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Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour

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ABSTRACT

Background: There is growing appreciation for the importance of bacteria in shaping brain development and behaviour. Adolescence and early adulthood are crucial developmental periods during which exposure to harmful environmental factors can have a permanent impact on brain function. Such environmental factors include perturbations of the gut bacteria that may affect gut–brain communication, altering the trajectory of brain development, and increasing vulnerability to psychiatric disorders. Here we assess the effects of gut bacterial depletion from weaning onwards on adult cognitive, social and emotional behaviours and markers of gut–brain axis dysfunction in mice. *Methods:* Mice were treated with a combination of antibiotics from weaning onwards and effects on behaviours and potential brain–gut axis neuromodulators (tryptophan, monoamines, and neuropeptides) and BDNF expression were assessed in adulthood. *Results:* Antibiotic-treatment depleted and restructured gut microbiota composition of caecal contents and decreased spleen weights in adulthood. Depletion of the gut microbiota from weaning onwards reduced anxiety, induced cognitive deficits, altered dynamics of the tryptophan metabolic pathway, and significantly reduced BDNF, oxytocin and vasopressin expression in the adult brain. *Conclusions:* Microbiota depletion from weaning onwards by means of chronic treatment with antibiotics in mice impacts on anxiety and cognitive behaviours as well as key neuromodulators of gut–brain communication in a manner that is similar to that reported in germ-free mice. This model may represent a more amenable alternative for germ-free mice in the assessment of microbiota modulation of behaviour. Finally, these data suggest that despite the presence of a normal gut microbiome in early postnatal life, reduced abundance and diversity of the gut microbiota from weaning influences adult behaviours and key neuromodulators of the microbiota–gut–brain axis suggesting that dysregulation of this axis in the post-weaning period may contribute to the pathogenesis of disorders associated with altered anxiety and cognition.

1. Introduction

Studies in germ-free mice have been crucial to our growing understanding of specific health benefits conferred by the commensal microbiota on host physiology, and more recently on brain function and development (Stilling et al., 2014). Specifically, the absence of bacteria from birth not only alters immune system function and our capacity to fight infection (Hapfelmeier et al., 2010), but also interferes with normal brain function and behaviours including anxiety (Diaz Heijtz et al., 2011; Neufeld et al., 2011; Clarke et al., 2013), sociability (Desbonnet et al., 2014), and memory (Gareau et al., 2011). Clinical studies reporting altered composition of the microbiota in disorders such as depression (Naseribafrouei et al., 2014; Dinan and Cryan, 2013), irritable bowel syndrome (Kennedy et al., 2014; Bonfrate et al., 2013; Jeffery et al., 2012), and autism (Finegold et al., 2010; Finegold, 2011; Mayer et al., 2014) give further credence to the theory that equilibrium of the microbial milieu in the gut, based on the specific nature, relative abundance and diversity of the various microbe

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constituents, is essential to brain development in the host. These findings have also opened up new avenues of opportunity for the development of effective therapies not only for disorders affecting the gut (Clarke et al., 2012), but also in the treatment of psychiatric conditions (Dinan, 2013).

Adolescence and early adulthood are important periods for brain development (Sturman and Moghaddam, 2011). Significant changes relating to neuronal architecture and function occur during adolescence that, from an evolutionary perspective, promote the maturation of behaviours and skills such as social and cognitive capabilities necessary to achieve independence from the secure family environment (Burnett et al., 2011). Environmental events have the capacity to shape and mould neuronal architecture and implement adaptations in brain function that in theory better equip an individual to cope with their environmental challenges. Nevertheless, aversive environmental factors experienced by an individual during this window of vulnerability can also result in maladaptive changes in brain development; the qualitative and quantitative nature of which depends on various factors (Spear, 2011). Hence, it is not surprising that many adult neuropsychiatric disorders, particularly schizophrenia, have their roots in this vulnerable period (Paus et al., 2008). The gut microbiota through associated metabolic and immune activities, afford significant advantages to the host throughout development (Selkrig et al., 2014). Recent studies have focused on the deleterious effects of early life antibiotic exposure on various health outcomes (Cho et al., 2012; Cox et al., 2014). To our knowledge, changes in brain development and behaviour as a result of gut microbiota depletion from early adolescence specifically, have not been assessed to date.

In this study we aim to examine the specific contributions of the microbiota from weaning onwards to brain development and behaviours by depleting the gut microbiota using a combination of antibiotics administered in high doses in the drinking water of mice. Thus by examining the effects of gut microbiota depletion on stress-responsivity, emotional and cognitive behaviours and neurochemical measures relevant to the microbiota–gut–brain axis (monoamines, tryptophan, kynurenine, BDNF, neuropeptides), we can assess the specific role bacteria play in brain development from weaning onwards.

2. Methods

2.1. Animals

First-generation offspring from NIH Swiss breeding pairs obtained from Harlan (UK) were used in all experiments. NIH Swiss mice were housed 4–5/cage in standard mouse cages in our barrier laboratory animal housing facility under a strict 12-h light/dark cycle. Antibiotic-treated and control mice received the same autoclaved pelleted diet (Special Diet Services, product code 801010). All mice were tested in adulthood (postnatal day 55–80). Experiments were conducted in accordance with the European Directive 86/609/EEC and the Recommendation 2007/526/65/EC, and were approved by the Animal Experimentation Ethics Committee of University College Cork.

2.2. Antibiotic treatment

To deplete the gut microbiota in adolescence [postnatal day (P) 21 onwards], a combination of antibiotics was chosen based on a previous report that this combination reduced the faecal bacterial DNA load by 400-fold while ensuring the animals' health (Reikvam et al., 2011). To avoid any confounding effects resulting from chronic stress induced by oral gavage (Branchi et al., 2005), antibiotics were administered in the drinking water and bottles were changed every second day. The dose of antibiotics was adjusted according to the mean volume of water consumed per mouse on each day. Water was autoclaved and water intake was monitored daily for the first week to adjust antibiotic dose and ensure the health of the animals, and subsequently monitored twice a week until termination of the study. The antibiotic cocktail consisted of ampicillin (1 mg/ml), vancomycin (5 mg/ml), neomycin (10 mg/ml), metronidazol (10 mg/ml), and supplemented with amphotericin-B (0.1 mg/ml). Groups consisted of non-treated control males ($n = 14$), and antibiotic-treated males ($n = 15$).

2.3. Body/tissue weight

To monitor the general health of the animals, body weights were recorded twice a week until commencement of the behavioural tests and also on the day of sacrifice. Post-mortem weights of spleen and adrenal glands were also measured and spleen/body and adrenal/body weight ratios were calculated for each mouse.

2.4. Behavioural assessments

Behavioural testing commenced approximately 4 weeks following the first dose of antibiotics (postnatal day 55) and where possible behaviour was scored using automated Ethovision video tracking software (XT 7.0; Noldus, The Netherlands). Details relating to the behavioural tests are provided in the Supplementary methods. Exploration and non-spatial cognition was assessed in the novel object recognition test. Anxiety was assessed using the light/dark box test by measuring time spent in the light chamber and faecal pellet excretion. The social transmission of food preference (Clipperton et al., 2008) test was adopted to assess social interaction and social memory in mice. Behavioural tests were conducted in the order above over a 2 week period in adulthood (postnatal day 55–70) with a 1 week interval between testing to minimise any stress associated with repeated testing. Antibiotic treatment was continued throughout testing (Fig. 1).

2.5. Acute restraint stress and tissue dissection

The absence of bacteria in mice is reported to increase the neuroendocrine response to acute restraint stress in adulthood (Sudo et al., 2004). Therefore, the effects of antibiotic-induced microbiota depletion on the corticosterone response to acute stress were assessed. One week after behavioural testing (postnatal day 77–80) and 30 min before sacrifice, antibiotic-treated and control mice were assigned to non-stress and stress groups such that half of each treatment group was subjected to a 30 min restraint stress to assess effects of antibiotic treatment on corticosterone response to an acute stressor. Mice were sacrificed, blood samples were taken and brains were dissected as described in the Supplementary methods.

2.6. Corticosterone assay

Serum corticosterone levels were assayed in duplicate using a Corticosterone Immunoassay Kit according to the manufacturers' instructions (Enzo Life Sciences, UK). The assay has a sensitivity of 26.99 pg/ml.

2.7. DNA extraction and high throughput DNA sequencing

Total DNA was isolated from caecal contents and processed for analysis of microbiota composition in line with 454 protocols at the Teagasc high throughput sequencing centre as described in Supplement 1. Phylum counts for each sample were extracted from MEtaGenome ANalyzer (MEGAN). α and β diversity indices and

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