Table 1. Species Whose Abundance Is Reduced in the Gut Microbiota of MHFD Offspring

Species of Interest	MRD Representation	MHFD Representation	Fold Change MRD/MHFD
Lactobacillus reuteri	7.49 ± 3.0	0.879 ± 0.21	9.24 ± 0.65
Parabacteroides distasonis	0.00709 ± 0.0055	0.00126 ± 0.0011	5.63 ± 1.17
Helicobacter hepaticus	7.35 ± 2.4	2.58 ± 1.3	2.84 ± 0.61
Bacteroides uniformis	5.49 ± 2.2	2.07 ± 0.78	2.65 ± 0.56
Olsenella unclassified	0.230 ± 0.064	0.121 ± 0.031	1.90 ± 0.38
Collinsella unclassified	0.0866 ± 0.031	0.0494 ± 0.016	1.75 ± 0.48
Bifidobacterium pseudolongum	19.4 ± 3.3	11.3 ± 2.4	1.71 ± 0.27
Lactobacillus johnsonii	24.5 ± 6.2	17.1 ± 5.2	1.43 ± 0.40

oxytocin-mediated synaptic adaptations in the VTA that underlie social behaviors.

DISCUSSION

Both genetic and environmental factors, and their interactions, play a crucial role in the etiology of neurodevelopmental disorders including ASD (Hallmayer et al., 2011). There is growing epidemiological evidence that maternal obesity heightens the risk of neuropsychiatric disorders in offspring (Krakowiak et al., 2012; Sullivan et al., 2014). Indeed, a recent study reported that mothers with obesity were 1.5 times more likely to have a child with ASD, and the increased risk of children with ASD was two-fold greater for pregnant mothers with both obesity and gestational diabetes (Connolly et al., 2016).

While most of the focus in the field has been on inflammation (Bolton and Bilbo, 2014) or epigenetic changes (Mathers and McKay, 2009), the biological mechanism by which maternal obesity affects offspring neurodevelopment remains to be determined. Here, we show that that the behavioral dysfunction associated with MHFD-induced obesity is induced by alterations in the offspring gut microbiota. Several lines of evidence support this idea. First, some individuals diagnosed with ASD present dysbiosis of the gut microbiota and gastrointestinal issues (Bresnahan et al., 2015; Mayer et al., 2014; Parracho et al., 2005). Second, maternal obesity leads to alterations in the offspring's gut microbiome in humans and non-human primates (Galley et al., 2014; Ma et al., 2014). Third, in mice, the gut microbiota of MHFD offspring is altered (Figure 1J) by the reduction in specific bacterial species (Table 1). Fourth, manipulation of the microbiome community by co-housing MHFD with MRD offspring rescues MHFD-induced social deficits and corrects their microbial phylogenetic profile (Figures 2 and S3). Fifth, GF mice are socially impaired and fecal microbiota transplanted from MRD (but not MHFD) offspring rescues GF social behavior (Figures 3 and S4). Finally, treatment with a single bacterial species, *L. reuteri,* which is dramatically reduced in MHFD offspring (Table 1), selectively restores social behavior in MHFD mice (Figures 4 and S5A–S5C).

We propose a model in which *L. reuteri* improves social behavior by promoting oxytocin-mediated functions. Consistent with this model, *L. reuteri*-treatment enhances oxytocin levels in the PVN of MHFD mice (Figures 4I and 4J) and direct oxytocin-treatment normalizes the social behavior of MHFD offspring (Figure 6). Although the precise mechanism by which *L. reuteri* promotes oxytocin in the brain remains to be determined, we favor the idea that the vagus nerve (Davari et al., 2013) could be the main pathway of communication between the gut/*L. reuteri* and changes in oxytocin in the PVN. It is known that vagal nerve fibers project to the PVN (Sabatier et al., 2013; Uvnäs-Moberg et al., 2014). In addition, neuronal activity in the PVN induced by bacterial colonization is blocked by subdiaphragmatic vagotomy (Wang et al., 2002). Especially relevant are the reports that the *L. reuteri*-mediated increase in oxytocin depends on the vagus nerve (Poutahidis et al., 2013) and that another *Lactobacillus* species, *L. rhamnosus*, reduced stress-induced anxiety in mice in a vagus-dependent manner (Bravo et al., 2011).

Our results provide new insight into the mechanism by which a marked shift in microbial ecology, caused by MHFD, can negatively impact social behaviors and related neuronal changes in offspring. These neuronal adaptations, which underlie social behavior by enhancing the salience and rewarding value of social stimuli, are surprisingly impaired by maternal diet-induced changes in the gut microbiome (Figure 5). Interestingly, according to a recent study, probiotic-based restoration of gut permeability in a mouse model of ASD can improve some behavioral abnormalities, but not social behaviors (Hsiao et al., 2013). Given that we identified a different probiotic candidate, *L. reuteri*, that rescues social behavior (Figures 4 and 5), but not other behavioral endophenotypes associated with ASD (Figure S6), in MHFD mice, we propose that a carefully selected combination of probiotics may be useful as a potential non-invasive treatment for patients suffering from neurodevelopmental disorders including ASD.

Figure 3. Fecal Microbiota from MRD, but not MHFD, Offspring Im
(A–D) GF mice show reduced reciprocal social interaction (A, $p < 0.0001$, $t = 22$
GF $p > 0.99$, $t = 0.39$; main group effect $F_{1,24} = 21.98$, $p < 0.0001$) and preferen
effect $F_{1,24} = 5.29$, $p < 0.05$).
(E and F) Schematic of fecal microbiota transplant (FMT) at 4 (E) and 8 week
(G and H) FMT from MRD, but not MHFD, offspring at weaning restored both
effect $F_{1,28} = 32.44$, $p < 0.0001$) and preference for social novelty (H, GF_{MRDCo}
(I and J) At 8 weeks, FMT from either MRD or MHFD donors failed to improve
$F_{1,12} = 0.07$, $p = 0.79$) or preference for social novelty in GF mice (J, GF_{MRDCol}
(K and L) PCoA of unweighted UniFrac distances based on the 16S rRNA gene
at four (K, $p = 0.001$, $R^2 = 0.83$; n = 1,000 rarefactions; 4,628 reads/sample) or
age. Plots show mean ± SEM.
See also Figure S4.