" of improving IQ with development correlates with decreasing "infectious diseases burden", even when controlling for GDP per capita, education, temperature and malnutrition; i.e. the recognized correlation of nutritional deficiencies with IQ is lost when the effects of infectious diseases are removed which they interpret as suggesting that the malnutrition-IQ link appears to occur through infectious diseases (*Eppig* et al., 2010). Whether there is an effect of infections on cognitive development that is independent of their effects on malnutrition is less clear. Although this is a controversial area, our data on diarrhoeal illness correlations also appear to be independent of stunting (<1HAZ) (which is itself also correlated with diarrhoea in the first 2 years of life) (*Pinkerton* et al., 2012).

INFECTION OR STUNTING IMPAIRS COGNITION

Convincing support for the effects of infection on child development came from albendazole trials in Kenya and Jamaica, suggesting that intestinal helminths impair growth and cognitive development. These were the basis of our studies of heavy early childhood diarrhoea burdens in Fortaleza. Stephenson and colleagues reported that, among Kenyan schoolchildren, even a single dose of albendazole showed a benefit in fitness, appetite and weight and height gains within 2 to 4 months when compared with double blinded placebo-treated controls (*Stephenson* et al., 1989, 1990, 1993). Similarly, in studies of schoolchildren in Jamaica, *Nokes* et al. (1992) showed improved fluency (long term memory and retrieval) and digit span backwards/forwards (from WISC, involves attention and distractibility) 2 months after a 3-day course of albendazole (vs. placebo). Since HAZ-2 may be a surrogate for early childhood diarrhoea burdens (*Dillingham* and *Guerrant*, 2004), it may also be relevant that Chang and co-workers described better arithmetic scores at 10 years old with higher HAZ-2 (height for age Z scores at 2 years old) (*Chang* et al., 2011). Although these brief anti-helminthic treatments did not necessarily eradicate intestinal geohelminths (and certainly did not prevent common re-infections), the 2-3 log reduction in major geohelminths provide impressive evidence for the importance of heavy intestinal nematode infections in the physical and cognitive development of schoolchildren. Our own data from Northeast Brazil also suggests that geohelminth infections in early childhood (i.e. from birth to 2 years old) have important, lasting effects on subsequent child growth and development (*Moore* et al., 2001). We and others are now exploring whether stunted children may also be at greater risk for later obesity, diabetes and metabolic syndrome in what we have called a 'collision of bad water with bad diets' (*DeBoer* et al., 2012).

The importance of stunting on cognitive development is clear from several studies (*Grantham-McGregor*, 1995). In studies of nonverbal intelligence at 8-11 years old in the Philippines, Mendez and Adair's group has shown that moderate to severe stunting (i.e. HAZ-2 <-2) at 2 years is associated with a 0.25 to 0.61 standard deviation lower mean cognitive test Z score by age 8 ($p<0.001$) (*Mendez* and *Adair*, 1999). They also noted that stunting (i.e. progressing from -1.5 to -3.3 HAZ) at 2 years correlated with progressive delays in age at starting school from 5 to 8+ years, and that, even

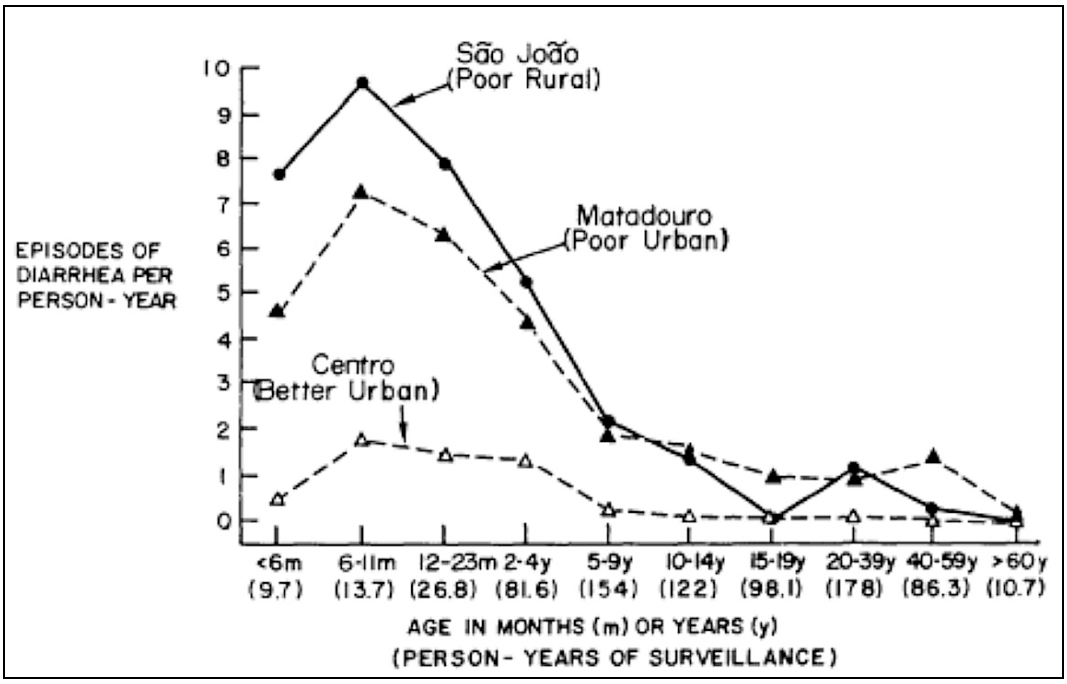


Figure 1: Age specific diarrhoea attack rates in rural and urban communities (Pacatuba and Goncalves Dias) in Northeast Brazil (Guerrant et al., 1983; Lima et al., 2000).

though schooling (achieving 2-6 years of education) improves mean cognitive test scores, there remains a 30% decrement in the stunted children at 11 years; i.e. stunting limits what education can accomplish!

THE CRITICAL 4-24 MONTH AGE "WINDOW" FOR CHILD DEVELOPMENT

Leonardo Mata, in studying The Children of Santa Maria Cauque (*Mata*, 1978) described how even impoverished children often start off on their growth curves reasonably well, only to fall progressively off with the onset of diarrhoeal and other common early childhood infections over the critical first 2 years of life. Precisely the same pattern of fall-off in linear growth (i.e. HAZ) occurs worldwide, with remarkably similar findings in Asia, Africa and Latin America described by Victora and Shrimpton (*Victora* et al., 2010) in 2010, much as they had shown nearly a decade earlier in 2001 (*Shrimpton* et al., 2001).

Feeding studies also confirm the importance of early childhood (3 years) in cognitive development. For example, in the two Guatemalan villages given an "atole" vegetable-protein supplement (163kcal with 11.5g protein) vs. "fresco" sugar sweetened beverage (59kcal and no protein) in two control villages in INCAP studies from 1969-1977. A follow-up 25-35 years later showed that those in the supplemented villages had a 10% better IQ, even after, adjusting for schooling (*Stein* et al., 2008), and that the 25-42 year old males had 46% higher wages, with the females having 8-20% higher reading, Raven scores and schooling if they had

been in the supplemented villages compared with the non-supplemented villages some 40 years before. However these benefits were seen only in those who had been in the supplement program in their first 2-3 years of life, showing the crucial timing of early life in cognitive development (*Hoddinott* et al., 2008).

We also have found increasingly impressive correlations in our Fortaleza studies of early childhood diarrhoea (ECD) that heavy diarrhoea burdens in the first two years of life are associated with impairments in cognitive function (TONI, WISC-III coding and digit span and WRAML mazes) several years later (*Guerrant* et al., 1999; *Niehaus* et al., 2002). Furthermore, these heavy diarrhoea burdens in the first 2 years of life also correlate with impaired schooling (both age at starting school and age-four-grade) several years later (*Lorntz* et al. 2006). Early heavy diarrhoea burdens are also associated with stunting at 2 years of age in our as well as in other multi-country studies (*Moore* et al. 2001; *Checkley* et al., 2008) and, as noted above, stunting at the second birthday (HAZ-2) is clearly associated with impaired cognition as assessed later in life. Although some suggest that the effects of diarrhoea on cognition may be only through its effects on stunting, as noted above, like Eppig, we find that the association of diarrhoea with cognitive impairment remains even when controlling for anthropometry. Thus heavy early childhood diarrhoea burdens clearly associate with lasting effects on stunted growth, and, in turn on impaired cognition. Whether the cognitive impact of early childhood diarrhoea is independent of (i.e. even greater than) the also significant effects of diarrhoea on stunting remains controversial. Nevertheless, the huge effects of early childhood diarrhoea on child growth and development are critical to recognize and ameliorate.

The reasons for the first 2 years of life being so critical are likely at least two-fold: first is the obvious vulnerability to the heaviest burdens of diarrhoea and enteric infection (with rates clearly highest in that age range) (*Guerrant* et al., 1983; *Lima* et al., 2000; *Fischer Walker* et al., 2012) (Figure 1) Second is the rapid development of brain growth and synaptogenesis and myelination in humans in this window from birth to 2 years. Unlike some other species in which brain development occurs predominantly *in utero*, human infants are born with small brains and sparse synapses relative to their near adult levels of brain weight-for-height and even synaptic density by 2 years of life (*Dobbing* and *Sands*, 1973; *Corel*, 1975; *Rice* and *Barone*, 2000; *Thompson* and *Nelson*, 2001) (Figure 2). Not only does the human brain double in size in the first year of life, by 3 years of age it reaches 80% of its adult volume (The Urban Child Institute: Baby's brain begins now, Conception to age 3. URL: www.theurbanchildinstitute.org/why-0-3/baby-and-brain, 2012; Nowakowski, 2006). Furthermore, synapse formation occurs predominantly between birth and 2-3 years of age (by which time the human brain has nearly 200% of its adult number of synapses); after which "blooming and pruning" gradually eliminate nearly half of these synapses throughout childhood and adolescence (*Corel*, 1975; *Huttenlocher*, 2002). These anatomic observations are even more apparent when one considers the impressive incapacity of the human new-born infant compared with the remarkable appearance of motor and cognitive skills including walking, running, talking and personality which develop by the child's second birthday.

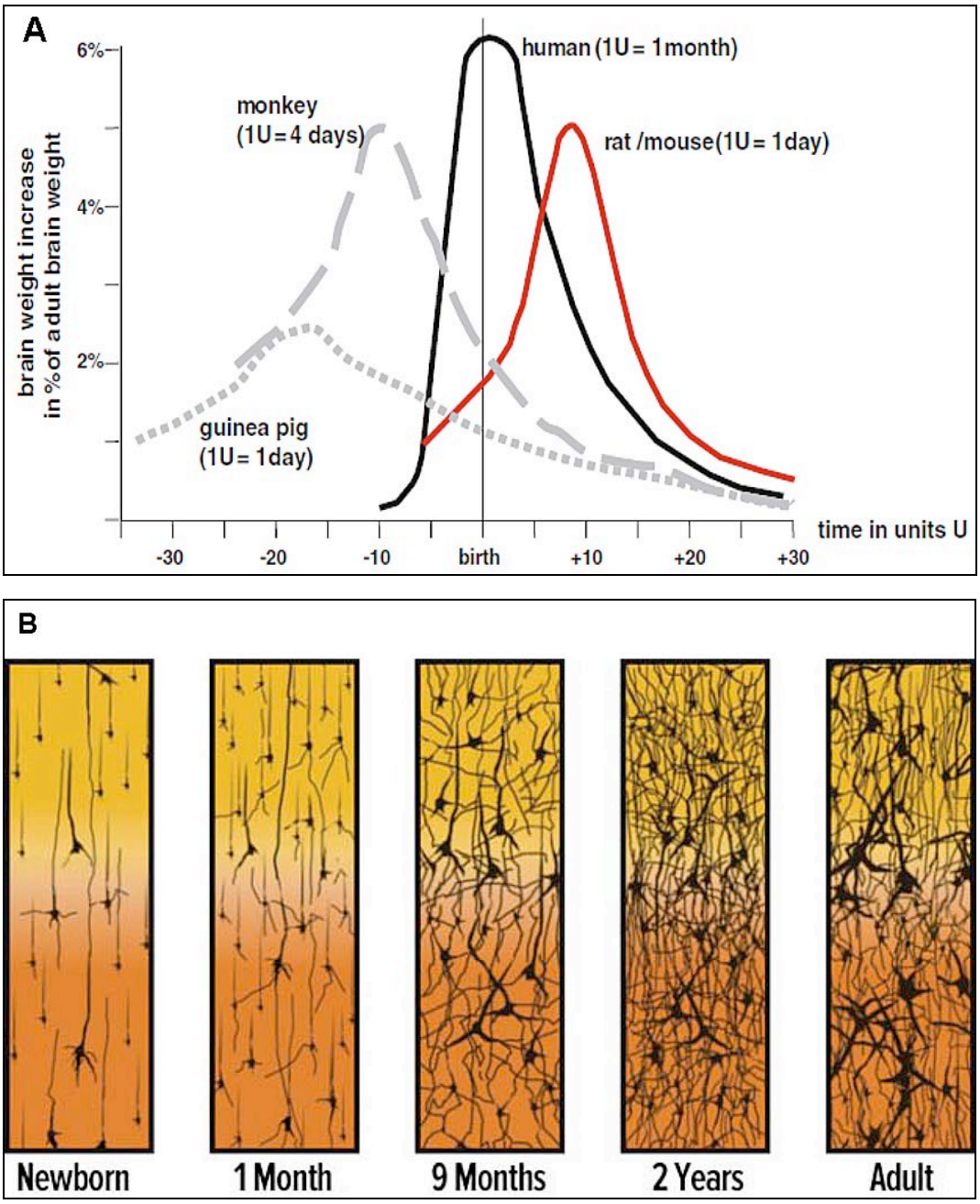
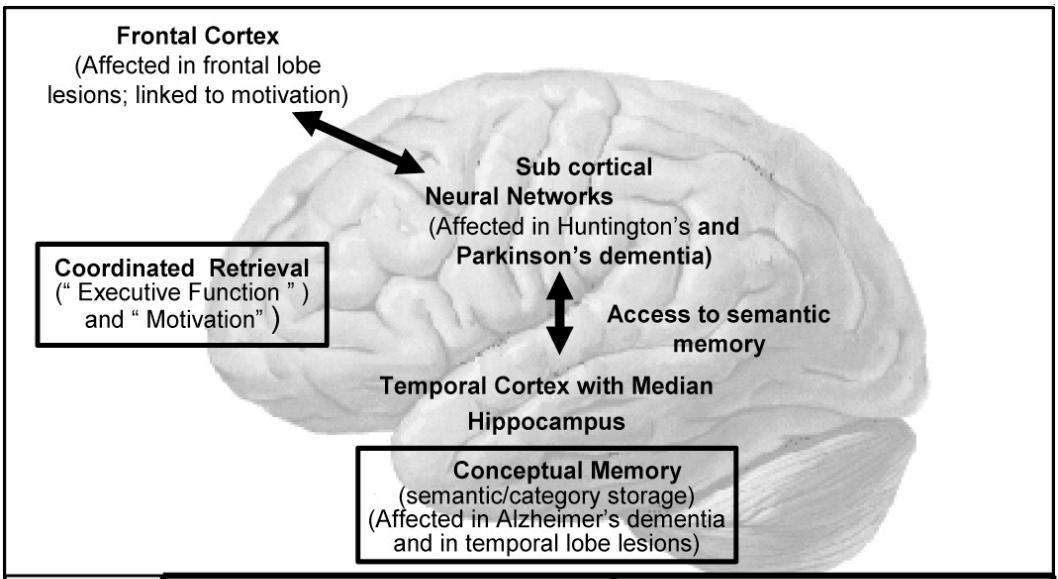


Figure 2: Age of brain development in humans and other animals, showing that most human brain development (by A: weight or by B: synapse formation) occur in the first 2 years of life. Adapted respectively from *Dobbing* and *Sands* (1973) and from *Corel* (1975).



Function	Coordinated Retrieval ("Executive Function" and Motivation")	Conceptual Memory (Long-term semantic/category storage)
Site	Frontal Cortex Sub cortical Networks	Temporal Cortex (with median Hippocampus)
Main Diseases	Huntington's/Parkinson's diseases Frontal lobe or sub cortical lesions	Alzheimer's disease Temporal lobe lesions ***? Early Childhood Diarrhea***
Test(s)	Phonemic and semantic performance	Semantic performance only

Figure 3: Brain regional development and function.

EARLY CHILDHOOD DIARRHOEA AND GIARDIASIS IMPAIR SEMANTIC FLUENCY (LIKE ALZHEIMER'S DISEASE) AND ApoE4 PROTECTS

When we examined the specific areas most affected by early childhood diarrhoea, it was striking that these were predominantly functions similar to those lost in early Alzheimer's disease. These include higher executive function and semantic (vs. phonetic) fluency (*Patrick* et al., 2005; *Oria* et al. 2009), with brain regions that likely differ from the predominant ones involved in phonemic fluency or in Parkinson's dementia (Figure 3).

Because of the semantic fluency predominance of the deficits, we examined the ApoE4 allele frequencies and found, to our initial surprise, that ApoE4 was protective of the cognitive function and against diarrhoea, the former only in the children with heavy diarrhoea burdens (*Oria* et al., 2005, 2007, 2010). Some early investigations into this link are beginning to support a host benefit of ApoE4 against enteric protozoa. The positive correlation

between *Giardia lamblia* and diarrhoea ($p<0.01$) in ApoE4 negative children in our study was lost in the presence of an ApoE4 allele ($p=0.53$) (*Oria* et al., 2005). Moreover, the ApoE4 allele appears to be protective against cognitive impairments due to early *Giardia* infections in Egypt (*Yahya* et al., 2009a, 2009b), although these findings should be further confirmed with larger numbers and better control for confounding factors. The association of potential protection against symptoms with this parasite by the host ApoE4 allele may be related to the *Giardia*'s requirement to obtain host cholesterol for its own growth (since *Giardia* is unable to synthesize cholesterol) thus needing to divert host cholesterol to the parasite from the intestinal milieu, which may deleteriously affect the developing brain (*Oria* et al., 2007). These effects may require an evolutionarily conserved LDL-receptor pathway, which is found to be down-regulated in ApoE4 carriers (*Mahley* and *Rall*, 2000). Recently, a putative *Giardia lamblia* low-density lipoprotein receptor-related protein (GlLRP), a type I membrane protein, which shares the substrate N-terminal binding domain and an FXNPXY-type endocytic motif with human LRPs, was identified (*Mahley* and *Rall*, 2000; *Rivero* et al., 2011).

In addition, Colton and colleagues (*Colton* et al., 2001; *Czapiga* and *Colton*, 2003) have shown that ApoE4 increases NO production in microglial macrophages by stimulating arginine uptake via an arginine-selective cationic amino acid transporter (CAT1). Hence we examined the ability of arginine to enhance parasite killing with *Cryptosporidium* infections in our murine model (*Castro* et al., 2012) and also the ability of targeted transgenic C57Bl6 mice with the human ApoE4 allele to resist cryptosporidial infections or their growth impairment in our murine model. We found that arginine is the most effective "anticryptosporidial drug" we have encountered in our murine model, with a 10-fold reduction in the number of parasites per milligram of intestinal tissue. These effects were only partially blocked by L-NAME (NG-nitro-arginine methyl ester) (*Castro* et al. 2012) or by BEC (S-(2-boronoethyl)-L-cysteine) (*Castro* et al., unpublished data), suggesting that the protection from arginine was through both the iNOS and the arginase pathways to kill the parasite and repair epithelial cell injury respectively. Furthermore, in studies that are being submitted for publication elsewhere, C57BK6J ApoEko mice expressing the human ApoE4/4 gene under murine ApoE promoter lost less weight, had better villus-to-crypt ratios and shed fewer parasites than ApoE 3/3 target replacement and wild-type mice, suggesting that understanding how ApoE4 may be protective can open novel approaches to better controlling protozoal infections. Interestingly, supporting the concept that ApoE4 may improve innate immunity against enteric pathogens, data from the Tsimane population in lowland Bolivia, (an indigenous forager-farmer population living under conditions resembling pre-industrial European populations with high infectious morbidity, high infection and inflammation, and shortened life expectancy) when bearing ApoE4 show lower serum C-reactive protein levels (*Vasunilashorn* et al., 2011), suggesting that ApoE4 bearers had lower rates of environmental-related infections.

Our data support that ApoE4 behaves as antagonistic pleiotropy, where the presence of ApoE4 in our gene pool was associated with increased needs for fat energy storage and improved innate immunity adaptations against enteric

infections in times where those were critical for human survival in the pre-industrialized era. However this once beneficial gene becomes potentially quite detrimental in the presence of longer life expectancy, reduced physical exertion and westernized diets. There are other postulates and literature evidence for an antagonistic pleiotropy described for ApoE4 during human early development and in adulthood (*Prentice* et al., 2005; *Alexander* et al., 2007; *Finch* and *Morgan*, 2007; *Beeri* et al., 2009; *Chang* et al., 2011).

EFFECTS OF MALNUTRITION AND OF MICRONUTRIENTS ON BRAIN ANATOMY AND FUNCTION

Especially vulnerable in the first two years of life in humans (analogous to the first few weeks of life in rodent models) is the postnatal brain plasticity, particularly in dynamic hippocampal, neocortical and cerebellar regions (*Frankova* and *Barnes*, 1968; *Rice* and *Barone*, 2000). It is these areas that are potentially altered by nutrients or micronutrients in critical developmental windows (*Georgieff*, 2007; *Pinero* et al., 2001; *de Souza* et al., 2011). Hence we examined the effects of malnutrition and of zinc and glutamine therapy in our murine model of litter clustering induced malnutrition. There we found that malnourished mice (with breast-milk restriction by increased litter size) had growth impairment and deficits in early post-natal behaviour ontogeny, associated with reduced serum and brain zinc levels. In addition, we found reduced hipocampal GABA levels and reductions in hippocampal synaptophysin (as a marker for synaptic density) expression, associated with malnutrition. Furthermore, malnutrition-induced CA-1 neuronal hypertrophy was found with litter size clustering, likely related to CA-1 cell death changes. All effects improved with glutamine and/or zinc treatment (Ladd et al., 2010).

We next examined the effects of zinc, vitamin A and glutamine supplementation on the growth and cognitive responses in 213 undernourished children from the favela in Fortaleza and found that lower vitamin A and glutamine levels were associated with disrupted intestinal barrier function (by lactulose/mannitol absorption ratios). There was also a significant correlation between vitamin A supplementation of apolipoprotein E4(+) children and improved lactulose/mannitol absorption ratios. In addition, only the ApoE4 positive children (37/213, 13.9%) who received glutamine supplementation (10 day glutamine treatment either with or without zinc or vitamin A supplementation) showed significant positive Pearson correlations between the changes in height-for-age z-scores over four months with delayed verbal learning scores, along with correlated changes over the same period in weight-for-age z-scores and weight-for-height z-scores that were associated with non-verbal intelligence quotients (*Mitter* et al., 2012).

CONCLUSIONS

In conclusion, we find profound and lasting effects of early childhood enteric infections and diarrhoea on children's growth and cognitive development. While these "vicious cycles of poverty" are only now being dissected

at the levels of molecular causality, genetic predispositions and potential novel interventions can already be designed that hold promise for interrupting these vicious cycles of diarrhoea, stunting, cognitive impairment and poverty.

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LITERATURE

Alexander, D.M., Williams, L.M., Gatt, J.M., Dobson-Stone, C., Kuan, S.A., Todd, E.G., Schofield, P.R., Cooper, N.J., and Gordon, E.: The contribution of apolipoprotein E alleles on cognitive performance and dynamic neural activity over six decades. Biol. Psychol. 75, 229-238 (2007).

Beeri, M.S., Schmeidler, J., Haroutunian, V., West, R., Ostad, D., Grossman, H.T., Rosendorff, C., and Silverman, J.M.: Better cognitive performance associated with worse cardiac functioning suggests antagonistic pleiotropy in very elderly subjects. Am. J. Geriatr. Psychiatry 17, 911-912 (2009).

Castro, I.C., Oliveira, B.B., Slowikowski, J.J., Coutinho, B.P., Siqueira, F.J., Costa, L.B., Sevilleja, J.E., Almeida, C.A., Lima, A.A., Warren, C.A., Oria, R.B., and Guerrant, R.L.: Arginine decreases Cryptosporidium parvum infection in undernourished suckling mice involving nitric oxide synthase and arginase. Nutrition 28, 678-685 (2012).

Chang, L., Andres, M., Sadino, J., Jiang, C.S., Nakama, H., Miller, E., and Ernst, T.: Impact of apolipoprotein E epsilon4 and HIV on cognition and brain atrophy: antagonistic pleiotropy and premature brain aging. Neuroimage 58, 1017-1027 (2011).

Checkley, W., Buckley, G., Gilman, R.H., Assis, A.M., Guerrant, R.L., Morris, S.S., Molbak, K., Valentiner-Branth, P., Lanata, C.F., and Black, R.E.: Multi-country analysis of the effects of diarrhoea on childhood stunting. Int. J. Epidemiol. 37, 816-830 (2008).

Colton, C.A., Czapiga, M., Snell-Callanan, J., Chernyshev, O.N., and Vitek, M.P.: Apolipoprotein E acts to increase nitric oxide production in macrophages by stimulating arginine transport. Biochim. Biophys. Acta 1535, 134-144 (2001).

Corel, J.L.: The postnatal development of the human cerebral cortex. Harvard University Press, Cambridge, MA, USA (1975).

Czapiga, M. and Colton, C.A.: Microglial function in human APOE3 and APOE4 transgenic mice: altered arginine transport. J. Neuroimmunol. 134, 44-51 (2003).

de Souza, A.S., Fernandes, F.S., and do Carmo, M.G.: Effects of maternal malnutrition and postnatal nutritional rehabilitation on brain fatty acids, learning, and memory. Nutr. Rev. 69, 132-144 (2011).

DeBoer, M.D., Lima, A.A.M., Oria, R.B.,

Scharf, R.J., Moore, S.R., Luna, M.A., and Guerrant, R.L.: Early childhood growth failure and the development origins of adult disease: Do enteric infections and undernutrition increase risk for the metabolic syndrome? Nutr. Rev. 70, 642-653 (2012).

Dillingham, R. and Guerrant, R.L.: Childhood stunting: Measuring and stemming the staggering costs of inadequate water and sanitation. Lancet 363, 94-95 (2004).

Dobbing, J. and Sands, J.: Quantitative growth and development of human brain. Arch. Dis. Child 48, 757-767 (1973).

Eppig, C., Fincher, C.L., and Thornhill, R.: Parasite prevalence and the worldwide distribution of cognitive ability. Proc. Biol. Sci. 277, 3801-3808 (2010).

Finch, C.E. and Morgan, T.E.: Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: A position paper. Curr. Alzheimer Res. 4, 185-189 (2007).

Fischer Walker, C.L., Lamberti, L., Adair, L., Guerrant, R.L., Lescano, A.G., Martorell, R., Pinkerton, R.C., and Black, R.E.: Does childhood diarrhea influence cognition beyond the diarrhea-stunting pathway? PLoS ONE 7, e47908 (2012).

Frankova, S. and Barnes, R.H.: Effect of malnutrition in early life on avoidance conditioning and behavior of adult rats. J. Nutr. 96, 485-493 (1968).

Georgieff, M.K.: Nutrition and the developing brain: nutrient priorities and measurement. Am. J. Clin. Nutr. 85, 614S-620S (2007).

Grantham-McGregor, S.: A review of studies of the effect of severe malnutrition on mental development. J. Nutr. 125, 2233S-2238S (1995).

Grantham-McGregor, S., Cheung, Y.B., Cueto, S., Glewwe, P., Richter, L., and Strupp, B.: Developmental potential in the first 5 years for children in developing countries. Lancet 369, 60-70 (2007).

Guerrant, R.L., Kirchhoff, L.V., Shields, D.S., Nations, M.K., Leslie, J., de Sousa, M.A., Araujo, J.G., Correia, L.L., Sauer, K.T., McClelland, K.E., Trowbridge, F.L., and Hughes, J.M.:. Prospective study of diarrheal illnesses in northeastern Brazil: Patterns of disease, nutritional impact, etiologies, and risk factors. J. Infect. Dis. 148, 986-997 (1983).

Guerrant, D.I., Moore, S.R., Lima, A.A., Patrick, P.D., Schorling, J.B., and Guerrant, R.L.: Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. Am. J. Trop. Med. Hyg. 61, 707-713 (1999).

Hoddinott, J., Maluccio, J.A., Behrman, J.R., Flores, R., and Martorell, R.: Effect of a nutrition intervention during early childhood on economic productivity in Guatemalan adults. Lancet 371, 411-416 (2008).

Huttenlocher, P.R.: Neural plasticity: The effects of the environment on the development of the cerebral cortex. Perspectives in cognitive neuroscience. Harvard University Press, Cambridge, MA, USA (2002).

Ladd, F.V., Ladd, A.A., Ribeiro, A.A., Costa, S.B., Coutinho, B.P., Feitosa, G.A., de Andrade, G.M., de Castro-Costa, C.M., Magalhaes, C.E., Castro, I.C., Oliveira, B.B., Guerrant, R.L., Lima, A.A., and Oria, R.B.: Zinc and glutamine improve brain development in suckling mice subjected to early postnatal malnutrition. Nutrition 26, 662-670 (2010).

Lima, A.A., Moore, S.R., Barboza, M.S., Jr., Soares, A.M., Schleupner, M.A., Newman, R.D., Sears, C.L., Nataro, J.P., Fedorko, D.P., Wuhib, T., Schorling, J.B., and Guerrant, R.L.: Persistent diarrhea signals a critical period of increased diarrhea burdens and nutritional shortfalls: A prospective cohort study among children in northeastern Brazil. J. Infect. Dis. 181, 1643-1651 (2000).

Lorntz, B., Soares, A.M., Moore, S.R., Pinkerton, R., Gansneder, B., Bovbjerg, V.E., Guyatt, H., Lima, A.A.M., and Guerrant, R.L.: Early Childhood Diarrhea Predicts Impaired School Performance. Ped. Inf. Dis. J. 25, 513-520 (2006).

Mahley, R.W. and Rall, S.C., Jr.: Apolipoprotein E: Far more than a lipid transport protein. Annu. Rev. Genomics Hum. Genet. 1,

507-537 (2000).
Mata, L.J.: The Children of Santa Maria Cauque: A prospective field study of health and growth. MIT Press, Cambridge, MA, USA (1978).
Mendez, M.A. and Adair, L.S.: Severity and timing of stunting in the first two years of life affect performance on cognitive tests in late childhood. J. Nutr. 129, 1555-1562 (1999).
Mitter, S.S., Oria, R.B., Kvalsund, M.P., Pamplona, P., Joventino, E.S., Mota, R.M., Goncalves, D.C., Patrick, P.D., Guerrant, R.L., and Lima, A.A.: Apolipoprotein E4 influences growth and cognitive responses to micronutrient supplementation in shantytown children from northeast Brazil. Clinics (Sao Paulo) 67, 11-18 (2012).
Moore, S.R., Lima, A.A., Conaway, M.R., Schorling, J.B., Soares, A.M., and Guerrant, R.L.: Early childhood diarrhoea and helminthiases associate with long-term linear growth faltering. Int. J. Epidemiol. 30, 1457-1464 (2001).
Niehaus, M.D., Moore, S.R., Patrick, P.D., Derr, L.L., Lorntz, B., Lima, A.A., and Guerrant, R.L.: Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. Am. J. Trop. Med. Hyg. 66, 590-593 (2002).
Nokes, C., Grantham-McGregor, S.M., Sawyer, A.W., Cooper, E.S., and Bundy, D.A.: Parasitic helminth infection and cognitive function in school children. Proc. Biol. Sci. 247, 77-81 (1992).
Nowakowski, R.S.: Stable neuron numbers from cradle to grave. Proc. Natl. Acad. Sci. USA 103, 12219-12220 (2006).
Oria, R.B., Patrick, P.D., Zhang, H., Lorntz, B., de Castro Costa, C.M., Brito, G.A., Barrett, L.J., Lima, A.A., and Guerrant, R.L.: APOE4 protects the cognitive development in children with heavy diarrhea burdens in Northeast Brazil. Pediatr. Res. 57, 310-316 (2005).
Oria, R.B., Patrick, P.D., Blackman, J.A., Lima, A.A., and Guerrant, R.L.: Role of apolipoprotein E4 in protecting children against early childhood diarrhea outcomes and implications for later development. Med. Hypotheses 68, 1099-1107 (2007).
Oria, R.B., Costa, C.M., Lima, A.A., Patrick, P.D., and Guerrant, R.L.: Semantic fluency: A sensitive marker for cognitive impairment in children with heavy diarrhea burdens? Med. Hypotheses 73, 682-686 (2009).
Oria, R.B., Patrick, P.D., Oria, M.O., Lorntz, B., Thompson, M.R., Azevedo, O.G., Lobo, R.N., Pinkerton, R.F., Guerrant, R.L., and Lima, A.A.: ApoE polymorphisms and diarrheal outcomes in Brazilian shanty town children. Braz. J. Med. Biol. Res. 43, 249-256 (2010).
Patrick, P.D., Oria, R.B., Madhavan, V., Pinkerton, R.C., Lorntz, B., Lima, A.A., and Guerrant, R.L.: Limitations in verbal fluency following heavy burdens of early childhood diarrhea in Brazilian shantytown children. Child Neuropsychol. 11, 233-244 (2005).
Pinero, D., Jones, B., and Beard, J.: Variations in dietary iron alter behavior in developing rats. J. Nutr. 131, 311-318 (2001).
Pinkerton, R.C., Oria, R.B., Oria, M.O.B., Patrick, P.D., Wiseman, B.L., Lima, A.A.M., Moore, S.R., Niehaus, M.D., and Guerrant, R.L.: Early childhood diarrhea predicts cognitive delays in later childhood independently of malnutrition. (2012).
Prentice, A.M., Rayco-Solon, P., and Moore, S.E.: Insights from the developing world: thrifty genotypes and thrifty phenotypes. Proc. Nutr. Soc. 64, 153-161 (2005).
Rice, D. and Barone, S. Jr.: Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. Environ. Health Perspect. 108, Suppl. 3, 511-533 (2000).
Rivero, M.R., Miras, S.L., Quiroga, R., Ropolo, A.S., and Touz, M.C.: *Giardia lamblia* low-density lipoprotein receptor-related protein is involved in selective lipoprotein endocytosis and parasite replication. Mol. Microbiol. 79, 1204-1219 (2011).
Shrimpton, R., Victora, C.G., de, O.M., Lima,

R.C., Blossner, M., and Clugston, G.: Worldwide timing of growth faltering: implications for nutritional interventions. Pediatrics 107, E75 (2001).

Stein, A.D., Wang, M., DiGirolamo, A., Grajeda, R., Ramakrishnan, U., Ramirez-Zea, M., Yount, K., and Martorell, R.: Nutritional supplementation in early childhood, schooling, and intellectual functioning in adulthood: A prospective study in Guatemala. Arch. Pediatr. Adolesc. Med. 162, 612-618 (2008).

Stephenson, L.S., Latham, M.C., Adams, E.J., Kinoti, S.N., and Pertet, A.: Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. J. Nutr. 123, 1036-1046 (1993).

Stephenson, L.S., Latham, M.C., Kurz, K.M., Kinoti, S.N., and Brigham, H.: Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infections. Am. J. Trop. Med. Hyg. 41, 78-87 (1989).

Stephenson, L.S., Latham, M.C., Kinoti, S.N., Kurz, K.M., and Brigham, H.: Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. Trans. R. Soc. Trop. Med. Hyg. 84, 277-282 (1990).

Thompson, R.A. and Nelson, C.A.: Developmental science and the media. Early brain development. Am. Psychol. 56, 5-15 (2001).

Vasunilashorn, S., Finch, C.E., Crimmins, E.M., Vikman, S.A., Stieglitz, J., Gurven, M., Kaplan, H., and Allayee, H.: Inflammatory gene variants in the Tsimane, an indigenous Bolivian population with a high infectious load. Biodemography. Soc. Biol. 57, 33-52 (2011).

Victora, C.G., de, O.M., Hallal, P.C., Blossner, M., and Shrimpton, R.: Worldwide timing of growth faltering: Revisiting implications for interventions. Pediatrics 125, e473-e480 (2010).

Yahya, R.S., Awad, S.I., and El-Gayar, E.K.: Apolipoprotein E4 effect on development and cognitive function in Giardia-infected children. Parasitologists United Journal 2, 111-118 (2009a).

Yahya, R.S., Awad, S.I., Elgayar, E.K., Elborii, H., Ragab, A., and Al-Sawah, G.A.: Impact of Apolipoprotein E4 On Development And Cognitive Function In Giardia-Infected Children. The Internet Journal of Parasitic Diseases Vol. 4, No. 1, DOI: 10.5580/16a3 (2009b).

THE GUT MICROBIOME AND THE NERVOUS SYSTEM: SUMMARY OF THE SEMINAR

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When this symposium was being planned, very little information existed as to the potential role of commensal bacteria in the gut in modulation of the peripheral or central nervous systems. A limited number of reviews were extant (*Collins* and *Bercik*, 2009; *Forsythe* et al., 2010) and to the knowledge of the planners, a symposium devoted to this subject was novel if not unique. In the course of 2011-2012 a significant number of reviews of the subject appeared and since June 2012, there has been an explosion of interest in this area so that the numbers of reviews may now equal or even be in excess of original articles in the field (*Grenham* et al., 2011; *Bravo* et al., 2012; *Collins* et al., 2012; *Cryan* and *Dinan*, 2012; *Dinan* and *Cryan*, 2012; *Forsythe* and *Kunze*, 2012, *Saulnier* et al., 2012)! Many of the participants in this symposium have been contributing authors to these reviews.

The concept of the gut microbiome as a "forgotten organ" and the complexity of the effects on the host of the more than 3 million genes which it contains have opened biologists' eyes to how little we know in this area. Initial major emphasis has been placed on the interactions between bacteria in the gut and the immune and endocrine systems and this has recently been extended to the nervous system. These pathways have been loosely termed the gut-brain or microbiome-gut-brain axis, however they have largely failed to take into account the enormous contribution of the fungal and viral genomes, which also contribute, to these interkingdom communications. The fungal contributions have recently been highlighted by *Iliev* et al. (2012) who termed this the "mycobiome" and showed that this "eukaryotic fungal community ....coexists with bacteria and substantially expands the repertoire of organisms interacting with the intestinal immune system to influence health and disease". The virome has also just started receiving attention in this respect (*Reyes* et al., 2012).

The fact that the microbiome may influence animal behaviour has been known for a number of years and was recently reviewed by *Ezenwa* et al. (2012). The choice of mates by *Drosophila* was shown to depend on specific gut Lactobacilli which, when eliminated, restored typical random mating. This was thought to depend on the influence of cuticular pheromone production (*Sharon* et al., 2010). The swarming of locusts depends upon the production of an aggregation pheromone, which in turn depends on the presence of 2 or 3 specific gut bacteria (*Dillon* et al., 2002). The nerves responsible for this phenomenon have been identified, as has serotonin as the neurotransmitter involved (*Anstey* et al., 2009). Several other animal behaviours have been associated with specific characteristics of the gut microbiome (*Ezenwa* et al., 2012).

The symposium began with an overview by Bud Craig who summa-

rized his relevant work on interoception which has focused attention on the way in which a particular neuroanatomical area of the brain, the insula, integrates sensory input from the body (*Craig*, 2002, 2010, 2011). The activation of the anterior insula correlates with subjective feelings and thereby emotions and the right and left insulae are probably asymmetrically organized, and possibly this is done in an opponent fashion. This is particularly evident in primates and presumably humans, and the data are striking and indicate that the non-dominant (i.e. mostly left) insula contains an answer to the question "how do you feel?". While this work has not yet identified the role of the gut microbiome, the evidence in rodents of commensal effects on brain neurochemistry and behaviour strongly predict a neuroanatomical target, possibly via the vagus nerve (*Bravo* et al., 2011a). Emeran Mayer, another symposium speaker, has published together with Bud Craig a major review on the brain-gut axis, incorporating modern concepts of how gut feelings are generated and integrated with special reference to functional gastrointestinal disorders such as irritable bowel syndrome (IBS) and gut pain (*Mayer* et al., 2006).

Paul Forsythe drew especial attention to the bidirectional interaction between the gut microbiome and the brain and stressed the fact that while the vagus nerve carried much of the information generated in the viscera to the brain, additional pathways exist, ranging from hormonal to possibly direct effects of bacterial products such as GABA or results of fermentation such as fatty acids. A recent publication showed that feeding of *L. rhamnosus* (JB-1) resulted in anxiolytic behavioural changes accompanied by alterations in specific GABA receptors in the brain and a blunted corticosterone response to stress, all of which were abrogated by prior vagotomy (*Bravo* et al., 2011a). The same bacterium causes increased electrical activity in the vagus nerve (*Perez-Burgos* et al., 2012). While the efferent vagal pathway has been identified by Tracey and colleagues (*Olofsson* et al., 2012) as part of a cholinergic anti-inflammatory reflex, the extent to which the afferent and efferent pathways are part of a reverberating network is not known. Commensal bacteria such as probiotics may well stimulate immediate functional immunoregulatory activity locally as well as through efferent vagal pathways and therefore in turn influence stress and low-grade inflammation associated conditions such as depression (*Raison* et al., 2010; *Dantzer*, 2012). Wolfgang Kunze was more concerned with how bacteria may be communicating with the enteric nervous system and thereby the brain. Several approaches were identified, both *in vivo* (long-term) and *ex vivo* (short-term). Bacteria placed in the lumen of a small bowel segment communicate within seconds with sensory afferent nerve fibres in the myenteric plexus and within minutes, decrease the amplitude of gut motor contractions. These effects are accompanied by inhibition of a specific calcium activated potassium channel (KCa 3.1) in AH cells of the myenteric plexus. Interestingly, mast cells from rats fed *Lactobacillus rhamnosus* (JB-1) also showed inhibition of this channel (*Forsythe* et al., 2012). These data clearly point to a generalized systemic effect of an ingested probiotic. At the same time, activation of vagal fibres by luminal JB-1 but not by another lactobacillus (*L. salivarius* that does not affect gut motor contractions) supports the vagal activation by these bacteria en route to the brain. While components of bacteria responsible for these effects are not

totally characterized, an exopolysaccharide of *B. fragilis*, PSA, recapitulated the effects of the parent bacteria. A very recent important report from Mazmanian's laboratory suggests that this may be occurring through outer membrane micro-vesicles shed by the bacteria, offering a novel bacteria-host communication pathway (*Shen* et al., 2012).

Michiel Kleerebezem drew attention to the molecular consequences of interactions between the microbiome and the host at the transcriptomic and metabonomic levels. The complexity of these interactions was highlighted by the fact that in a double-blind placebo controlled cross-over design with 3 different probiotics in young healthy adults, each bacterium induced a different pattern of response in regulatory and other drug-like pathways in host tissues (*van Baarlen* et al., 2011). An additional problem facing the whole field is to define the "normal" microbiome in health and the emerging definition of a molecular 'bandwidth of human health' might provide an important window for further exploration (*Bron* et al., 2012). Recent just published papers have identified surprising and dynamic patterns of transcriptional signatures of intestinal adaptive and innate immunity, metabolism and neuronal development in response to a changing microbiome in conventionalized germ-free mice (*El Aidy* et al., 2012a, 2012b, 2012c).

Elaine Holmes focused on metagenomic and metabonomic approaches to studying the role of the gut microbiome in human health and disease and highlighted the value of studying microbial metabolites as specific biomarkers in urine and serum (*Holmes* et al., 2012). These may reflect primary or secondary changes in microbial communities or their consequent effects on host pathways. For example, antibiotic induced effects on gut microbial composition can be analysed in this way and valuable information obtained on relative dysbiosis through a non-invasive approach (*Swann* et al., 2011). Extensive studies are building an archive of data in health and disease and demonstrate the power of combining different molecular approaches in microbe-host interactions (*Kinross* et al., 2008; *El Aidy* et al., 2012b). The application of urinary metabolic phenotyping to patients with and without autism have shown differences in certain microbial co-metabolites and suggest intriguing gut microbial disturbances in this developmental disorder (*Yap* et al., 2010).

Many chronic diseases are thought to be associated or initiated by stress. Chronic stress is correlated with subsequent low-grade inflammatory changes and is thought to cause or exacerbate conditions as varied as depression, anxiety and IBS. John Cryan offered examples and experimental models and the ways in which gut microbes could influence the hypothalamic-pituitary-adrenal (HPA) axis and how this in turn could change microbial composition (*Bravo* et al., 2011b; *Cryan* and *Dinan*, 2012; *Dinan* and *Cryan*, 2012). Exploration of such changes in the germ-free state and the effects of when (neonatal, post-weaning or adult) they occurred in conventionally housed animals has provided new insights into the programming of immune, endocrine and nervous systems. Such experiments have also revealed significant gender differences that may have their human counterparts in chronic disorders that may involve the nervous system (*Clarke* et al., 2012).

Major depressive disorder is accompanied by increases in serum of inflammatory markers such as C-reactive protein and TNF (*Dantzer* et al., 2011; *Dantzer*, 2012) and Graham

Rook persuasively argued that this was associated with a change in composition of the gut microbiome in the last few decades (*Raison* et al., 2010). This imbalance may have involved the loss of beneficial commensal bacteria (*Rook* and *Lowry*, 2008). This concept is an important variation on what is commonly termed "the hygiene hypothesis" and raises serious questions while offering possible answers as to why there is an increased prevalence of diseases such as depression, autism and autoimmunity in recent times (*Rook*, 2010; *Rook* et al., 2012).

IBS is co-morbid with psychiatric manifestations (*Whitehead* et al., 2002) and Premysl Bercik discussed experimental evidence linking dysbiosis with changes in the brain and behaviour including germ-free models and effects of antibiotic or probiotic treatment (*Bercik* et al., 2012; *Collins* et al., 2012; *Saulnier* et al., 2012). He adduced evidence that changes in the microbiome could be accompanied by alterations in the neurochemistry of the brain and behaviour in both vagotomised and non-vagotomised mice (*Bercik* et al, 2011, 2012). Therefore not all psychoactive effects of bacteria are mediated via the vagus nerve. Perhaps the most striking examples involved demonstration that faecal transplants from donor mice with defined phenotypic behavioural characteristics to others with equally stereotypic, but different behaviours resulted in the hosts displaying the behaviour of the donor animals (*Bercik* et al., 2011).

Emeran Mayer summarized information from many of the speakers listed above and integrated these into clinical knowledge. He stressed the fact that clinical infections may lead to alterations in behaviour and brain neurochemistry without necessarily involving changes in the gut microbiome (*Mayer*, 2011). These alterations may in turn influence, and be influenced by antibiotic and possibly probiotic treatment. IBS may represent a combination of these effects and the outcome and presentation not only may be influenced by these factors, but also by early life trauma (*Berman* et al., 2012). It is therefore not surprising that patients' symptoms may be treated by psycho-educational intervention (*Labus* et al., 2012). Many of these behavioural and neurochemical brain changes are reflected in the use of non-invasive functional magnetic resonance imaging (fMRI) and many of the important studies in this respect emanate from Emeran Mayer's laboratory. It is therefore fitting that a recent study by *Larsson* et al. (2012) involves Bud Craig as a co-investigator, and finally, that evidence of fMRI changes has been observed in as yet unpublished studies of probiotic ingestion.

Richard Guerrant brought the symposium down to earth with a practical clinical reminder of the major potentially permanent detrimental effect on cognition of repeated childhood dehydrating or malnourishing infections, with and without diarrhoea (*Guerrant* et al., 2012). Prior to this presentation, little discussion had occurred about the role of diet and nutrition on the gut microbiome and consequent effects on the host. Indeed, this area has to date received scant attention in the literature. Because of its importance to health and well-being in developing countries as well as in the industrialized world, it will be crucial to extend research efforts into this subject. Possession of the ApoE4 allele was shown to be a protective factor against the development of cognitive and growth defects in children with heavy diarrheal burdens (*Mitter* et al., 2012), and a murine model supporting this observation was described.

In conclusion, many aspects of the emerging evidence for a microbiome-gut-brain axis were energetically discussed during this symposium. They revealed many convergent pieces of evidence in support of the concept and importance of the subject. However, they also showed up areas in which little effort had yet been placed, such as the role of nutrition and diet and the lack of definition of what constitutes a normal microbiome in a healthy adult. A recent study on the composition of the microbiome in a healthy and elderly population has shown an interesting role of diet (*Claesson* et al., 2012). The effects of ethnicity, culture and gender are only just beginning to be explored. Furthermore, our ignorance of the possible roles of the mycobiome and virome is huge. We have not even touched upon the recent flurry of papers suggesting that the enteric nervous system may be involved in the earliest manifestations of Parkinson's disease, even before classical symptoms appear (*Forsyth* et al., 2011; *Natale* et al., 2011). Whether this represents an imbalance of organisms that together make up the normal microbiome or an example of an unusual pathogen, which enters the body through the enteric nervous system, remains unknown.

We greatly look forward to the next symposium on this subject.

LITERATURE

Anstey, M.L., Rogers, S.M., Ott, S.R., Burrows, M., and Simpson, S.J.: Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. Science 323, 627-630 (2009).

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., and Collins, S.M.: The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141, 599-609 (2011).

Bercik, P., Collins, S.M., and Verdu, E.F.: Microbes and the gut-brain axis. Neurogastroenterol. Motil. 24, 405-413 (2012).

Berman, S., Suyenobu, B., Naliboff, B.D., Bueller, J., Stains, J., Wong, H., Mandelkern, M., Fitzgerald, L., Ohning, G., Gupta, A., Labus, J.S., Tillisch, K., and Mayer, E.A.: Evidence for alterations in central noradrenergic signaling in irritable bowel syndrome. Neuroimage 63, 1854-1863 (2012).

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl, Acad. Sci. USA 108, 16050-16055 (2011a).

Bravo, J.A., Dinan, T.G., and Cryan, J.F.: Alterations in the central CRF system of two different rat models of comorbid depression and functional gastrointestinal disorders. Int. J. Neuropsychopharmacol. 14, 666-683 (2011b).

Bravo, J.A., Julio-Pieper, M., Forsythe, P., Kunze, W., Dinan, T., Bienenstock, J., and Cryan, J.F.: Communication between gastrointestinal bacteria and the nervous system. Curr. Opin. Pharmacol. 12, 667-672 (2012).

Bron, P.A., van Baarlen, P., and Kleerebezem, M.: Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. Nat. Rev. Microbiol. 10, 66-78 (2012).

Claesson, M.J., Jeffery, I.B., Conde, S., Power, S.E., O'Connor, E.M., Cusack, S., Harris, H.M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G.F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace,

M., Brennan, L., Stanton, C., Marchesi, J.R., Fitzgerald, A.P., Shanahan, F., Hill, C., Ross, R.P., and O'Toole, P.W.: Gut microbiota composition correlates with diet and health in the elderly. Nature 488, 178-184 (2012).

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., and Cryan, J.F.: The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol. Psychiatry, doi: 10.1038/mp.2012.77. (Epub ahead of print) (2012).

Collins, S.M. and Bercik, P.: The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology 136, 2003-2014 (2009).

Collins, S.M., Surette, M., and Bercik, P.: The interplay between the intestinal microbiota and the brain. Nat. Rev. Microbiol. 10, 735-742 (2012).

Craig, A.D.: How do you feel? Interoception: the sense of the physiological condition of the body. Nat. Rev. Neurosci. 3, 655-666 (2002).

Craig, A.D.: Once an island, now the focus of attention. Brain Struct. Funct. 214, 395-396 (2010).

Craig, A.D.: Significance of the insula for the evolution of human awareness of feelings from the body. Ann. NY Acad. Sci. 1225, 72-82 (2011).

Cryan, J.F. and Dinan, T.G.: Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701-712 (2012).

Dantzer, R., O'Connor, J.C., Lawson, M.A., and Kelley, K.W.: Inflammation-associated depression: From serotonin to kynurenine. Psychoneuroendocrinology 36, 426-436 (2011).

Dantzer, R.: Depression and inflammation: an intricate relationship. Biol. Psychiatry 71, 4-5 (2012).

Dillon, R.J., Vennard, C.T., and Charnley, A.K.: A note: Gut bacteria produce components of a locust cohesion pheromone. J. Appl. Microbiol. 92, 759-763 (2002).

Dinan, T.G. and Cryan, J.F.: Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. Psychoneuroendocrinology 37, 1369-1378 (2012).

El Aidy, S., Kunze, W., Bienenstock, J., and Kleerebezem, M.: The microbiota and the gut-brain axis: Insights from the temporal and spatial mucosal alterations during colonisation of the germfree mouse intestine. Benef. Microbes 3, 251-259 (2012a).

El Aidy, S., Merrifield, C.A., Derrien, M., van Baarlen, P., Hooiveld, G., Levenez, F., Dore, J., Dekker, J., Holmes, E., Claus, S.P., Reijngoud, D.J. and Kleerebezem, M.: The gut microbiota elicits a profound metabolic reorientation in the mouse jejunal mucosa during conventionalisation. Gut, Epub ahead of print (2012b).

El Aidy, S., van Baarlen, P., Derrien, M., Lindenbergh-Kortleve, D.J., Hooiveld, G., Levenez, F., Dore, J., Dekker, J., Samsom, J.N., Nieuwenhuis, E.E., and Kleerebezem, M.: Temporal and spatial interplay of microbiota and intestinal mucosa drive establishment of immune homeostasis in conventionalized mice. Mucosal Immunol. 5, 567-579 (2012c).

Ezenwa, V.O., Gerardo, N.M., Inouye, D.W., Medina, M., and Xavier, J.B.: Microbiology. Animal behavior and the microbiome. Science 338, 198-199 (2012).

Forsyth, C.B., Shannon, K.M., Kordower, J.H., Voigt, R.M., Shaikh, M., Jaglin, J.A., Estes, J.D., Dodiya, H.B., and Keshavarzian, A.: Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PLoS One 6, e28032 (2011).

Forsythe, P., Sudo, N., Dinan, T., Taylor, V.H., and Bienenstock, J.: Mood and gut feelings. Brain Behav. Immun. 24. 9-16 (2010).

Forsythe, P. and Kunze, W.A.: Voices from within: gut microbes and the CNS. Cell. Mol. Life Sci., Epub ahead of print (2012).

Forsythe, P., Wang, B., Khambati, I., and

Kunze, W.A.: Systemic effects of ingested *Lactobacillus rhamnosus*: Inhibition of mast cell membrane potassium (IKCa) current and degranulation. PLoS One 7, e41234 (2012).

Grenham, S., Clarke, G., Cryan, J.F., and Dinan, T.G.: Brain-gut-microbe communication in health and disease. Front. Physiol. 2:94, doi: 10.3389/fphys.2011.00094, Epub (2011).

Guerrant, R.L., Deboer, M.D., Moore, S.R., Scharf, R.J., and Lima, A.A.: The impoverished gut-a triple burden of diarrhoea, stunting and chronic disease. Nat. Rev. Gastroenterol. Hepatol. doi: 10.1038/nrgastro.2012.239, (Epub ahead of print) (2012).

Holmes, E., Li, J.V., Marchesi, J.R., and Nicholson, J.K.: Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. Cell. Metab. 16, 559-564 (2012).

Iliev, I.D., Funari, V.A., Taylor, K.D., Nguyen, Q., Reyes, C.N., Ström, S.P., Brown, J., Becker, C.A., Fleshner, P.R., Dubinsky, M., Rotter, J.I., Wang, H.L., McGovern, D.P., Brown, G.D., and Underhill, D.M.: Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science 336, 1314-1317 (2012).

Kinross, J.M., von Roon, A.C., Holmes, E., Darzi, A., and Nicholson, J.K.: The human gut microbiome: Implications for future health care. Curr. Gastroenterol. Rep 10, 396-403 (2008).

Labus, J., Gupta, A., Gill, H.K., Posserud, I., Mayer, M., Raeen, H., Bolus, R., Simren, M., Naliboff, B.D., and Mayer, E.A.: Randomised clinical trial: Symptoms of the irritable bowel syndrome are improved by a psycho-education group intervention. Aliment. Pharmacol. Ther. doi: 10.1111/apt.12171, (Epub ahead of print) (2012).

Larsson, M.B., Tillisch, K., Craig, A.D., Engström, M., Labus, J., Naliboff, B., Lundberg, P., Ström, M., Mayer, E.A., and Walter, S.A.: Brain responses to visceral stimuli reflect visceral sensitivity thresholds in patients with irritable bowel syndrome. Gastroenterology 142, 463-472 (2012).

Mayer, E.A., Naliboff, B.D., and Craig, A.D.: Neuroimaging of the brain-gut axis: From basic understanding to treatment of functional GI disorders. Gastroenterology 131, 1925-1942 (2006).

Mayer, E.A.: Gut feelings: The emerging biology of gut-brain communication. Nat. Rev. Neurosci. 12, 453-466 (2011).

Mitter, S.S., Oria, R.B., Kvalsund, M.P., Pamplona, P., Joventino, E.S., Mota, R.M., Goncalves, D.C., Patrick, P.D., Guerrant, R.L., and Lima, A.A.: Apolipoprotein E4 influences growth and cognitive responses to micronutrient supplementation in shantytown children from northeast Brazil. Clinics (Sao Paulo) 67, 11-18 (2012).

Natale, G., Pasquali, L., Paparelli, A., and Fornai, F.: Parallel manifestations of neuropathologies in the enteric and central nervous systems. Neurogastroenterol. Motil. 23, 1056-1065 (2011).

Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., and Tracey, K.J.: Rethinking inflammation: Neural circuits in the regulation of immunity. Immunol. Rev. 248, 188-204 (2012).

Perez-Burgos, A., Wang, B., Mao, Y.K., Mistry, B., McVey Neufeld, K.A., Bienenstock, J., and Kunze, W.A.: Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. Am. J. Physiol. Gastrointest. Liver Physiol., Epub ahead of print (2012).

Raison, C.L., Lowry, C.A., and Rook, G.A.: Inflammation, sanitation, and consternation: Loss of contact with coevolved, tolerogenic microorganisms and the pathophysiology and treatment of major depression. Arch. Gen. Psychiatry 67, 1211-1224 (2010).

Reyes, A., Semenkovich, N.P., Whiteson, K., Rohwer, F., and Gordon, J.I.: Going viral: Next-generation sequencing applied to phage populations in the human gut. Nat.

Rev. Microbiol. 10, 607-617 (2012).

Rook, G.A. and Lowry, C.A.: The hygiene hypothesis and psychiatric disorders. Trends Immunol. 29, 150-158 (2008).

Rook, G.A.: 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin. Exp. Immunol. 160, 70-79 (2010).

Rook, G.A., Raison, C.L., and Lowry, C.A.: Can we vaccinate against depression? Drug Discov. Today 17, 451-458 (2012).

Saulnier, D.M., Ringel, Y., Heyman, M.B., Foster, J.A., Bercik, P., Shulman, R.J., Versalovic, J., Verdu, E., Dinan, T.G., Hecht, G.A., and Guarner, F.: The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. Gut Microbes **4**, Epub ahead of print (2012).

Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I., and Rosenberg, E.: Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 107, 20051-20056 (2010).

Shen, Y., Torchia, M.L., Lawson, G.W., Karp, C.L., Ashwell, J.D., and Mazmanian, S.K.: Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell. Host Microbe 12, 509-520 (2012).

Swann, J.R., Tuohy, K.M., Lindfors, P., Brown, D.T., Gibson, G.R., Wilson, I.D., Sidaway, J., Nicholson, J.K., and Holmes, E.: Variation in antibiotic-induced microbial recolonization impacts on the host metabolic phenotypes of rats. J. Proteome Res. 10, 3590-3603 (2011).

van Baarlen, P., Troost, F., van der Meer, C., Hooiveld, G., Boekschoten, M., Brummer, R.J., and Kleerebezem, M.: Human mucosal *in vivo* transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. Proc. Natl. Acad. Sci. USA 108, Suppl. 1, 4562-4569 (2011).

Whitehead, W.E., Palsson, O., and Jones, K.R.: Systematic review of the comorbidity of irritable bowel syndrome with other disorders: What are the causes and implications? Gastroenterology 122, 1140-1156 (2002).

Yap, I.K., Angley, M., Veselkov, K.A., Holmes, E., Lindon, J.C., and Nicholson, J.K.: Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. J. Proteome Res. 9, 2996-3004 (2010).