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Gut Microbiome and Brain-Gut Axis in Autism — Aberrant Development of Gut-Brain Communication and Reward Circuitry

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<http://dx.doi.org/10.5772/55425>

1. Introduction

The function of the gut microbiome and the bidirectional communication between the gastrointestinal tract (GIT) and the brain is increasingly recognized in health and disease and disruption in its composition is not unique to the autistic pathology. However, the bidirectional communication between the gut and the brain, “the gut-brain/brain-gut axis” in autism has been relatively understudied. In general, this communication between gut and brain occurs through a direct neuronal pathway via the vagus nerve, the hormonal pathway of several hormones involved in the regulation of food intake, such as cholecystokinin (CCK), ghrelin, leptin and insulin, and by the immunological signaling pathway involving cytokines. Recent studies indicate that the vagus nerve is involved in immunomodulation as suggested by its ability to attenuate the production of proinflammatory cytokines in experimental models of inflammation (de Jonge and Ullola, 2007). Furthermore, the gut microbiome emerges as a major player not only in the maturation of GIT tissue and the gut brain axis but also in brain maturation, through its effect on both the immune and endocrine systems. Many toxins, toxicants, infectious agents, diet or stress, affect an individual’s gut microbiome, which may be especially sensitive during the critical developmental period. Disruption of the developing microbiome may have profound consequences on the developing gut-brain axis including the brain as well as long-term effects on both the physical and psychological development.

This chapter attempts to bridge basic animal studies with clinical findings pertaining to the brain-gut and gut microbiome in autism, and includes a discussion of various strategies in managing autistic symptoms. The discussion also includes possible changes in the reward

system(s) in autism as a consequence of altered gut microbiome. It is possible that aberrant regulation of the reward system(s) underlines behavioral abnormalities in ASD that could be targeted by future microbiome-targeting therapies.

2. Effect of perinatal infection and toxicants on the developing brain

In a continuing quest to understand the nature of gene-environment interactions in ASD, we have recently completed two animal studies examining the effect of perinatal exposure of thimerosal (TM) and lipopolysaccharides (LPS) on the developing rat central nervous system (CNS). Both TM and infections (modeled by LPS exposure) have been implicated in autistic pathology.

Organic mercury compounds are powerful toxicants with a range of harmful neurological effects in humans and animals. TM, which is metabolized to ethyl mercury, has been discontinued as a preservative from infant vaccines but continues to be used in several vaccines including a flu vaccine administered to pregnant and lactating mothers (Sulkowski et al., 2012). Perinatal maternal exposure of two strains of rats, Sprague Dawley (SD) and spontaneously hypertensive (SHR) rats, to thimerosal (200 ug/kg body weight) resulted in both sex- and strain-specific abnormalities in the neonatal rats (Sulkowski et al., 2012). Behavioral abnormalities included delayed startle response and decreased motor learning, with the effects being both sex- and strain-specific. TM exposure also resulted in a significant increase in cerebellar levels of an oxidative stress marker (3-nitrotyrosine) and a decrease in cerebellar type 2 deiodinase, responsible for local intrabrain conversion of thyroxine to the active hormone, 3', 3,5,-triiodothyronine (T3). These effects were associated with an increased expression of several genes negatively regulated by T3 (Sulkowski et al., 2012); Khan et al., 2012) suggesting that perinatal exposure to TM impacts the developing brain at the genetic level. As TM exposure during the postnatal phase coincided with lactation, some of the TM was delivered through the milk to the GIT and may have had an effect on the developing gut microbiome known to be sensitive to heavy metal exposure (Lapanje et al., 2007). This effect may be in part due to competition with zinc resulting in a disturbance in metallothionein function and general chelating capacity for other metals. Thus, at least part of the neonatal impact of TM/mercury could be mediated via its action on the gut microbiome.

In a related study (Xu et al., submitted) we examined the effect of *E.coli* lipopolysaccharides (LPS) exposure during critical developmental periods on the developing brain employing the animal model of infection. Clinical and epidemiological data suggest that maternal infection during pregnancy and nursing increases the probability of neonatal brain injury and may have a long-lasting impact on brain functions. Maternal infection during pregnancy has been linked to neurological and neurobehavioral disorders in humans such as cerebral palsy (Schendel, 2001; Schendel et al., 2002), neonatal strokes (Ferriero, 2004), schizophrenia (Watson et al., 1999; Pearce, 2001) and affective disorders (Watson et al., 1999). Animal studies implicate bacterial infection in the pathology of Parkinson's disease (Carvey et al., 2003), and notably, schizophrenia and autism (Patterson, 2002). The triggering signals for cytokine production are endotoxins, major components of the outer membrane of Gram-negative bacteria.

LPS exposure is one of the most acceptable models of infection; LPS is a sufficient trigger for cytokine production. LPS administered to the pregnant mother are transferred to the fetus through the placenta (Kohmura et al., 2000), and result in increased cytokines levels in the amniotic fluid (Urakabo et al., 2001; Gayle et al., 2004) and the fetal brain (Urakabo et al., 2001). Bacterial infection of lactating mothers also results in an increased level of cytokines in milk (Bannerman et al., 2004). Pretreatment of suckling rats with LPS (10 mg/kg-day × 5 days – the dose which produces weak, transient signs of endotoxemia) results in reduced pancreatic secretion and attenuates acute pancreatitis at adult age due to an increased concentration of the antioxidative enzyme SO in the pancreatic tissue, and to the modulation of cytokines production (Jaworek et al., 2007a, b). This late-effect of LPS is accompanied by dose-dependent reduction of mRNA signal for CCK1 receptor on pancreatic acini as well as modified expression of acinar pro-apoptotic heat shock protein-60 (HSP60) and Bax proteins (Jaworek et al., 2007b, 2008). Early postnatal LPS exposure results in increased expression of toll-like receptor 4 (TLR4) and caspase-3 and 9- proteins in the pancreatic tissue of adult rats (Bonior et al., 2012). These studies clearly indicate that perinatal exposure to LPS may have long lasting consequences on the GIT function, and as expected, though not studied in detail, on the brain-gut axis.

Perinatal maternal exposure of two strains of rats, SHR or SD rat dams to LPS (200 µg/kg body weight) resulted in increased rollover time, delayed startle, and decreased motor learning, with the effects being both strain- and sex-specific. LPS challenge also resulted in a trend towards an increase in cerebellar levels of 3-NT and a decrease in D2 activities in LPS-exposed pups (Xu et al., submitted). Several genes were affected by LPS. Notably Type 2 deiodinase 2 (DIO2) and brain derived neurotrophic factor (BDNF) expression was significantly elevated, while transthyretin (TTR) expression was decreased following LPS exposure. *In vitro*, acute exposure of cerebellar cultures to LPS resulted in a decreased size of the dendritic area of Purkinje cells. Our data thus demonstrate that perinatal infection impacts the developing cerebellum in a sex- and strain-dependent manner via mechanisms involving oxidative stress, enzymes involved in maintaining local TH homeostasis, and downstream gene expression. Interestingly, gene changes observed in the brains of LPS-exposed rats were distinct from TM-associated gene effect suggesting that the underlying macromolecular mechanism may be trigger-specific.

Perinatal LPS exposure could have a profound effect on the gut microbiome similar to the effect of repeated treatment with antibiotics. Experiments in healthy mice have shown that disrupting the normal balance of the gut microbiome with antibiotics caused changes in mice behavior and was accompanied by changes in BDNF which has been linked to depression and anxiety (Bercik et al., 2011; Neufeld et al., 2011). Perinatal LPS exposure most likely affects gut motility as suggested by studies of irritable bowel syndrome (IBS), where mild bacterial overgrowth-associated motility disorder can be reversed by antimicrobials (Scarpignato and Pelosini, 1999). Animal studies have also shown that stress can change the composition of the microbiome, where the changes are associated with increased vulnerability to inflammatory stimuli in the GIT. Could gut dysbiosis be induced by recurrent infections? We have observed an increase in neurotrophin levels in the cerebella of rats exposed to LPS (Sajdel-Sulkowska et

al, unpublished observation) and brain region-specific changes in neurotrophin levels in ASD (Sajdel-Sulkowska et al., 2011). Together these observations suggest that a bacterial infection could trigger the gut microbiome to induce cytokine overproduction leading to an imbalance of brain neurotrophins and contribute to developmental abnormalities.

3. Effect of environmental perturbations on the developing components of the brain-gut axis: Intestinal permeability, inflammation and gut microbiome

As indicated above the perinatal development of the CNS structure and function greatly depends on the gastrointestinal GIT function. Little is known about the regulation of embryonic gut epithelium or the effect of prenatal infection. The two key developmental time-points in the regulation of the GIT both occur postnatally, the first few days after birth when all gut digestive functions are launched by first colostrum ingestion and the second at weaning when the digestive system has to modify its function following a switch from mother's milk to solid food. The first time-point is particularly relevant for all mammalian species since it is associated with a complex of dynamic changes in the GIT structure and function leading to a temporary drop in the gut permeability barrier. The secretion of digestive juices (e.g., gastric and pancreatic juice secretion) is obviously close to null before birth. Our studies indicate that in neonatal calves the exocrine pancreas secreted low but measurable amounts of pancreatic juice from the first postnatal day. The secretion responded to colostrum feeding showing a clear-cut cephalic phase associated with plasma pancreatic polypeptide (PP) elevation but no gastric or intestinal phase. Further studies involving vagal blockades and pharmacological cholecystokinin receptor antagonists indicated that in neonatal calves pancreatic response to first colostrum feeding is already controlled by a neuro-hormonal mechanism involving CCK and long vago-vagal reflex (Zabielski et al., 2002). Thus, the brain-gut axis control of the exocrine pancreas observed in one- and two-month old milk-fed calves is already present at birth, only the magnitude of the response increases with age reaching its peak at four weeks. The other GIT function in neonatal calves closely associated with the brain-gut axis control include periodic activity of GIT motility (migrating motor complex, MMC) and secretion (pancreatic juice periodic secretion) observed already two-three days after birth along with plasma PP oscillations (Zabielski et al., 2002). Plasma PP, a marker of efferent vagal activity, increased with age indicating that brain-gut axis further develops after birth (and may be potentially sensitive to any environmental modifications).

Intestinal functions in neonates are far more complex than in adults due to intensive developmental processes. The small intestine is one of the fastest body organs to grow in size postnatally as well as the fastest organ in rebuilding its structure. Relatively little is known regarding the development of the large intestine, a major organ inhabited by gut bacteria. At birth, the small intestinal mucosa is lined by enterocytes ready for rapid uptake of colostrum macromolecules (open gut). These enterocytes, so called fetal type enterocytes, are equipped with a system of vesicles and cisterns (apical canalicular system, ACS) which

form large size mobile vacuoles in the upper part of the cell enabling the transfer of intact colostral molecules into the blood (Baintner 2002). Approximately two days after birth, following substantial intake of colostral bioactive substances, the permeability of the gut epithelium is dramatically reduced to macromolecules due to the rapid replacement of fetal type enterocytes by adult type enterocytes, a phenomenon known as gut closure; the cell replacement is made by a receptor-mediated apoptosis involving TGF- β 1 and TNF- α as mediators (Godlewski et al., 2005, Strzałkowski et al., 2007). Consequently, adult type enterocytes do not contain ACS and large vacuoles. Interestingly, in the gut of neonatal pigs the cells undergoing apoptosis, which is followed by unzipping-zipping events markedly disrupting epithelial cell continuity, are located on the entire length of the villi (Godlewski et al., 2005; see also Fig. 1). In contrast, in adult animals the apoptotic cells are observed only on the villi top, forming a so called extrusion zone. Therefore, in neonates there is a much wider absorptive surface that is potentially subject to environmental stimuli as compared to adults. Though, one population of fetal type enterocytes disappears within the first few days after birth, there is still another population of fetal type enterocytes existing in the lower small intestine, in piglets observed until approximately three weeks after birth. These enterocytes are important for the intracellular digestion of nutrients by lysosomal enzymes, and form digestive vacuoles as a result of non-selective macromolecule uptake. Their massive loss in piglets is observed 2-3 weeks after birth. Nevertheless the protection by intestinal mucus and colostral biologically active peptides and proteins, extensive apoptosis and unzipping-zipping of a great number of epithelial cells at the same time may potentially open epithelial gates for any xenobiotics and harmful bacteria, and thereby facilitate their transfer into blood circulation.

Studies of preterm piglets and intrauterine growth retarded (IUGR) piglets demonstrated that the gut barrier in both groups of animals is open for a longer time than in full-term-appropriate weight piglets. Namely, the lower part of the small intestine of 28 day-old IUGR piglets still contained fetal type enterocytes expressing digestive vacuoles indicating marked delay in gut mucosa development (Mickiewicz et al. 2012 JPP). The gut epithelium continuity in IUGR and preterm neonates is not as finely controlled as in control rats; abnormalities of the gut epithelium may facilitate exposure of the gut and in turn the whole organism to external factors or xenobiotics. It is possible that gut permeability is altered in critically ill children and predispose them to bacterial translocation via a mechanism that creates a hostile environment in the gut and alters the gut microbiome favoring the growth of pathogens that promote bacterial translocation (Papoff et al., 2012).

Recent studies indicate that the vagus nerve is involved in immunomodulation as suggested by its ability to attenuate the production of proinflammatory cytokines in experimental models of inflammation (de Jonge and Ullola, 2007). Furthermore, functional development of the vagus nerve occurs at two stages with the neuronal population in the dorsal motor nucleus of the vagus (DMNV) maturing ahead of the sensory neuron population of the vagal sensory nucleus NTS (Islami et al., 2008). There appears to be an important link between the vagus nerve and memory recall in infancy suggesting that social learning, modulated by autonomic nervous system, may be jeopardized in preterm infants (Haley et al., 2010)

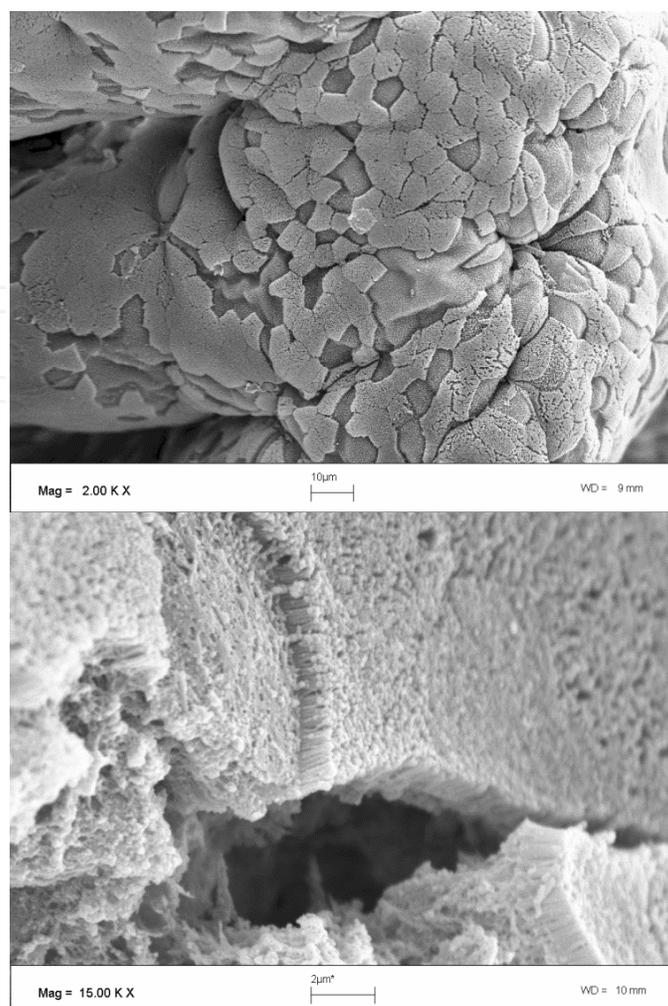


Figure 1. Packetts of apoptotic cells in the epithelium of neonatal piglets. Massive apoptosis is evidenced by scanning electron microscope (SEM) image by shortened microvilli, several yet unzipped spaces between cells are present. (SEM images generously supplied by dr. Tomasz Skrzypek, Catholic University of Lublin, Poland)

In conclusion, maturation of the autonomic nervous system may be delayed in preterm and IUGR animals. Furthermore, delayed development of the GIT in preterm and IUGR animals, including longer gut permeability, facilitates the toxic effect of external factors including bacterial translocation. Furthermore, the immature gut seemingly fails to stimulate the development of the vagus nerve. Importantly, there is some evidence pointing to altered gut permeability (leaky gut) in autism and possibly genetic predisposition to abnormalities in tight junctions in ASD (White, 2003; de Magistris et al, 2010).

4. Determinants of individual sensitivity of brain-gut axis and gut microbiome to environmental toxins; intrinsic and extrinsic components

Studies of the human microbiome revealed that even healthy individuals differ remarkably in the microbes of the gut. The gut microbiome is regulated by both extrinsic and intrinsic factors.

While much of this biodiversity remains unexplained, extrinsic factors such as diet, environment, and early microbial exposure, and the intrinsic factors such as host genetics have been implicated (Human Microbiome Project Consortium, 2012); our own studies (Sulkowski et al., 2012) suggest that sex may play an important role. Diet-derived carbohydrates that are not fully digested in the upper gut are metabolized by bacteria in the human large intestine. These nondigestible carbohydrates influence microbial fermentation and total bacterial number in the colon. Human milk, unlike milk of other mammalian species, contains high amounts of oligosaccharides of yet unknown function, but one can speculate that dietary oligosaccharides may play an important function in the development of the microbiome in human neonates. Evidence exists that the amount and type of nondigestible carbohydrates influence the species composition of the intestinal microbiome. Individual variation in the gut microbiome may, in part reflect differences in dietary intake, but the response of the gut microbiome to dietary change can also differ among individuals (Flint, 2012)

Furthermore, an outcome of the exposure to infectious microbes or their toxins is also influenced by both microbial and host genes. Some host genes encode defense mechanisms, whereas others assist pathogen function. Extensive human diversity in cell lethality dependent on toxin binding and uptake has been observed (Martchenko et al., 2012). Furthermore, there is evidence that individuals may evolve their own specific microbiome (Clayton, 2012).

Results of our recent animal studies (Sulkowski et al, 2012; Khan et al, 2012, Xu et al, submitted; see also Fig. 2) indicate that the sensitivity of the developing CNS to both environmental toxins and infection, are both sex- and rat strain-dependent. It can be extrapolated that the sensitivity of the human microbiome is also sex-dependent. Because of this individual variability in host response it is not surprising that the results of human postmortem studies of ASD brains are difficult to interpret.

5. Microbiome

The human GIT harbors a large number (1000 to 1150) of bacterial species and is involved in maintaining homeostasis and well-being. Functions of this microbiome include the regulation of the mucosal immune system, GIT motility, epithelial barrier regulation, gut secretion, digestion and metabolism (Grenham et al., 2011). One of the main functions of gut microbes is to extract nutrients from otherwise indigestible fibers (Tremaroli and Backhed, 2012). The microbiome, absent at birth, is gradually colonized by facultative bacteria and anaerobic bacteria (Grenham et al., 2011).

Several lines of evidence point to both brain-gut axis and gut microbiome abnormalities in autism which are summarized in Fig 3. Children with ASD frequently present a variety of gastrointestinal (GI) symptoms, although some claim that the data supporting increased GI symptomology in autistic children not to be rigorous enough (Erickson et al., 2005). The so-called “bacterial theory” of autism proposes the GIT symptoms are associated with changes in microbial composition and that these changes could be involved in the pathogenesis or progression of several childhood diseases including autism (Somma et al., 2010).

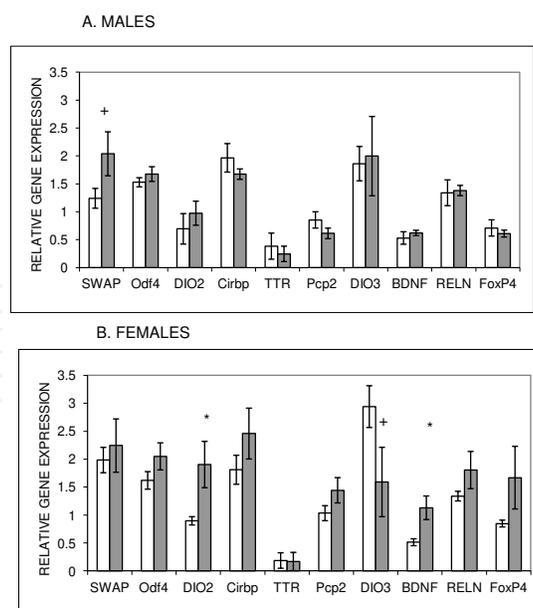


Figure 2. The effect of LPS exposure on cerebellar gene expression. Gene expression was measured by quantitative RT-PCR in cerebellar tissue of rat pups exposed perinatally to LPS (200 μ g/kg BW) and was normalized to cyclophilin A. Panel A: males, Panel B: females. Data are presented as relative gene expression (mean \pm S.E.M.; *, $p < 0.05$; +, $p < 0.1$; Xu et al., submitted).

It has been suggested that an abnormal gut microbiome in some ASD children may be due to certain antimicrobial drugs that play a key role in modifying the intestinal bacterial flora and selecting potentially harmful bacteria normally kept at bay by the innate intestinal flora. And so, both *Clostridia* (Finegold, 2011b) and *Desulfovibrio* (Finegold, 2011a) have been implicated in autistic pathology. *Clostridia* form spores and the spores could likely survive antibiotic treatment and subsequently flourish. *Desulfovibrio* is an anaerobic bacillus that does not produce spores and is resistant to some antibiotics such as cephalosporins used in treatment of common childhood diseases such as ear infections (Finegold, 2011a). An increase in *Bacteroides*, a decrease in *Firmicutes* with an overall increase in biodiversity has been observed in IBD, celiac disease and autism (Iebba et al., 2011). An increase in *Clostridium histolyticum*, a recognized toxin producer with systemic effects, has been observed in fecal samples of ASD children (Parracho et al., 2005). A strong correlation of gastrointestinal symptoms with autism, and a decrease in *Bifidobacteria* and increase in *Lactobacilli*, was observed in fecal samples of ASD children (Adams et al., 2011). An association between high levels of intestinal, mucopit-thelial-associated *Sutterella* species and GI disturbances has been detected in intestinal biopsy samples in children with autism (Williams et al., 2012). This latter study may provide the most accurate picture of the gut microbiome as the data were derived directly from the gut.

A response to oral treatment with vancomycin, not absorbed from the GI tract, in autism suggests the importance of gut flora in a disease (Finegold, 2011a). Evidence suggests that ASD may be associated with altered innate immune response; thus children with GI problems may reflect inflammation as a reaction to an endotoxin produced by gut bacteria (Jyonouchi et al., 2002).

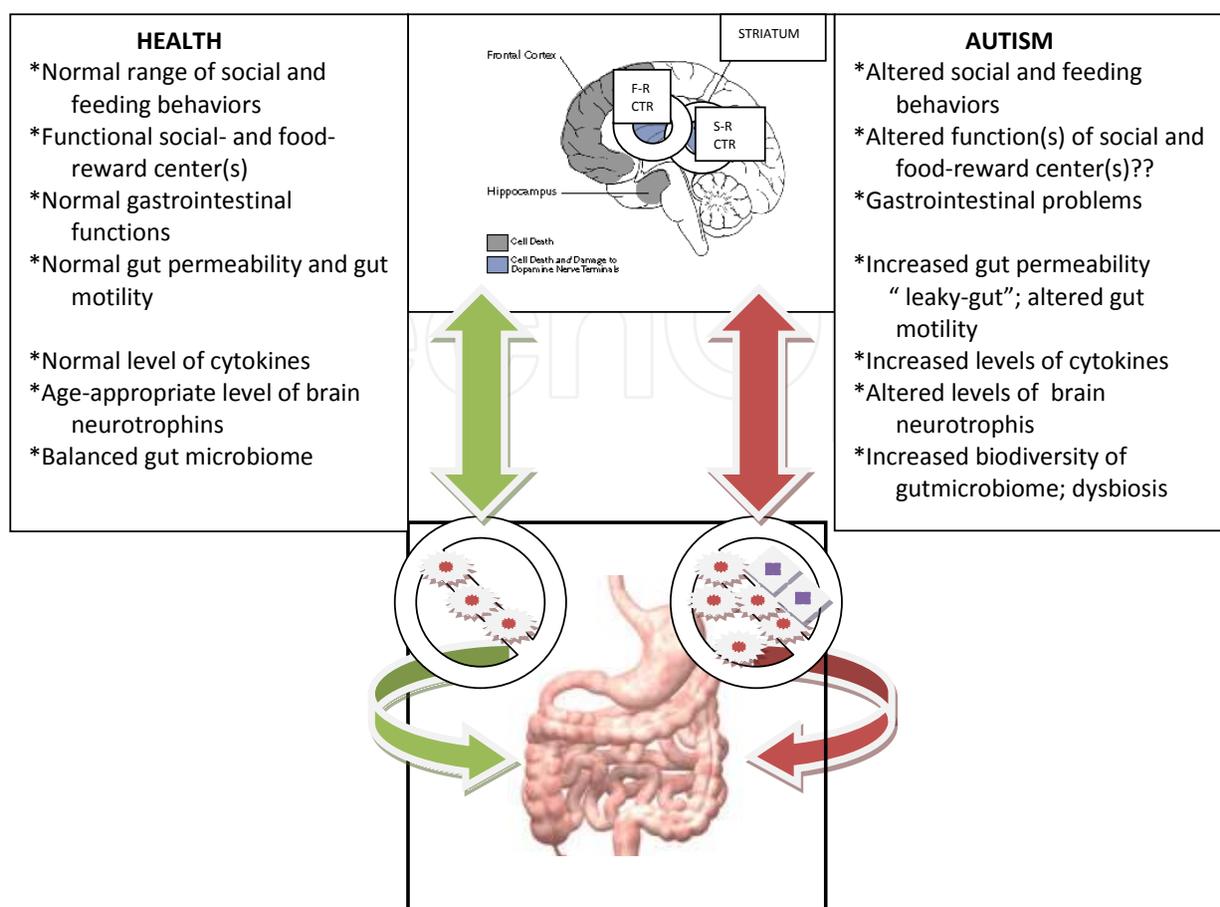


Figure 3. Altered gut microbiome and the brain gut axis in autism. S-R-CTR, social reward center; F-R CTR, food reward center.

Our most recent studies suggest altered expression of ghrelin, the activating enzyme (ghrelin O-acyltransferase, GOAT) and the receptor in several brain areas of autistic children (Sajdel-Sulkowska, unpublished observation). A decrease in ghrelin mRNA has been also observed in the temporal gyrus of Alzheimer patients (Gahete et al., 2010) suggesting ghrelin may contribute to the severity of AD pathology. Since we have measured the levels of ghrelin mRNA, it can be assumed that the changes observed were due to the altered levels of brain ghrelin.

The majority of circulating ghrelin is synthesized by gastric mucosa X/A-like cells in response to negative energy status. These cells are not typical endocrine cells since the oxyntic mucosa cells produce HCl in the stomach lumen and ghrelin as a hormone. Ghrelin is the most potent orexigenic peptide, and plays an important role in glucose metabolism and also in GIT cytoprotection. In addition to its ability to stimulate appetite, ghrelin stimulates the release of growth hormone release via the growth secretagogue, GHS-R1a receptor. Ghrelin O-acyltransferase, GOAT, is the enzyme that activates ghrelin. The ghrelin/GHS-R/GOAT system may play an important role in metabolic disorders in children (Lim and Korbonits, 2012). In addition to the ghrelin of GIT origin, and the hypothalamus being the main source of brain ghrelin, ghrelin has been detected in the midbrain, hindbrain, hippocampus, spinal cord and

several organs outside the brain. While the systemic endogenous ghrelin exerts a tonic stimulating effect on hypothalamic CRH (Rucinski et al., 2012), its function in the brain includes the modulation of membrane excitability, control of neurotransmitter release, neuronal gene expression, and neuronal survival and proliferation (Ferrini et al., 2009).

It has been reported that ghrelin of GIT origin interacts with bacterial toxins (Tiaka et al., 2011) and exerts a protective role in experimental colitis; is it possible that the ghrelin of brain origin plays a protective role as well? If so, changes in the level of brain-derived ghrelin could be detrimental to the developing brain.

6. Existing and emerging therapeutic strategies in autism targeting the gut-brain axis and gut microbiome: Role of individual microbes and dietary amino acids in maintaining gut-brain homeostasis

Existing therapies targeting the gut microbiome include diet, antibiotics, and probiotics. Dietary restriction, including the removal of dairy casein-containing products, wheat and gluten sources, sugar, chocolate, preservatives, and food coloring have all been found to be therapeutic in autism. Interestingly, dairy casein-containing products stimulate ghrelin (a hunger hormone) and reduce CCK (a satiety agent) production in the periphery and in the brain. Gastrointestinal problems in autism appear to respond to antimicrobial agents. Treatments targeting *Candida*, and probiotics have been used to reduce dysbiosis and control gut permeability (Kidd, 2002). Other strategies include the removal of heavy metals (including mercury) by chelation and sulfur-sulphydryl repletion. Supplementation with dimethylglycine, vitamin B6, magnesium, vitamin B3, C, folic acid, calcium and zinc, cod liver, digestive enzymes, all appear to be beneficial in a number of autistic children (Kidd, 2002). Immune therapies, including pentoxifyllin, immunoglobulin, transfer factors and colostrums appear to work in a limited number of cases,

The initial promising use of secretin, a triggering factor for digestion, in the treatment of autism has been more recently disclaimed. In multiple randomized controlled trials secretin offered no significant benefit (Krishnaswami et al., 2011; Williams et al., 2012).

Abnormalities in the primary pathway for carbohydrate digestion and transporters, involving disaccharidases and hexose transporters, have been reported and found to be accompanied by dysbiosis as evidenced by a decrease in Bacteroidetes and an increase in the ratio of Firmicutes to Bacteroidetes (Williams et al., 2011). These abnormalities respond to probiotic and dietary responses (Williams et al., 2011). Probiotic therapy appears to influence microbiome composition, intestinal barrier function and mucosal immune responses (Critchfield et al., 2011). There is evidence to support alterations of fecal microbiome in autism, and in the majority of cases treatment with vancomycin, an antibiotic that targets gram positive anaerobes and is minimally absorbed by the gut, can improve symptoms (Sandler et al., 2000).

Recently therapies targeting the gut microbiome are emerging as a viable strategy in the treatment of CNS disorders (Forsythe et al., 2010). Preclinical studies of selected probiotics in

healthy volunteers (Messaoudi et al., 2011) provided encouraging results for further studies exploring the concept of microbial targeting of the GIT under pathological conditions including autism. Individually tailored probiotic formulations, enriched in specific strains of gut bacteria, could one day be used in treatments of ASD even as an adjuvant to other treatments.

7. Possible connection of gut microbiome and behavior; microbiome and behavioral abnormalities in ASD

The intestinal microbiome participates in the development of the HPA axis (Sudo et al., 2004) and is critical to the development of appropriate stress response later in life, which occurs during a narrow, critical developmental window. This process involves both the regulation of the levels of brain derived neurotrophic factor (BDNF) and NMDA receptors (Sudo et al., 2004). The microbiome also plays an important role in anxiety-like behavior (Messaoudi et al., 2011), depressive behaviors (Neufield et al., 2011; Messaoudi et al., 2011), but the effects are diminished in vagatomized animals, suggesting either the direct communication between the bacteria and the brain (Bravo et al., 2011) or through the brain-gut axis. The latter possibility is an indirect action of bacteria on an afferent vagal pathway via gut immune, endocrine and enteric nervous system (ENS) controlling mechanisms.

Animal studies have also shown that stress can change the composition of the microbiome, where the changes are associated with increased vulnerability to inflammatory stimuli in the GIT (Gareau et al., 2006); here the microbiome plays an important role in memory dysfunction (Gareau et al., 2011). Stress is known to inhibit gut contraction, one of the crucial defense strategies against bacterial colonization of gut mucosa. Early psychological trauma of maternal separation resulted in persistent mucosal barrier dysfunction in neonatal rats, including host defense to luminal bacteria, by mechanisms involving peripheral CRH receptors (Gareau et al., 2006).

Oral antibiotics disrupt the microbiome and favor environment for opportunistic bacteria. *Clostridium tetani*, an anaerobic bacillus produces a potent neurotoxin, tetanus neurotoxin (TeNT) that is transported by the vagus nerve from the GI to the CNS. In the brain TeNT disrupts the release of neurotransmitters by the proteolytic cleavage of synaptobrevin, a synaptic vesicle membrane protein. This inhibition may be related to a variety of behavioral deficits characteristic of autism. Some children with autism treated with anti-clostridia antibiotics have shown a reduction in stereotyped behavior (Bolte, 1998).

8. The role of the reward system in gut-brain communication, the interaction between food-reward and social-reward systems; altered gut-microbiome regulation of the reward loop in autism?

Autism is characterized by both severe deficits in social interaction and communication and significant eating difficulties with a highly restricted range of food choices (Williams et al.,

2000). It seems logical to hypothesize that altered composition of the gut microbiome under a “leaky gut” condition in autism interferes with the normal activity of the reward circuitry including both social and feeding behavior, as illustrated in Fig. 3. In support of this hypothesis are the neuroimaging, electrophysiological and neurochemical data suggesting a disruption in reward seeking tendencies in ASD, and especially in social contexts (Kohls et al., 2012). It has been proposed that this disruption is caused by abnormalities of the dopaminergic-oxytocinergic “wanting circuitry” that includes the ventral striatum, amygdale, and the ventromedial prefrontal cortex (Kohls et al., 2012). Indeed, Individuals with ASD are characterized by low responsiveness to social rewards (Dawson et al., 2005; Schultz, 2005; Neuhaus et al, 2010). Recent studies of the left amygdala and orbito-frontal cortex, which are the main components of the social brain, showed neuronal dysfunctions in these structures in autism (Mori et al, 2012). Furthermore, brain levels of serotonin, the “happy hormone” are regulated by gut bacteria as evidenced by studies involving germ-free animals (Clarke et al., 2012). Abnormalities in blood serotonin levels are consistently altered in a subset of children with ASD.

It is also possible that the abnormalities in vagus nerve functions may further contribute to social deficits in autism (Goetz et al., 2010).). It is thus of interest (Ito and Craig, 2008) that there is a possibility that the viscerosensory information is sent via the vagus nerve directly to the reward centers. The vagus nerve is involved in our emotional responses and in feelings of compassion as shown in vagal stimulation, suggesting that the social bond is related to the gut-brain axis (Goetz et al., 2010). Studies utilizing single-photon emission tomography (SPET) provide evidence for the limbic system-vagal nerve connection (Barnes et al., 2003). Vagotomy was for decades a method of choice in treating a number of gastric diseases in adults; it would be of interest to address it in context of autistic pathology.

Furthermore, the intestinal microbiome regulates the HPA during both development and adulthood (Sudo et al., 2004) and plays an important role in the stress response. Activation of the HPA axis involves the release of endogenous opioids which are components of the brain reward system (Adam et al., 2007).

In humans, sensory factors, such as taste and smell, have an important role in reward-related feeding (Rolls, 2011); gustatory, olfactory, visual and somatosensory aspects of food are regulated by the orbitofrontal cortex. Environmental cues, as well as cognitive, reward, and emotional factors play an important role in food intake which may override the homeostatic requirements (Berthoud, 2006). Environmental cues regulate endocannabinoid and opioid systems which play an important role in reward-related feeding and have wide receptor distributions within the CNS (Cota et al., 2006). Hypothalamic endocannabinoids increase food intake through a leptin-regulated mechanism. The nucleus accumbens is a key limbic pathway and may be implicated in regulation of hedonistic and homeostatic feeding (Berthoud, 2006). Dopamine appears to be associated with reward-related food intake and with behaviors required to maintain feeding essential for survival (Di Marzo et al., 2001).

The neural circuit mediating reward-related behavior is a complex network that includes the midbrain, substantia nigra, the amygdala, the ventral striatum, the ventromedial prefrontal

cortex and ventral anterior cingulated cortex with the central relay located in ventral striatum (Kohls et al., 2012).

It is interesting, that the ventral striatum is associated with both social-reward and food-reward circuitry (Adam et al., 2007). Although it is generally assumed that the two centers are separate, the observation of altered sucrose preference and positive correlation with ventral striatum dopamine levels under conditions of social isolation stress in perinatal rats lends support to the speculation of inter-connectivity of the two centers (Brenes and Fornaguera, 2008).

9. Conclusions

The “leaky gut” during development may be potentially more vulnerable to environmental insults than the normally developing GIT. Consequently, alterations in the gut microbiome may play an important role in autistic pathology. Evidence is growing that points to an early developmental abnormality in establishing GIT and innate microbial milieu. The gut microbiome, regulated by both intrinsic and extrinsic factors, may be further jeopardized by recurrent infections and/or recurrent use of antibiotics. A developmentally abnormal gut microbiome may in turn affect both the gut-brain axis and brain development and contribute to the etiology of ASD. Abnormalities in the gut-brain axis may further lead to the aberrant development of both the social and the food reward system(s) in autism. Future studies targeting the gut-brain/brain-gut axis in autism and the gut microbiome are warranted, but must take into consideration individual variation in gut microbiomes and intrinsic and extrinsic sensitivities and sex. Results of these studies will likely contribute to our understanding of ASD and advance new and viable therapies.

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