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Thinking with your stomach? Gut feelings on microbiome modulation of brain structure and function (Commentary on Luczynski et al.)

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Is that a rumbling I feel in my stomach? Or perhaps it is the trillions of bacteria down there who are in revolt, fomenting (or fermenting!) a microbial revolution? No active biologist or medical researcher can easily ignore the revolution in gut microbiome research in recent years. It seems that the gut microbiome, the entire microbial ecosystem occupying the gastrointestinal ecological niche, can impact almost every organ in the human body, not least of which being the brain. The gut microbiome has been found to signal to both the developing and adult mammalian brain, modulating both health and disease states (Cryan and Dinan, 2012; Mayer et al., 2014).

The latest interesting research article in this issue of the *European Journal of Neuroscience* (Luczynski et al., 2016), provides new insight into the relationship between the gut microbiome and the mammalian brain. There are a number of different ways in which the roles of the gut microbiome can be experimentally investigated. One of the ways in which this is done is to compare germ-free (GF) animals with conventionally colonized (CC) controls. The GF animals are bred and raised under germ-free conditions in which the gastrointestinal tract is not populated by the maternal (and other) microbial populations during and after birth, as occurs in the CC control animals. This allows relatively simple questions to be asked regarding the role of the gut microbiome in specific aspects of developing and adult biology.

In the present study (Luczynski et al., 2016), the focus of the investigations was the total tissue volume, and morphology of specific neuronal cell populations, in selected brain regions (the amygdala and hippocampus) of adult (9-10 weeks of age) laboratory mice raised under GF conditions (with ostensibly no gut microbiome) versus CC conditions (a control gut microbiome). Strikingly, the authors found that the amygdala and hippocampus of the GF mice was larger, and the dendritic and spine morphologies in specific amygdalar and hippocampal neuronal cell populations were altered. The morphological changes observed were different in the amygdala and hippocampus, which is interesting in light of prior findings that stress responses, anxiety and other behaviours can be altered in GF mice. The findings provide new insight into the role of the gut microbiome on brain development and structure, particularly with respect to synaptogenesis and synaptic morphology.

There are a number of questions which arise from this new study (Luczynski et al., 2016). One technical question is whether all of the observed effects are due only to the germ-free status, or are there other environmental factors (not involving microbial differences) which differ

between the GF and CC housing conditions? It is stated in this study (Luczynski et al., 2016) that the housing conditions (apart from the presence/absence of gut microbiota) were highly similar, so the authors appear to have controlled for such potential environmental confounds. Another possible explanation for the results could have been that the behaviour of the GF and CC mice in their home cages (which is not described in this study) could have been different. If so, any change in home-cage behaviour could have caused the observed changes in the amygdala and hippocampus (and these specific neuronal cell populations), rather than vice versa. A further possibility is that the GF mothers were providing a different in utero environment (and possibly also differential lactation and postnatal maternal care) that was impacting brain development very early in the GF offspring.

An additional potential confound in such GF studies of gut microbiome function is the fact that littermate control animals cannot be used. As the GF and CC colonies of mice must, by definition, be maintained separately, it is possible that there are subtle genetic drift and/or epigenetic effects which could lead to differences in such colonies (independent of germ-free status) over time. Recent discoveries regarding the somewhat Lamarckian spectre of transgenerational epigenetic inheritance (both maternal and paternal) affecting brain development and function (Bale, 2015), means that any animal experiments which do not (or in this case cannot) utilise littermate controls must be interpreted carefully in the light of such potential confounds.

However, these questions aside, and assuming that the differences between the GF mice were entirely due to the absence of gut microbiota, the study raises a number of new questions for this field. How does the gut microbiome regulate the morphology of amygdala and hippocampal neurons? Does this occur primarily via effects on synaptogenesis and neuronal differentiation, or were the effects observed occurring at a later stage of neuronal maturation? One way to answer such questions would be to perform a developmental timecourse study, to establish at what stage the amygdala, hippocampus, and possibly other brain structures, begin to differ between GF and CC mice. Furthermore, what are the functional consequences (including cognition and behaviour) of the amygdalar and hippocampal changes in the GF mice?

More general questions regard how specific bacterial populations in the microbiome signal from the gut to the developing and adult brain. A range of direct, and less direct, mechanisms have been proposed, including roles for the vagus nerve, immune activation, and

molecules release from gut bacterial populations which reach the CNS directly (e.g. Clarke et al., 2013; Dinan and Cryan, 2016; Kennedy et al., 2016).

This burgeoning field, exploring the effects of the gut microbiome on the brain, has major implications for our understanding of cognition, behaviour and various brain disorders, as well as their potential future treatment. Changes in the gut microbiome have been linked to a range of different neurological and psychiatric disorders. Major brain disorders proposed to have causative links to the gut microbiome include autism, schizophrenia, depression and anxiety disorders (Ochoa-Repáraz et al., 2011; Foster et al., 2013; Mayer et al., 2014; Nemani et al., 2015; McVey et al., 2016). It has been proposed that this knowledge could be used in the development of novel therapeutic approaches which target the gut microbiome, in order to treat symptoms which are primarily manifested in the brain and other organs outside the gut (Dinan et al., 2013; Borre et al., 2014). In order to harness our knowledge of the 'microbiome-gut-brain axis' for therapeutic purposes, much more mechanistic information is required regarding the exact bacterial populations which mediate neural effects, the molecular and cellular intermediates between gut and brain, and the extent to which the microbial modulators are overlaid on genetic and environmental factors which impact primarily within, and upon, the brain.

The far-reaching implications for the microbiome-gut-brain axis biology extend well beyond human health and disease. As demonstrated by these experiments in laboratory mice, as well as many other studies in a variety of other species, the microbiota-gut-brain axis has implications for agriculture, ecology and species conservation. Furthermore, the gut provides a prime potential mediator for environmental factors which are known to act on the brain. For example, there are a large number of studies which demonstrate effects of diet on brain development, adult function, cognition and behaviour. It has been demonstrated that diet can significantly alter the gut microbiome and it is possible that this could mediate neuromodulatory effects of diet. Similarly, stress also have a range of effects on the brain and some of these effects could involve the gut microbiome, as stress has also been reported to impact relevant microbiota (e.g. De Palma et al., 2015).

These trillions of bacteria within our guts are neither bystanders nor freeloaders. It appears that mice and men and their intestinal flora have co-evolved in symbiotic relationships, with the gut microbiome mediating many environmental impacts on brain structure and function.

However, humans are experiencing recent environmental changes for which our ‘hunter-gatherer’ genomes, bodies and brains are maladapted. The ‘experience-expectant’ nature of developmental algorithms may lead to ‘decanalization’ of brain maturation under shifting population environmental exposures (McGrath et al., 2011), and it is possible that the gut microbiome could contribute to such decanalized development and associated predispositions to various brain disorders (Burrows and Hannan, 2013).

In conclusion, the new findings in this article (Luczynski et al., 2016) add novel pieces to a complex puzzle of bidirectional signalling between the gut microbiome and the brain, during development and throughout life. This revolutionary new field of biology has the potential to transform how we understand the human brain and body, in health and disease.

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