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Sex-specific modulation of the gut microbiome and behavior in Siberian hamsters

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ABSTRACT

The gut microbiome is a diverse, host-specific, and symbiotic bacterial environment that is critical for mammalian survival and exerts a surprising yet powerful influence on brain and behavior. Gut dysbiosis has been linked to a wide range of physical and psychological disorders, including autism spectrum disorders and anxiety, as well as autoimmune and inflammatory disorders. A wealth of information on the effects of dysbiosis on anxiety and depression has been reported in laboratory model systems (e.g., germ-free mice); however, the effects of microbiome disruption on social behaviors (e.g., aggression) of non-model species that may be particularly important in understanding many aspects of physiology and behavior have yet to be fully explored. Here we assessed the sex-specific effects of a broad-spectrum antibiotic on the gut microbiome and its effects on social behaviors in male and female Siberian hamsters (*Phodopus sungorus*). In Experiment 1, we administered a broad-spectrum antibiotic on a short-term basis and found that antibiotic treatment altered the microbial communities in the gut in male and female hamsters. In Experiment 2, we tested the effects of single versus repeated antibiotic treatment (including a recovery phase) on behavior, and found that two, but not one, treatments caused marked decreases in aggressive behavior, but not other social behaviors, in males; aggression returned to normal levels following recovery. Antibiotic-treated females, in contrast, showed decreased aggression after a single treatment, with all other social behaviors unaffected. Unlike males, female aggression did not return to normal during either recovery period. The present findings demonstrate that modest antibiotic treatment results in marked disruption of the gut microbiome in hamsters, akin to research done in other rodent species and humans. Further, we show that treatment with a broad-spectrum antibiotic, which has dysbiotic effects, also has robust, sex-specific effects on aggression, a critical behavior in the survival and reproductive success of many rodent species.

1. Introduction

The large intestine of the gastrointestinal (GI) tract contains about 100 trillion microorganisms, an amount ten times greater than the total number of cells in the body (Wallace et al., 2011). Though much of our attention was not focused on these microorganisms until recently. The gut microbiome is not only a diverse, host unique, and symbiotic bacterial environment, but it is critical for mammalian survival (Clarke et al., 2014), and further, exerts a surprisingly powerful influence on brain and behavior.

Information from the gut can communicate with the central nervous system (CNS) and thus microbes living in the gut can influence memory, emotions, and affective behaviors in many species (Dinan and Cryan, 2012). For example, dysbiosis has been linked to various physical and psychological disorders, including autism spectrum disorders (ASD) and anxiety, as well as autoimmune and inflammatory disorders, such as allergies and asthma (Clarke et al., 2014; Mueller et al., 2015). Individuals of all species must have the ability to express the appropriate types of behaviors in context. Therefore studying how disruption of the microbiome might affect these behaviors is important to understand the fitness of an individual across a range of species, including humans, and with such insights we can begin to connect ecological and translational research.

A wealth of information has been reported in model systems (e.g., germ-free mice) (Clarke et al., 2014; Collins et al., 2012; O'Mahony et al., 2009); however, the effects of microbiome disruption on social behaviors particularly important in the success of non-model species (e.g., aggression) have yet to be fully explored. In studying these model systems, researchers are only partially

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able to determine the role that the microbiota play in the natural function of the mammalian system. Although they have therapeutic benefits, antibiotics can alter the structure, function, and ultimately evolution of host microbial communities in the gut (Archie and Theis, 2011). Therefore antibiotics are a useful tool to manipulate the gut microbiome, and further, doing so in a non-model species provides insight into the potential cause of the microbiota-dependent changes we see in physiology and behavior and will help complement the important strides the field has taken in understanding the gut-brain axis thus far.

Recent work in BALB/c mice has shown that disruption of the microbiota via antibiotics increases exploration, a non-social behavior, yet the same change in behavior is not seen in germ-free mice, unless the germ-free mice are colonized with the microbiota from other strains (Bercik et al., 2011). Because most social behaviors are essential for the maintenance of appropriate interactions with conspecifics, both in reproductive and non-reproductive contexts, understanding the ways in which they are affected are important to our understanding of behavior and physiology. The influence of the gut microbiome on social behavior has become of recent interest, yet the effects on aggression, an important behavior for a number of rodent species and humans alike, has not yet been determined. Aggressive behavior varies greatly across species, however, a number of indicators can be used to identify it. Often, an aggressive act involves two or more individuals competing for resources (e.g., food, mates, territory), and these acts of aggression may result in severe injury, or even death (Gould and Zeigler, 2007; Soma et al., 2015). Aggressive behaviors include attacking and wrestling, chasing and biting (Jasnow et al., 2002; Nelson et al., 1995), as well as pushing and jump-fighting. Many of these aggressive behaviors are important in signaling reproductive condition to potential mates (Gould and Zeigler, 2007).

To provide information about identity, sex, and reproductive state, which are important for survival, many rodents, including male and female hamsters, have a sexually dimorphic gland on the midline of their ventral surface that they use for scent-marking, in which they rub their ventral gland on a protruding surface (Johnston, 1993; Lai and Johnston, 1994; Reasner and Johnston, 1987; Rendon et al., 2016b). These types of behaviors are essential for recognition of conspecifics, and are particularly important in a social context. When animals are first introduced, they will investigate one another, via nose-to-nose and nose-to-anogenital sniffing, in order to use the chemical signals to identify sex, age, reproductive status, quality, and other characteristics of their conspecifics (Pellis and Pellis, 1988; Rendon et al., 2016a,b). Because investigative behaviors can influence how often other social behaviors (e.g., exploration, aggression, reproduction) may occur, they are critical to our understanding of the behavioral phenotype (Wynne-Edwards and Lisk, 1987; Wynne-Edwards, 2003). Investigating how the microbiome affects these important behaviors may help us to determine a more thorough understanding of psychiatric disorders often associated with unusual social interactions, such as autism or schizophrenia (Scattoni et al., 2011).

The goal of the present study was to examine if the broad-spectrum antibiotic, enrofloxacin, can be used as a tool to manipulate the gut microbiome and whether this tool also has the potential to affect social behaviors in male and female Siberian hamsters. We hypothesized that a 7-day antibiotic treatment would be sufficient to alter the microbial composition of the gut as well as to initiate changes in both aggressive and investigative behaviors, therefore altering aspects of the adult behavioral phenotype. The results of these studies will help to further our understanding of the influence of the gut microbiome on physiology and behavior, and potential sex differences seen in response to disruption of the gut microbiome.

2. Materials and methods

2.1. Experiment 1: Effect of antibiotics on the gut microbiome

2.1.1. Animal housing conditions

Male and female adult (>60 days of age) hamsters were reared and maintained under long days (light:dark, 16:8 h), and individually housed in polypropylene cages (28 × 17 × 12 cm). Males and females were run together in cohorts in the same animal holding room. All procedures were the same for all cohorts. Ambient temperature was maintained at 20 ± 2 °C, and relative humidity was maintained at 55 ± 5%. Hamsters were given *ad libitum* access to purified tap water and standard laboratory rodent chow (Lab Diet 5001, PMI Nutrition). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee at Indiana University.

2.1.2. Antibiotic treatment

To determine how modest antibiotic treatment affects the microbial communities in the gut and potential sex differences in the gut microbiome in Siberian hamsters, 12 males and 12 females were assigned to either a control group (n = 6 males, n = 6 females), in which animals received sterilized water administered via sterile pipette orally once daily, or an experimental group (n = 6 males, n = 6 females), in which animals received a broad-spectrum antibiotic [Abx: 0.3 μl of enrofloxacin (Baytril, Bayer Animal Health) 10% oral solution per gram of body mass] administered via sterile pipette orally once daily (Romick-Rosendale et al., 2009). Enrofloxacin is a fluoroquinolone antimicrobial agent, frequently used for treatment in many domesticated animals and does not easily cross the blood brain barrier (BBB) (Alvarez et al., 2010; Ooie et al., 1997a,b; Slate et al., 2014). Enrofloxacin inhibits DNA synthesis and has been given orally to hamsters numerous times at varying doses and has been documented as safe and effective in our species (Martorell et al., 2010; Romick-Rosendale et al., 2009; Slate et al., 2014; Thomas et al., 2008). On days 1–7 (D1-7) of experimentation (Pre-Treatment), all animals were monitored and weighed regularly. During days 8–14 (Treatment), Abx animals received Abx treatment once daily and control animals received sterilized water once daily, during which animals were monitored and weighed regularly. During days 14–21 (Post-Treatment), both the experimental group and the control group were monitored and weighed regularly.

2.1.3. Fecal sampling

Following the Pre-Treatment, Abx/Control Treatment, and the Post-Treatment Recovery period, effects on the gut microbiome were assessed by taking fecal samples from each animal. To take fecal samples, animals were removed from their home cage and held over a sterile container once daily, after which the fecal samples were stored in −80 °C until the samples were processed. All animals were returned to their home cage until the following day. To determine whether restraint stress during fecal samples affected the gut microbiome, fecal samples were also taken from a subset of males and females (n = 3 males and n = 3 females) that received neither water (control) treatment nor antibiotic (experimental) treatment.

2.1.4. Microbiome analysis

Fecal samples were sequenced (IDEXX Bioresearch, Columbia, MO) to determine microbial composition in the gut (n = 9 males; n = 9 females). DNA was extracted from the fecal material and purified over a DNeasy spin column, and the extracted DNA was quantified via fluorometry for normalization when plating. Using the