



Do gut reactions to antibiotics lead to sex dependent changes in behavior following neonatal immune challenge?



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It has been established that early life perturbations to the developing brain, immune, and neuroendocrine systems play a significant role in the developmental origins of anatomy, physiology, and behavior (Bilbo, 2013; Fleming et al., 2018). These perturbations may take the form of a diverse array of stressors, both processive and systemic (Anisman and Merali, 1999), that impart critical influences on lifetime health as demonstrated by both human and animal studies (Fleming et al., 2018). Given that the gut-brain axis develops simultaneously alongside neural, endocrine and immune pathways suggests that early life challenges to this system may also have an impact on its maturational trajectory and be influential on later life health outcomes (Borre et al., 2014). It is these concepts, among others, that Sylvia et al (2018) explore in this issue of *Brain, Behavior, and Immunity*.

A renewed interest in the modulatory effects of the microbiome on both physiology and behavior has led to the evolution of our understanding of this system. The microbiome is now understood to have a pivotal role in organism functioning, a concept even emerging as a dominant topic in the popular press (e.g. Pontin, 2018). Studies investigating the microbiome frequently utilize germ free animals, however these animals do not adequately represent our natural environment where we are surrounded by a plethora of bacteria, fungi, and viruses. Alternatively, direct manipulation of the microbiome can be exploited by employing antibiotic treatments. Indeed, using antibiotics as a tool has revealed sex-dependent associations between the diversity of the gut microbiome and social behaviors in adult animals (Sylvia et al., 2017; Sylvia et al., 2018). Not surprisingly, early life exposure to antibiotics also affects the diversity of microbial colonization and has been implicated in later life anxiety-like behavior (Borre et al 2014), highlighting the role of the microbiome across the lifespan.

In their most recent work, Sylvia et al (2018) sought to determine whether exposure to early life inflammation affects the composition of the gut microbiome and its influences on behavior in response to an antibiotic ‘stressor’ exposure in adulthood. Using a dual-administration protocol, male and female Siberian hamsters were given 50 µg/kg of lipopolysaccharide (LPS) or sterile saline on postnatal days (P)3 and 5. In adulthood, animals were assigned to receive an oral administration of either sterile water or the broad spectrum antibiotic enrofloxacin (ABX; Baytril) daily between P72 and P78. Using 16 s rRNA sequencing

methods, fecal samples collected at defined time points across development (e.g. weaning, pre-, and post- ABX treatment) were evaluated. In parallel with others, Sylvia and colleagues did not find that microbial communities differed substantially between male and female animals, nor as a consequence of postnatal LPS challenge alone (Kentner et al., 2018; Sylvia et al., 2018). However, the authors extend upon these findings by demonstrating that a secondary challenge with ABX in adulthood significantly altered the composition of the gut microbiome as a function of early life inflammatory experience. Moreover, neonatal LPS and adult ABX treatments were associated with sex dependent effects on anxiety-like behaviors. Combined, these data suggest that early life inflammatory challenges may leave individuals ‘primed’ for altered gut-mediated behavioral responses to subsequent stressors encountered across the life span (Sylvia et al., 2018). This view is in line with previous work (see Bilbo, 2013) demonstrating that the underlying immune and behavioral programming effects of early life immune activation may not be apparent until there is a secondary challenge (in this case antibiotics) in later life.

The sex specific nature of the neurobehavioral outcomes that follow stressors, either in isolation or against a background of perinatal inflammation, contribute additional complexity to our understanding of brain and behavior. However, these differences inform us of important methodological considerations such as the developmental timing of stressor exposure. For example, Sylvia et al. (2018) observed that a single ABX treatment was sufficient to induce aggressive behavior in older female hamsters while two treatments were required for males. In the present study however (Sylvia et al., 2018), only male investigative and self-directed grooming behaviors were altered by antibiotics in younger adult animals, either in isolation or combined with neonatal inflammatory challenge. Other studies have also reported sexually dimorphic responses as a function of the developmental timing of stressor exposure (Giovanoli et al., 2013; Kapoor et al., 2009). Future investigations will need to evaluate how neuroendocrine, immune, and gut-brain mechanisms converge and how the developmental trajectory of these systems differ by sex.

Furthermore, Sylvia et al (2018) highlight the importance of considering an organism’s previous experience within their present context, when trying to understand and interpret biological outcomes associated

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with stressor exposure. Taking a developmental time course perspective across the lifespan may provide us with better insight into the interactions between multiple stressors (both homotypic and heterotypic) and body systems (e.g. CNS, immune, microbiome), compared to the typical research approach of focusing on one type of stressor or body system in isolation. While the clinical translation of this work can only be speculative at this point, the study by [Sylvia and colleagues \(2018\)](#) raises the intriguing possibility that early life immune activation may program our neurobiological responses to antibiotic exposure in adulthood, as mediated via the microbiome. However, this possibility is not exclusive to antibiotics as these findings are in line with previous evidence demonstrating early programming effects to stressors in general ([Giovanoli et al., 2013](#); [Kapoor et al., 2009](#); [Bilbo, 2013](#)). The evidence provided in the current study offers insight into the widely studied issue of how antibiotics affect not only our microbiome but also have further implications systemically on one's health and social behavior ([Sylvia et al., 2018](#)). There is a multitude of current research that confirms the relationship between the gut microbiome and other body systems. Yet, now, more studies are investigating further into the effects the gut microbiome can possibly have on host social behaviors ([Münger et al., 2018](#)). It is now apparent that a better understanding of the mechanisms underlying gut-brain axis development and its intricate interactions between different body systems will be fundamental to our understanding of health and behavior across the lifespan.

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