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The gut microbiome and the nervous system.
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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.

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