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

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**A R T I C L E   I N F O**

Article history:
Received 29 June 2016
Received in revised form 28 September 2016
Accepted 16 October 2016
Available online 2 November 2016

**Keywords:**
Aggression
Blood-brain barrier
Enrofloxacin
Gut-brain axis
Immune system
Investigation
Scent-marking

**A B S T R A C T**

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.

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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
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
purified DNA, a previously well-validated U515F/806R primer set was used to amplify a portion of the V4 region of the 16S rRNA gene using the Illumina MiSeq platform yielding a 300 base pair fragment from all bacterial species present in the sample (Ericsson et al., 2015). From each, 200–500 megabases of sequence information were obtained. Sequences were determined using QIIME (Quantitative Insights Into Microbial Ecology) v1.8 Pipeline, and once sequences were determined, they were BLASTed (Basic Local Alignment Search Tool) against the Greengenes database (Second Genome, Inc.: University of Colorado, University of Queensland) to identify operational taxonomic units (OTUs) and taxonomic classification. We found a mean of 32,335 sequences per sample. We conducted a principal component analyses (PCA) on the 14 bacterial phyla that were present in fecal samples for both sexes to examine the effects of antibiotic treatment on microbial communities across groups.

2.1.5. Tissue collection
At the end of the experiment (D21), all animals were euthanized using a lethal intraperitoneal (i.p.) injection of a ketamine and xylazine cocktail in 0.9% saline. Following euthanasia, livers, spleens, and reproductive organs were dissected and weighed to determine the effects of antibiotic treatment on gross anatomy.

2.2. Experiment 2: Effects of repeated antibiotic treatment on the microbiome and social behavior

2.2.1. 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).

2.2.2. Antibiotic treatment
We housed 18 males and 18 females, and assigned them to either a control group (n = 9 males; n = 9 females), in which animals received sterilized water administered via sterile pipette orally once daily for two treatment courses, or an experimental group (n = 9 males; n = 9 females), in which animals received a broad-spectrum antibiotic [Abx: 0.3 μL of enrofloxacins per gram of body mass administered via sterile pipette orally once daily] for two treatment courses (Romick-Rosendale et al., 2009). On days 1–7 (D1-7) of experimentation (First Treatment Period), Abx animals received Abx treatment and control animals received sterilized water. On days 8–14 (First Recovery Period), all animals were monitored and weighed. Again on days 15–21 (Second Treatment Period) Abx animals received Abx treatment and control animals received sterilized water. Finally, on days 22–28 (Second Recovery Period), all animals were monitored and weighed.

2.2.3. Fecal sampling
Following Treatment Period 2, effects on the gut microbiome were assessed by taking fecal samples from each animal. To collect fecal samples, we used the same protocol described in Experiment 1. All fecal samples were stored in −80°C until the samples were processed.

2.2.4. Microbiome analysis
Fecal samples collected at the end of Treatment Period 2 were sequenced (IDEXX Bioresearch, Columbia, MD) to determine microbial composition in the gut (n = 6 males, n = 6 females). DNA was extracted from the fecal material, purified, and quantified according to the previously described protocol. The DNA was then used to amplify a portion of the V4 region of the 16S rRNA gene using the Illumina MiSeq platform yielding a 300 base pair fragment from all bacterial species present in the sample (Ericsson et al., 2015). From each, 200–500 megabases of sequence information were obtained. Sequences were determined using QIIME v1.8 Pipeline and BLASTed against the Greengenes database to identify operational taxonomic units (OTUs) and taxonomic classification. We found a mean of 81,313 sequences per sample.

2.2.5. Behavioral interactions and analyses
We recorded and analyzed aggressive behavior, investigative behavior, scent-marking behavior, and grooming using the resident-intruder paradigm with same-sex social partners per previously outlined methods for this species (Jasnow et al., 2000; Rendon et al., 2016b, 2015). On D7 (Treatment 1), D14 (Recovery 1), D21 (Treatment 2) and D28 (Recovery 2), behavioral testing was conducted during the first 2 h of the dark phase to control for circadian rhythm of behavior. We staged five-minute dyads such that they were composed of a resident hamster (n = 18 males and n = 18 females) and a same-sex intruder hamster (n = 9 males and n = 9 females). We paired animals of the same reproductive status, of approximately the same age, with comparable mass (±5%), and from different parents. All trials were video recorded (Sony HandyCam Digital Camcorder HDR-SR7) under low-illumination red lights.

We quantified aggressive, investigative, scent-marking, and grooming behaviors of the resident hamster using ODLg™ (Macropod Software, Eden Prairie, MN). For aggression, we quantified latency to first attack (s), and frequency and duration of attacks and chases (s). For investigation, we quantified the frequency and duration of nose-to-anogenital and nose-to-nose investigation (s). For scent-marking, we quantified the frequency and duration of scent depositing (s), and for grooming, we quantified the frequency and duration of the resident animal grooming him or herself (s). We used four separate principal component analyses (PCA) on all aggression variables (69.63–88.28% of the total variance explained, Table 1 in Supplementary material), investigative variables (84.61–95.23% of the total variance explained, Table 2 in Supplementary material), scent-marking variables (96.15–99.98% of the total variance explained, Table 3 in Supplementary material) and grooming variables (79.70–96.28% of the total variance explained, Table 4 in Supplementary material) for both sexes. All behaviors independently loaded strongly onto the first component; therefore the composite aggression score (PC-AGG), composite investigation score (PC-INV), composite scent-marking score (PC-SMK) and composite grooming score (PC-GRM) were used to examine the effects of antibiotic treatment on changes in social behavior of resident hamsters.

2.2.6. Tissue collection
At the end of the experiment (D28), all animals were euthanized via a lethal i.p. injection of a ketamine and xylazine cocktail in 0.9% saline. Following euthanasia, livers, spleens, reproductive organs and reproductive fat tissue were dissected and weighed to determine the effects of antibiotics on gross anatomy.

3. Statistical analyses
We performed all statistical analyses on microbiome data in R v. 3.2.2 (R Core Team 2015), and attributed statistical significance at p < 0.05. Principal component analyses (PCA) were conducted on microbiome data at the phylum level in Experiment 1 (Table 5 in Supplementary material). Two-way analyses of variance (ANOVAs)
were used to compare the effects of treatment (control and antibiotics) and time (Pre-Treatment, Treatment, and Post-Treatment) across groups in Experiment 1. If a two-way ANOVA reported a significant interaction of treatment and time, Tukey’s Honest Significant Difference post hoc tests were run to determine pair-wise relationships. To determine the diversity across groups, we calculated the Shannon-Wiener index and converted values to the effective number of species (Hill, 1973; Jost, 2006), and we also calculated Bray-Curtis dissimilarity scores across groups and time points. In Experiment 2, two-tailed t-tests were used to compare the relative abundance of each phylum across treatment groups following Treatment Period 2.

All behavioral analyses were performed in JMP v. 11.0.0 (SAS Institute, Inc., Cary, NC), and attributed statistical significance at \( p < 0.05 \). Data were log or square root transformed to attain normality and equal variances. PCAs were conducted on the social behaviors (Tables 1–4 in Supplementary material) to reduce the number of variables for analysis, retaining PCs with an eigenvalue greater than 1. Two-tailed t-tests were used to compare physiological and behavioral changes across treatment groups. Mixed model ANOVAs were calculated with treatment (control and antibiotics) as a between-groups variable and time (Treatment 1, Recovery 1, Treatment 2, Recovery 2) as a within-subjects variable. If a mixed model ANOVA reported a significant effect or interaction of effects pair-wise comparisons were conducted using two-tailed t-tests. Spearman’s rank correlations were run on the relative abundance of each phylum across treatment groups following Treatment Period 2.

In Experiment 2, two-tailed \( t \)-tests were used to compare physiological and behavioral changes across treatment groups. Mixed model ANOVAs were calculated with treatment (control and antibiotics) as a between-groups variable and time (Treatment 1, Recovery 1, Treatment 2, Recovery 2) as a within-subjects variable. If a mixed model ANOVA reported a significant effect or interaction of effects pair-wise comparisons were conducted using two-tailed t-tests. Spearman’s rank correlations were run on the relative abundance of each phylum across treatment groups following Treatment Period 2.

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4. Results

4.1. Experiment 1

4.1.1. Siberian hamsters have a diverse gut microbiome

The microbial community composition in fecal samples of male and female hamsters with and without antibiotics is shown in Fig. 1. The composition of the male gut microbiome was dominated by Firmicutes (~50%) and Bacteroidetes (~40%). In males, 95 unique operational taxonomic units (OTU) were identified from 14 phyla, including one phylum from the Archaea kingdom and a designated ‘Other’ category where no specific BLAST hit was found for the sequences. Thirteen OTUs were eliminated from this count, because they were only represented in 1 sample.

The composition of the female gut microbiome was dominated by Firmicutes (~50%) and Bacteroidetes (~30%). In females, 98 unique operational taxonomic units were identified from 14 phyla, including one phylum from the Archaea kingdom and a designated ‘Other’ category when there was no specific BLAST hit for the sequences. Thirteen OTUs were eliminated from this count, because they were only represented in 1 sample.

4.1.2. Antibiotics differentially affect the gut microbiota across the sexes

Three PCs were extracted for each sex. For males, 92.23% of the total variance was explained by PCs 1–3 (Table 5 in Supplementary material). Baseline measures were scattered for PCs 1 and 2 among groups, but clustered for PC 3, indicating a variable microbial composition for males. In contrast, control and antibiotic groups were clearly separated in opposite directions during both the treatment and recovery periods, which indicates that antibiotic treatment alters microbial diversity in the gut (Fig. 2a&b). In males, treatment affected 5 phyla: Cyanobacteria \((F_{1,12} = 4.91, p = 0.047)\), Elusimicrobia \((F_{1,12} = 6.24, p = 0.03)\), Euryarchaeota \((F_{1,12} = 5.89, p = 0.03)\), Proteobacteria \((F_{1,12} = 12.23, p = 0.004)\), and TM7 \((F_{1,12} = 4.85, p = 0.048)\). Time affected Proteobacteria \((F_{2,12} = 4.90, p = 0.03)\), and TM7 \((F_{2,12} = 4.57, p = 0.03)\). There was an interaction between treatment and time for Tenericutes \((F_{2,12} = 4.74, p = 0.03)\), and TM7 \((F_{2,12} = 5.51, p = 0.02)\) (Fig. 3a-f). There was no effect of treatment or time or their interaction for 8 phyla: ‘Other’, Actinobacteria, Bacteroidetes, Deferrribacteres, Firmicutes, Fusobacteria, Spirochaetes, and Verrucomicrobia \((p > 0.05)\) in all cases.

For females, 92.28% of the total variance was explained by PCs 1–3 (Table 5 in Supplementary material). Baseline measures were clustered for all 3 PCs among groups, indicating a homogenous

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**Fig. 1.** The microbial community composition in fecal samples of male and female hamsters across treatment groups. The composition of the male and female gut microbiome is made up of 14 phyla, including one phylum from the Archaea kingdom and a designated ‘Other’ category where no specific BLAST hit was found for the sequences. Both the male and female gut microbiome are dominated by Firmicutes and Bacteroidetes, yet antibiotic treatment does not affect these highly abundant phyla, and instead most strongly affects phyla in lower abundance in both male and female hamsters.
microbial composition for females. In contrast, control and antibiotic groups were clearly separated in opposite directions during both the treatment and recovery periods, indicating that antibiotic treatment alters the microbial composition of the gut (Fig. 2c&d). In females, treatment affected 3 phyla: Cyanobacteria ($F_{1,12} = 18.88, p = 0.001$), Proteobacteria ($F_{1,12} = 11.01, p = 0.006$), and Tenericutes ($F_{1,12} = 6.67, p = 0.02$). Time affected Proteobacteria ($F_{2,12} = 6.99, p = 0.01$). There was an interaction between treatment and time for Proteobacteria ($F_{2,12} = 4.55, p = 0.03$) (Fig. 3g-i). There was no effect of treatment, time, or their interaction for 11 phyla: ‘Other’, Actinobacteria, Bacteroidetes, Deferribacteres, Elusimicrobia, Euryarchaeota, Firmicutes, Fusobacteria, Spirochaetes, TM7, and Verrucomicrobia ($p > 0.05$ in all cases).

Analysis of the No-Treatment group validated that we did not introduce any new, unidentifiable bacteria into the antibiotic or control groups, therefore analysis and results were completed on the antibiotic- and water-treated groups only.

4.1.3. Antibiotics affect the diversity of the gut microbiome

In males, the effective number of species before treatment was 11.653 in control animals and 12.290 in Abx animals. Immediately following the treatment period, the effective number of species was 10.455 in control animals, and in Abx animals, the effective number of species decreased to 5.683, approximately 46% less diverse than the control group at this time point. Following the recovery period, the effective number of species was 8.840 in control animals, and in Abx animals, the value increased slightly, to 6.531 (Table 6 in Supplementary material). Similarly, in females, the effective number of species before treatment was 10.583 in control animals and 9.988 in Abx animals. Immediately following
In the treatment period, the effective number of species was 11.680 in control animals, and in Abx animals, the effective number decreased to 6.620, approximately 44% less diverse than the control group at this time point. Following the recovery period, the effective number of species was 12.317 in control animals, and in Abx animals, there was a small increase, to 7.547 (Table 7 in Supplementary material). In both male and female antibiotic groups, there was a decrease in alpha diversity following the treatment period, and the same decrease was not seen in control groups.

Further, in males across groups, the pre-treatment Bray-Curtis dissimilarity score was 0.322, the treatment period Bray-Curtis dissimilarity score was 0.374, and the post-treatment Bray-Curtis dissimilarity score 0.190 (Table 8 in Supplementary material). In females across groups, the pre-treatment Bray-Curtis dissimilarity score was 0.005, the treatment period Bray-Curtis dissimilarity score was 0.004, and the post-treatment Bray-Curtis dissimilarity score 0.003.

Fig. 3. Effects of antibiotics on the relative abundance of bacterial phyla in male (a-f) and female (g-i) hamsters. Although Bacteroidetes and Firmicutes are in the highest relative abundance in male and female hamster fecal samples, antibiotics did not affect these phyla, and instead those phyla in low relative abundance are most strongly affected by treatment in both sexes. In males and females, there was an effect between treatment, time, or their interaction for 3 phyla: Cyanobacteria (a,g), Proteobacteria (b,h), and Tenericutes (c,i). Male microbiome analysis showed an effect between treatment, time, or their interaction for 3 additional phyla: Elusimicrobia (d), Euryarchaeta (e), and TM7 (f). There was no effect of treatment or time or their interaction for 8 phyla in males: ‘Other’, Actinobacteria, Bacteroidetes, Deferribacteres, Firmicutes, Fusobacteria, Spirochaetes, and Verrucomicrobia, and there was no effect of treatment or time or their interaction for 11 phyla in females: ‘Other’, Actinobacteria, Bacteroidetes, Deferribacteres, Elusimicrobia, Euryarchaeta, Firmicutes, Fusobacteria, Spirochaetes, TM7, and Verrucomicrobia. Bar heights represent means ± S.E.M. Means with different letters are statistically different (p < 0.05).
score was 0.111, the treatment period Bray-Curtis dissimilarity score was 0.277, and the post-treatment Bray-Curtis dissimilarity score 0.206 (Table 9 in Supplementary material). In both males and females, the greatest dissimilarity was observed during the treatment period.

4.1.4. Antibiotics did not affect body mass or organ mass

Antibiotics did not affect male body mass ($F_{1,14} = 1.09, p = 0.91$), liver mass ($F_{1,14} = 0.91, p = 0.91$), or spleen mass ($F_{1,14} = 0.67, p = 0.53$), when compared with control males. Similarly, antibiotics did not affect female body mass ($F_{1,13} = 1.93, p = 0.42$), liver mass ($F_{1,13} = 1.31, p = 0.74$), or spleen mass ($F_{1,13} = 2.59, p = 0.12$), when compared with control females (Table 10 in Supplementary material).

4.2. Experiment 2

4.2.1. Repeated antibiotics affect the gut microbiota

Similar to Experiment 1, we assessed the effects of antibiotics on the male and female microbiome. In males, treatment significantly affected the relative abundance of Tenericutes ($t_2 = -5.539, p = 0.031$), and it moderately affected Cyanobacteria ($t_2 = -3.5572, p = 0.071$) and Proteobacteria ($t_{2,441} = -3.0512, p = 0.072$) (Fig. 4a and Fig. 1 in Supplementary material). There was no effect of treatment for 11 phyla: ‘Other’, Actinobacteria, Bacteroidetes, Deferrribacteres, Elusimicrobia, Euryarchaeota, Firmicutes, Fusobacteria, Spirochaetes, TM7, and Verrucomicrobia ($p > 0.05$ in all cases). In females, treatment affected TM7 ($t_{1,620} = -4.102, p = 0.018$) and Tenericutes ($t_2 = -4.092, p = 0.055$), and it moderately affected Cyanobacteria ($t_2 = -3.001, p = 0.095$) (Fig. 4b and Fig. 1 in Supplementary material). There was no effect of treatment for 11 phyla: ‘Other’, Actinobacteria, Bacteroidetes, Deferrribacteres, Elusimicrobia, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, and Verrucomicrobia ($p > 0.05$ in all cases).

4.2.2. Repeated antibiotics affect the diversity of the gut microbiome

Following the second treatment period in males, the effective number of species was 8.691 in control animals and 3.950 in Abx animals, approximately 55% less diverse than the control group; in females, the effective number of species was 8.601 in control animals and 5.011 in Abx animals, approximately 42% less diverse than in the control group (Table 11 in Supplementary material). In both male and female antibiotic groups, alpha diversity was lower than in the control group (Table 11 in Supplementary material). In males, the Bray-Curtis dissimilarity score was 0.267 following treatment period 2, and in females, the Bray-Curtis dissimilarity score was 0.156 (Table 12 in Supplementary material).

4.2.3. Antibiotics affect aggressive behavior differentially across the sexes

Male hamsters given a single, 7-day antibiotic treatment displayed no change in overall aggression (PC-AGG, $F_{1,14} = 4.80, p = 0.79$) when compared with males given a one-time, 7-day control treatment (Fig. 5c). Specifically, males given a one-time week-long antibiotic treatment displayed no change in number ($F_{1,14} = 4.20, p = 0.66$, Fig. 5a) or duration ($F_{1,14} = 4.26, p = 0.62$) of attacks and no change in number in females ($F_{1,14} = 4.15, p = 0.70$) or duration ($F_{1,14} = 3.62, p = 0.81$) of chases. In addition, latency to first attack did not differ across groups ($F_{1,14} = 2.64, p = 0.44$). In contrast to males, female hamsters given a single 7-day antibiotic treatment displayed a decrease in overall aggression (PC-AGG, $F_{1,16} = 7.80, p = 0.01$) when compared with females given a single 7-day control treatment (Fig. 5d). Specifically, females given a one-time week-long antibiotic treatment displayed fewer ($F_{1,16} = 7.48, p = 0.01$, Fig. 5b) and shorter attacks ($F_{1,16} = 7.04, p = 0.02$), as well as fewer ($F_{1,16} = 6.41, p = 0.02$) and shorter ($F_{1,16} = 4.23, p = 0.04$) chases. In addition, females given 7 days of antibiotic treatment exhibited a longer latency to the first attack ($F_{1,16} = 8.16, p = 0.01$).

Male hamsters given two 7-day treatments of antibiotics separated by a 7-day recovery period displayed a decrease in overall aggression (PC-AGG, $F_{1,14} = 12.78, p = 0.003$) when compared with males given two control treatments following the same timeline (Fig. 5c). Specifically, males given two week-long antibiotic treatments displayed decreases in number ($F_{1,14} = 7.18, p = 0.02$, Fig. 5a) and duration ($F_{1,14} = 2.44, p = 0.03$) of attacks and decreases in number ($F_{1,14} = 10.94, p = 0.005$) and duration ($F_{1,14} = 22.92, p = 0.0003$) of chases. However, latency to first attack did not differ across groups ($F_{1,14} = 12.49, p = 0.49$). Similar to males, female

Fig. 4. Effect of antibiotics on relative abundance of Tenericutes in male and female hamsters following treatment period 2. Similar to what was found in Experiment 1, in Experiment 2, in males (a) and females (b), treatment affected the relative abundance of Tenericutes (males: $t_2 = -5.539, p = 0.0310$; females: $t_2 = -4.092, p = 0.055$). Bar heights represent means ± S.E.M. An asterisk (*) indicates statistically significant differences between group means at $p < 0.05$. There was also an association between the relative abundance of Tenericutes and the number of attacks in male and female hamsters at the end of treatment period 2 (c). When separated by sex, the relative abundance of Tenericutes in male and female hamsters showed trends towards significant associations with number of attacks (see Table 13 in Supplementary material).
hamsters given two week-long antibiotic treatments displayed a decrease in overall aggression (PC-AGG, \( F_{1,16} = 24.35, p = 0.0001 \)) when compared with females given two week-long control treatments (Fig. 5d). Specifically, females given two week-long antibiotic treatments displayed fewer (\( F_{1,16} = 22.73, p = 0.0002 \), Fig. 5b) and shorter (\( F_{1,16} = 20.14, p = 0.0004 \)) attacks and fewer (\( F_{1,16} = 24.63, p = 0.0001 \)) and shorter (\( F_{1,16} = 22.24, p = 0.0002 \)) chases. In addition, the latency to the first attack was longer (\( F_{1,16} = 6.38, p = 0.02 \)) in repeated antibiotic-treated females.

4.2.4. Aggression is associated with relative abundance of bacteria in the gut

The number of attacks in male and female hamsters following treatment period 2 was associated with the relative abundance of Tenericutes (\( r_s = 0.764, n = 12, p = 0.007 \)) (Fig. 4c), Cyanobacteria (\( r_s = 0.717, n = 12, p = 0.014 \)), and TM7 (\( r_s = 0.667, n = 12, p = 0.046 \)). In contrast, the number of attacks was not associated with the 11 other phyla: 'Other', Actinobacteria, Bacteroidetes, Deferribacteres, Elusimicrobia, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, and Verrucomicrobia (\( p > 0.05 \) in all cases). For complete list of correlations and for separate correlations by sex, see Table 13 in Supplementary material.

4.2.5. Antibiotics did not affect investigative behavior

Male hamsters given antibiotic treatment displayed no change in overall investigative behavior (PC-INV, one-time: \( F_{1,16} = 2.17, p = 0.69 \); PC-INV, two-time: \( F_{1,14} = 2.04, p = 0.18 \)) when compared with males given control treatment (Fig. 6a). Specifically, males given antibiotic treatment displayed no change in number (one-time: \( F_{1,14} = 4.70, p = 0.80 \); two-time: \( F_{1,14} = 4.38, p = 0.85 \)) or duration (one-time: \( F_{1,14} = 4.49, p = 0.50 \); two-time: \( F_{1,14} = 4.97, p = 0.92 \)) of nose-to-anogenital investigation and no change in number (one-time: \( F_{1,14} = 4.37, p = 0.85 \); two-time: \( F_{1,14} = 2.40, p = 0.14 \)) or duration (one-time: \( F_{1,14} = 4.21, p = 0.65 \); two-time: \( F_{1,14} = 3.20, p = 0.10 \)) of nose-to-nose investigation (Table 1). Similar to males, female hamsters given antibiotic treatment displayed no change in overall scent-marking behavior (PC-SMK, one-time: \( F_{1,16} = 2.44, p = 0.14 \); PC-SMK, two-time: \( F_{1,14} = 2.35, p = 0.15 \)) when compared with males given control treatment. Specifically, antibiotic-treated males displayed no change in number (one-time: \( F_{1,14} = 1.81, p = 0.20 \); two-time: \( F_{1,14} = 2.46, p = 0.14 \)) or duration (one-time: \( F_{1,14} = 2.04, p = 0.18 \); two-time: \( F_{1,14} = 2.18, p = 0.92 \)) of ventral gland depositing (Table 1). Similar to males, female hamsters given antibiotic treatment displayed no change in overall scent-marking behavior (PC-SMK, one-time: \( F_{1,16} = 0.80, p = 0.38 \); PC-SMK, two-time: \( F_{1,16} = 1.33, p = 0.27 \)) when compared with females given control treatment. Specifically, antibiotic-treated females displayed no change in number (one-time: \( F_{1,16} = 0.88, p = 0.36 \); two-time: \( F_{1,16} = 1.37, p = 0.55 \)) or duration (one-time: \( F_{1,16} = 0.81, p = 0.38 \); two-time: \( F_{1,16} = 1.43, p = 0.25 \)) of ventral gland depositing (Table 2).

4.2.6. Antibiotics did not affect scent-marking behavior

Male hamsters given antibiotic treatment displayed no change in overall scent-marking behavior (PC-SMK, one-time: \( F_{1,14} = 2.44, p = 0.14 \); PC-SMK, two-time: \( F_{1,14} = 2.35, p = 0.15 \)) when compared with males given control treatment. Specifically, antibiotic-treated males displayed no change in number (one-time: \( F_{1,14} = 1.81, p = 0.20 \); two-time: \( F_{1,14} = 2.46, p = 0.14 \)) or duration (one-time: \( F_{1,14} = 2.04, p = 0.18 \); two-time: \( F_{1,14} = 2.18, p = 0.92 \)) of ventral gland depositing (Table 1). Similar to males, female hamsters given antibiotic treatment displayed no change in overall scent-marking behavior (PC-SMK, one-time: \( F_{1,16} = 0.80, p = 0.38 \); PC-SMK, two-time: \( F_{1,16} = 1.33, p = 0.27 \)) when compared with females given control treatment. Specifically, antibiotic-treated females displayed no change in number (one-time: \( F_{1,16} = 0.88, p = 0.36 \); two-time: \( F_{1,16} = 1.37, p = 0.55 \)) or duration (one-time: \( F_{1,16} = 0.81, p = 0.38 \); two-time: \( F_{1,16} = 1.43, p = 0.25 \)) of ventral gland depositing (Table 2).

4.2.7. Antibiotics did not affect grooming behavior

Antibiotic-treated males displayed no change in overall grooming behavior (PC-GRM, one-time: \( F_{1,14} = 0.16, p = 0.70 \); PC-GRM, two-time: \( F_{1,14} = 2.45, p = 0.14 \)) when compared with males given control treatment. Specifically, antibiotic-treated males displayed no change in number (one-time: \( F_{1,14} = 0.51, p = 0.49 \); two-time: \( F_{1,14} = 1.49, p = 0.12 \)) or duration (one-time: \( F_{1,14} = 1.57, p = 0.46 \); two-time: \( F_{1,14} = 4.85, p = 0.14 \)) of self-grooming (Table 1). Similar to males, antibiotic-treated females displayed no change in overall grooming behavior (PC-GRM, one-time: \( F_{1,16} = 0.85, p = 0.37 \); PC-GRM, two-time: \( F_{1,16} = 1.80, p = 0.39 \)) when compared with females given control treatment. Specifically, antibiotic-treated females displayed no change in number (one-time: \( F_{1,16} = 0.54, p = 0.43 \); two-time: \( F_{1,16} = 1.35, p = 0.26 \)) or duration (one-time:
Experiment 2: Means ± SEM of investigation, scent-marking, and grooming behaviors in male hamsters across treatment groups. No values were significantly different across treatment groups.

<table>
<thead>
<tr>
<th>Time</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Control</td>
<td>Abx</td>
<td>Control Abx</td>
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<tr>
<td>0.75</td>
<td>1.44 ± 0.36</td>
<td>1.11 ± 0.25</td>
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<tr>
<td>1.44</td>
<td>1.78 ± 0.50</td>
<td>1.44 ± 0.24</td>
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<tr>
<td>2.17</td>
<td>1.56 ± 0.38</td>
<td>1.56 ± 0.38</td>
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<tr>
<td>2.89</td>
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Table 1

**Table 2**

Experiment 2: Means ± SEM of investigation, scent-marking, and grooming behaviors in female hamsters across treatment groups. No values were significantly different across treatment groups.

<table>
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<tr>
<th>Time</th>
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Table 2

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<td>2.89</td>
<td>1.78 ± 0.50</td>
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F<sub>1,16</sub> = 1.07, p = 0.32; two-time: F<sub>1,16</sub> = 1.09, p = 0.31 of self-grooming (Table 2).

4.2.8. Antibiotics did not affect body mass or reproductive physiology

Antibiotics did not affect body mass (F<sub>1,16</sub> = 1.35, p = 0.56), testes mass (t<sub>16</sub> = -1.68, p = 0.52), liver mass (t<sub>16</sub> = 1.78, p = 0.10), or spleen mass (t<sub>16</sub> = 1.40, p = 0.70), when compared with control males. Similarly, antibiotics did not affect female body mass (F<sub>1,16</sub> = 3.19, p = 0.86), ovaries mass (t<sub>16</sub> = -1.13, p = 0.90), uterine horn mass (t<sub>16</sub> = -1.14, p = 0.89), parametrial white adipose tissue mass (t<sub>16</sub> = -0.98, p = 0.35), liver mass (t<sub>16</sub> = -1.05, p = 0.96), or spleen mass (t<sub>16</sub> = -1. 64, p = 0.54), when compared with control females (Table 14 in Supplementary material).

5. Discussion

The gut microbiome is a diverse symbiotic community that acts as a vital part of the body's ecosystem, and its disruption can have lasting effects. Previous studies have shown that disrupting the gut microbiome can influence anxiety- and depression-like behaviors, as well as learning and memory (Foster and Neufeld, 2013; Mayer et al., 2015), yet the effects of gut dysbiosis via antibiotics on aggressive behaviors had yet to be explored. Here we tested the effects of antibiotic use on social behaviors, including aggression, investigation, scent-marking, and grooming. In Experiment 1, we administered antibiotics on a short-term basis, and assessed the effects on the microbial communities in the gut; we found that antibiotic treatment altered the gut microbiome in both male and female Siberian hamsters. In Experiment 2, we administered repeated doses of antibiotics and assessed social behaviors, and discovered an interesting sex difference in response to antibiotic treatment. Specifically, we found that male aggressive behavior decreased after two antibiotic treatments, whereas investigation, scent-marking, and grooming remained unaffected. Aggression returned to normal following a “wash-out” period. In contrast to males, females given a single antibiotic treatment showed decreased aggression, with all other behaviors remaining the same.
same; females were unable to recover behaviorally from the initial treatment. Here we provide data suggesting that antibiotic treatment with a fluoroquinolone antimicrobial agent that results in marked disruption of the microbiome, may also have lasting, sex-specific effects on social behavior in hamsters, and likely other rodent species. These robust antibiotic-induced changes in behavioral patterns across sexes may have potential implications for sex-specific treatment for many physiological as well as psychological disorders.

5.1. The symbiotic community in the gut is vital to mammalian health

Despite inter-individual variability, short-term treatment with antibiotics, as well as repeated doses of antibiotics, altered the bacterial composition of the gut in male and female Siberian hamsters, at the phylum level. Although Bacteroidoetes and Firmicutes were in the highest relative abundance in the microbiome of male and female hamsters, antibiotics did not affect these phyla; instead, those phyla in relatively low abundance (e.g., Cyanobacteria, Proteobacteria, and Tenericutes) were most strongly affected by treatment in both sexes. Much of the current research focuses explicitly on phyla that are present in high abundance in the microbiome (e.g., Bacteroidoetes and Firmicutes) and not those in low relative abundance (Ley et al., 2006). In our studies, we have shown that when Bacteroidoetes and Firmicutes remain unchanged, other phyla in the gut are altered, and we provide evidence suggesting that the gut microbiota may influence the brain and behavior.

In particular, in male and female hamsters in Experiment 1, there was a significant change in relative abundance of Cyanobacteria, Proteobacteria, and Tenericutes following antibiotic treatment. Cyanobacteria, often found in the normal gut flora of mammals (Sukenik et al., 2015), disappeared from the microbiota in both males and females. In both sexes, the relative abundance of Cyanobacteria began to increase after 7 days of recovery, though never reaching Pre-Treatment levels. Following antibiotic treatment in males and females, the relative abundance of Proteobacteria also decreased, however, Proteobacteria showed greater recovery in both male and female microbiota 7 days post-treatment. In contrast, the relative abundance of Tenericutes decreased after antibiotic treatment in both males and females, and it did not recover in either sex after the wash-out period, suggesting that this phylum is less capable of recovering after perturbation.

In addition to the three phyla discussed above, the male microbiota also showed significant changes in relative abundance of Elusimicrobia, Euryarchaeota, and TM7 in Experiment 1. Following antibiotic treatment in males, both Elusimicrobia and Euryarchaeota disappeared from the gut, and neither phylum reappeared following recovery. In contrast, TM7, a recently described subgroup of gram-positive bacteria (Kuehbacher et al., 2008), decreased in relative abundance after antibiotic treatment, and recovered following the 7-day recovery period. TM7 has been shown to play a possible role in inflammatory bowel disease (IBD) and other similar diseases (Kuehbacher et al., 2008).

Although there were some shifts in other phyla in male and female microbiota, we found no significant change in the relative abundance of seven of the thirteen phyla in males and ten of the thirteen phyla in females. Most phyla identified were comprised of common genera often seen in other studies. Within the Firmicutes, we observed Ruminococcus, Clostridium, Lactobacillus, Eubacteria and Roseburia, often critical in gut motility and bowel disorders, yet the mechanisms behind these relationships are not well understood (Machiels et al., 2013; Tremaroli and Bäckhed, 2012). Within the Bacteroidoetes, Actinobacteria, Proteobacteria, and Euryarchaeota phyla, we identified common genera, including Prevotella, Bifidobacterium, Desulfovibrio, and Methanobrevibacter, respectively, which aid in degradation of complex glycans, reduction of sulfate, and production of intestinal methane (Tremaroli and Bäckhed, 2012). Similar to Experiment 1, following the second treatment period in Experiment 2, in males, Tenericutes were affected by antibiotic treatment and Cyanobacteria and Proteobacteria, though not significant, were affected by the second treatment of antibiotics as well. In females, treatment affected TM7 and Tenericutes, and though not significant, the second treatment with antibiotics also affected Cyanobacteria.

Further, analysis of alpha and beta diversity in both experiments suggests that, in addition to the changes in relative abundance of specific phyla, antibiotic treatment also produced a decrease in diversity in both sexes, though to varying degrees. Maintaining a diverse microbial composition in the gut may be a vital part of eliciting appropriate, species-typical behavior. The precise role that each bacterium plays in the community may not be the most important factor in maintaining homeostasis within the body. Instead, the way in which each organism interacts with others within the community may be most critical. We provide evidence that suggests bacteria in low relative abundance in the gut microbiome may have potential long-term consequences on physiology and behavior.

5.2. Antibiotics affect behavior in a sex-dependent manner

Previous studies have suggested that what is lacking in much of the microbiome work to date is consideration of the role of sex in the gut-brain axis (Foster and Neufeld, 2013). We demonstrate here that there is a strong sex difference in response to the same antibiotic treatment, and that females were more strongly affected by a single antibiotic-treatment period, with seemingly long-lasting effects on behavior. We also present data suggesting that specific behaviors (i.e., attacking) may be associated with the relative abundance of particular bacteria in the gut. These findings suggest that females may be more sensitive to stressors, and therefore with further insult, they will exhibit an even more robust change in behavior.

The sex-specific results that we present here provide evidence that while antibiotics are an important therapy for many infectious diseases in both sexes, the physiological and behavioral consequences of antibiotic-use in males and females should be further investigated. It is of further importance to focus on these sex differences because, in humans, women are twice as likely to suffer from anxiety and depression as men, and exactly why we see this is yet to be determined (Klein and Corwin, 2002; Kornstein et al., 2000). Notably, sex hormones (e.g., estradiol and testosterone) affect regulation of motor and sensory function in the gastrointestinal (GI) tract, and estrogens plays a particularly important role in these physiological changes. Further, bowel disorders and mental diseases related to these disorders are more common in women than in men as well (Mulak et al., 2014), suggesting the role that sex plays in regulation of the gut-brain axis. In a recent study, men who self-reported feeling depressed also exhibited higher rates of aggression, substance abuse, and risk taking when compared with women reporting the same feelings of depression (Martin et al., 2013). This study suggests that the behavioral symptoms often seen associated with depression and potentially other psychiatric diseases, may vary across sexes.

5.3. Potential mechanisms mediating the influence of the gut microbiota on behavior

There are many potential neuroendocrine-immune mechanisms mediating microbiome–CNS cross-talk, and we are beginning to understand the role that each of those play in regulation of physiology and the resulting behavioral phenotype. Antibiotics
are a suitable tool to understand the cause of the microbiota-dependent changes we see in physiology and behavior (Fröhlich et al., 2016). A wide range of antibiotics has been used in recent microbiome research, often administered in cocktails of three or more antibiotics (e.g., ampicillin, neomycin, vancomycin) (Fröhlich et al., 2016), forcefully fighting off microbial activity. Though this is successful in knocking down many of the bacterial communities in the gut, the effects of an individual antibiotic on the microbiome and downstream effects on behavior in rodent species, is lacking. Previous studies have shown that application of many common antibiotics (e.g., clindamycin, ampicillin, vancomycin, erythromycin, and gentamicin) cause lethal enterocolitis in hamster species; the fluoroquinolone antibiotic used in the present study, enrofloxacin, does not produce the same lethal effects, and unlike some other antibiotics, it does not cross the blood brain barrier (Alvarez et al., 2010; Bartlett et al., 2015; Ooie et al., 1997a, b). Thus, the effects of enrofloxacin on aggressive behavior in both males and females in this study may be due to the communication between the gut microbiota and the brain, rather than a direct effect of antimicrobial use on behavior, yet we still do not know the specific mechanisms underlying this effect. As with previous studies utilizing a range of antibiotics, it is not known if the antibiotic’s effects on behavior are due to changes in the gut microbiota alone. Many commonly used antibiotics have the ability to thin the mucosal layer of the epithelium, increasing permeability of drugs, other molecules of the immune system, and even infectious molecules (reviewed in Ubeda and Pamer, 2012). The current studies shed light on the potential role that the microbiota play in the behavioral phenotype and provide evidence of the correlation between microbes and behavior. However, further studies could focus on more direct assessments of the influence of microbial communities on the brain and behavior.

Behavior may be strongly influenced by the gut microbiota via direct communication with the CNS. For example, mice challenged with the common gastrointestinal pathogen, Campylobacter jejuni (C. jejuni), exhibit reduced exploration in the open arms of the plus maze, and the brains of these mice show increased expression of c-Fos labeling in autonomic brain regions, without activation of an immune response (Goehler et al., 2008). The lack of immune response suggests that the vagus nerve may play an important role in sending information from the gut microbiota to particular regions of the brain (Goehler et al., 2008). Further studies have shown that challenging the gut microbiota with non-infectious bacteria and probiotics, increases c-Fos mRNA levels in specific regions of the brain without activation of the systemic immune response as well (Sudo et al., 2004). It is also possible that the effects of antibiotics on behavior are due to changes in the microbiota on other surfaces in addition to the gut. Future work will help determine whether the effects on behavior seen here are directly related to gut microbial changes or if the effects are indirect, acting via other organ systems of the body.

Alternatively, the immune system, and particularly cytokines, may play a role in the cross-talk between the microbiota and the brain, and this may influence the changes we see in behavior. Studies suggest that gut dysbiosis is related to disturbances in adaptive immune cells and that some inflammatory bowel disorders may be the consequence these changes (Lee and Mazmanian, 2010). For example, germ-free mice exhibit altered development of many aspects of the immune system, including fewer, smaller and inactive lymph nodes, spleens, and Peyer’s patches, which thereby prohibit the immune system from responding appropriately (Hoshi et al., 1992; Pollard and Sharon, 1970). Germ-free mice are also deficient in particular cytokines, including IL-17, which aids in the production of T helper cells during an immune response; however, microbiota disturbance does not affect the levels of some other cytokines, suggesting that there are specific features of the immune response that may be sensitive to these changes (Ivanov et al., 2008).

6. Conclusion

The gut microbiome is a diverse, host unique, and symbiotic bacterial environment that works in concert with many other organisms in the body to influence the brain and behavior. Our work here suggests that antibiotic treatment alters the microbial communities in the gut in a sex-dependent manner, and this disruption is correlated with social behavior. Determining the role that the microbiota play in communication with the brain across the sexes is vital to our understanding of psychological disorders and the behavioral changes that are present in these diseases. More broadly, these data further our understanding of the behavioral consequences of antibiotic use in a non-model species and demonstrate robust, sex-specific effects on social behavior. Our data provide insight into a feature of the behavioral phenotype not yet discussed in this area of literature, and it may provide vast implications for human psychological disease and treatment.

Author contributions

K.E.S and G.E.D designed the research; K.E.S., N.M.R., and E.A.S. performed the research; C.P.J. and K.E.S. analyzed the microbiome data; N.M.R., K.E.S., and E.A.S. analyzed the behavioral and physiological data; and K.E.S., N.M.R., E.A.S., C.P.J., and G.E.D. wrote the paper.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgments

The authors thank Jim Pottebaum, DVM for his assistance in antibiotic treatment choice, and L.C. Beck, D.L. Boyes, and K.J. O’Malley for assistance in behavioral filming, necropsies, and general animal procedures. This work was supported by National Science Foundation Graduate Research Fellowship Program to N.M.R., Faculty Research Support Program Grant, and Indiana University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2016.10.023.

References


