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Review

The Microbiota, the Gut and the Brain in Eating and Alcohol Use Disorders: A 'Ménage à Trois'?

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Abstract

Aims: Accumulating evidence for the influence of the gut microbiota on the bidirectional communication along the gut-brain axis suggests a role of the gut microbiota in eating disorders (EDs) and alcohol and substance use disorders. The potential influence of altered gut microbiota (dysbiosis) on behaviors associated with such disorders may have implications for developing therapeutic interventions.

Methods: A systematic review of preclinical and clinical studies evaluating the gut microbiota, EDs and alcohol and substance use disorders was conducted using MEDLINE, Embase and Web of Science databases with the objective being to examine the role of the gut microbiota in behavioral correlates of these disorders. Original papers focused on the gut microbiota and potential behavioral implications were deemed eligible for consideration.

Results: The resulting 12 publications were limited to gut microbiota studies related to EDs and alcohol and substance use disorders. Some studies suggest that dysbiosis and gut microbial byproducts may influence the pathophysiology of EDs via direct and indirect interference with peptide hormone signaling. Additionally, dysbiosis was shown to be correlated with alcohol use disorder-related symptoms, i.e. craving, depression and anxiety. Finally, a mouse study suggests that manipulations in the gut microbiota may affect cocaine-related behaviors.

Conclusions: Promising, albeit preliminary, findings suggest a potential role of the gut microbiota in behavioral correlates of EDs and alcohol and substance use disorders.

Short summary: Preliminary evidence exists supporting the role of the gut microbiota in eating disorders and alcohol and substance use disorders, although additional investigation is needed to determine what is causative versus epiphenomenological.

INTRODUCTION

The gut microbiota is a collection of over 100 trillion microorganisms residing in the gastrointestinal tract (Savage, 1977). Their collective genome, the microbiome, encodes 100 times more genes than the human genome (Qin *et al.*, 2010). The gut microbiota plays several roles in digestion, inflammation and immunity (Li *et al.*, 2008;

Abt and Artis, 2009; Nicholson *et al.*, 2012; Leclercq *et al.*, 2017). The microbiota–host relationship is mutualistic. Imbalances or alterations in microbial composition or activity – dysbiosis – can influence health and is implicated in various diseases, e.g. irritable bowel disease, colorectal cancer and diabetes (Kim *et al.*, 2013). Gut dysbiosis is associated with damages to the intestinal barrier, which

negatively impacts the host's ability to respond to stressors (Mutlu *et al.*, 2012).

There is a bidirectional pathway of communication along the microbiome-gut-brain axis. On one hand, bottom-up signaling pathways influence health through vagal, neural, endocrine and/or immune pathways, and may impact behavior, brain activity and levels of neurotransmitters, receptors and neurotrophic factors (Cryan and Dinan, 2012; Forsythe *et al.*, 2012; Forsythe and Kunze, 2013). Additionally, microbial-derived metabolites can activate these pathways. On the other hand, neural signaling through top-down pathways can influence gut function, disrupt the intestinal barrier ('leaky gut') and alter the composition and function of the gut microbiota.

The influence of the microbiota on stress, autism, anxiety, depression, Parkinson's disease and schizophrenia has been shown in various preclinical and clinical work (Sudo *et al.*, 2004; Adams *et al.*, 2011; Bravo *et al.*, 2011; Diaz Heijtz *et al.*, 2011; Neufeld *et al.*, 2011; Bendtsen *et al.*, 2012; Cryan and Dinan, 2012; Forsythe *et al.*, 2012; Hsiao *et al.*, 2013; Burokas *et al.*, 2015). Similarly, recent research suggests that the gut microbiota may impact behaviors related to problematic alcohol drinking and eating.

Notably, eating disorders (EDs) and alcohol and substance use disorders share neurobiological mechanisms that regulate these pathological behaviors and both disorders involve altered reward processing and related neural circuitries (Goodman, 2008; Schreiber *et al.*, 2013). Behaviors related to EDs and alcohol and substance use disorders (e.g. urges, cravings, binge episodes) are associated with altered orbitofrontal and prefrontal cortex activity and reduced inhibition and self-control. EDs and alcohol and substance use disorders present with high mortality and morbidity (Table 1), but limited treatment options (Smink *et al.*, 2012; Grant *et al.*, 2015).

Recent preclinical studies have shown that the gut microbiota can influence key neural pathways involved in EDs and alcohol and substance use disorders. Mice lacking microbes have marked changes in striatal (Diaz Heijtz *et al.*, 2011), amygdala (Stilling *et al.*, 2015), hippocampal (Neufeld *et al.*, 2011; Clarke *et al.*, 2013) and cortical (Hoban *et al.*, 2016) gene expression, in addition to alterations at the morphological and spine density level (Luczynski *et al.*, 2016b). Such findings are coupled with an exaggerated hypothalamic–pituitary–adrenal axis response and changes in anxiety, cognitive and social behaviors (Luczynski *et al.*, 2016a). These brain

Table 1. Overview of the disorders of interest examined in this systematic review

Overview	Main features	Estimated prevalence
Eating disorders		
■ Serious and often fatal illnesses that cause severe disturbances to a person's eating behavior (National Institute of Mental Health (NIMH), 2016) ■ Stem from individuals' voluntary motivation to restrict eating or lose weight; transitions into involuntary, pathological behavior (Andersen, 2014) ■ Includes Anorexia nervosa and Bulimia nervosa	■ A persistent disturbance in eating behavior ■ May involve change in ingestion and absorption of food and nutrients ■ Impaired physical and psychosocial health (American Psychiatric Association, 2013); often comorbid psychological disorders (*i.e.* depression, anxiety) ■ Altered brain reward mechanisms (Barry *et al.*, 2009; Berridge *et al.*, 2010) ■ **Anorexia nervosa**—restriction of energy intake, fear of gaining weight, and/or behaviors interfering with weight gain; distorted or disturbed self-perception of size or weight (American Psychiatric Association, 2013) ■ **Bulimia nervosa**—shares some clinical features with Anorexia nervosa, involves recurrent binge-eating episodes, unhealthy behaviors to prevent weight gain, and self-evaluation excessively focused on body shape or weight	■ The 12-month prevalence is: ■ **Anorexia nervosa**: 0.4% in young females; less clear in males (American Psychiatric Association, 2013) ■ **Bulimia nervosa**: 1–1.5% in young females; less clear in males (American Psychiatric Association, 2013) ■ Eating disorders incidence is about ten times higher in females than males (Becker *et al.*, 1999; Lindvall Dahlgren and Wisting, 2016)
Alcohol and substance use disorders	Alcohol and substance use disorders	Substance use disorders
■ **Substance use disorders**—cluster of cognitive, behavioral and physiological symptoms indicating that the individual continues using a substance despite significant substance-related problems (American Psychiatric Association, 2013) ■ **Alcohol use disorders**—medical diagnosis for severe problem drinking that consists of both physical and behavioral symptoms (American Psychiatric Association, 2013)	■ Underlying change in brain circuits that persist beyond detoxification; behavioral effects of these changes can be seen in relapse and craving (American Psychiatric Association, 2013) ■ Symptoms include, but are not limited to craving, withdrawal, and tolerance ■ Share similar neural correlates with Eating disorders with regard to altered reward signaling; involve issues with impulse control (Schulte *et al.*, 2016)	■ 12-month prevalence and lifetime prevalence were 3.9% and 9.9%, respectively from 2012–2013 in the United States (Grant *et al.*, 2016) **Alcohol use disorders** ■ 12-month prevalence and lifetime prevalence were 13.9% and 29.1%, respectively (Grant *et al.*, 2015) ■ Harmful use of alcohol is leading cause of morbidity and mortality (Whiteford *et al.*, 2013; Grant *et al.*, 2015); accounts for approximately 2.5 million deaths per year worldwide (World Health Organization, 2011); fourth leading cause of disability worldwide (Whiteford *et al.*, 2013)

areas and behaviors are involved in ED and alcohol and substance use disorders pathology, thus it is plausible that the gut microbiota plays a role in such disorders. This systematic review examines the literature on, and discusses the relationship between, behaviors exhibited in these disorders and gut dysbiosis.

METHODS

A systematic review was conducted on the gut microbiota, EDs and alcohol and substance use disorders. We considered 'behaviors' in this context as any action or comportment correlated to the clinical manifestation of the disorder, and specifically we searched for *craving*. Both preclinical and clinical published studies were included without limitations for the year or language. Both reviews and original research articles were included in the search, but only full-length original articles were reviewed and included.

The MEDLINE, Embase and Web of Science databases were searched for records indexed up until April 2016 (see Table 2). In addition to the studies found by the systematic searches, citations within those articles were also evaluated, if relevant. The titles and abstracts were screened by two independent reviewers (J.E.T. and S.B.) for their relevance to the objective of this review. Articles focused on microbiota of body areas other than the gut, or on microbial profiles and/or metabolic factors, but not on host behaviors, were excluded. Inclusion criteria involved the use of behavioral outcomes or measures and a focus on the disorders of interest. In the event of a disagreement over relevance, the full texts of articles were examined and discussed to determine if the inclusion criterion for behavioral outcomes related to the disorders of interest was satisfied. The full texts of those deemed relevant were examined thoroughly. The selection followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al.*, 2009) (Fig. 1).

RESULTS

The selection process for the studies included in this review is detailed in Fig. 1. Consistent with the exclusion criteria involving metabolic outcomes, or microbial profiling without behavioral measures or focusing on microbiota from alternative body areas, all microbiome-related studies on substance use disorders without

Table 2. Search strategy of this systematic review

The terms were searched both as medical subject heading terms and text words in MEDLINE; the strategy was modified for the additional databases (Embase and Web of Science) to conform to their controlled vocabularies and database field structures:
(1) Microbiota OR microbiome OR metagenome
(2) Alcoholism OR alcohol-related disorders OR alcohol use disorder
(3) Smoking OR tobacco use OR tobacco use disorder OR tobacco products OR cannabis OR marijuana smoking OR marijuana abuse OR marijuana OR THC
(4) Opioid-related disorders OR amphetamine-related disorders OR cocaine-related disorders OR substance-related disorders OR cocaine OR heroin OR narcotics OR substance abuse OR drug addict* OR opioid* OR amphetamine OR methamphetamine
(5) Food
(6) ED OR anorexia nervosa OR binge eating OR bulimia nervosa
(7) Craving
(8) #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8
(9) #1 AND #8

behavioral correlations were deemed outside of the scope for this review. Because the only explicit behavioral outcome searched was 'craving,' many articles were read in full to assess for additional examples of behavioral outcomes, such as anxiety behavior. Of the initial 916 records, 10 studies satisfied the criteria of this systematic review: Two additional papers (Breton *et al.*, 2016a; Kiraly *et al.*, 2016) were identified and included following the culmination of the formal search due to their relevance to the review objective. Therefore, a total of 12 papers were included: 8 papers on EDs and 4 papers on alcohol and substance use disorders. These studies are reviewed next.

Gut microbiota, dysbiosis and EDs
Broad overview of the field
The impact of diet and nutrition on the gut microbiota led researchers to investigate the role of gut bacteria modulation in the central nervous system control of food intake (Flint *et al.*, 2012). Gut bacteria perform host metabolic functions, facilitate energy extraction from food, increase nutrient availability (Backhed *et al.*, 2005) and alter taste receptors (Duca *et al.*, 2012).

Investigations on the gut-brain axis in EDs are sparse, despite correlations with microbial changes. Indeed, Anorexia Nervosa (AN) patients have significantly lower amounts of total intestinal bacteria and higher prevalence of specific bacterial strains, which may reflect altered metabolic capacity resulting from the disorder (Armougom *et al.*, 2009; Morita *et al.*, 2015; Bulik, 2016; Carr *et al.*, 2016; Mack *et al.*, 2016). Gut bacteria may play a role in cravings for specific foods or induce dysphoria to motivate the ingestion of foods (Alcock *et al.*, 2014). An evolutionary pathway has been proposed, such that gut bacteria act to enhance their own survival or hinder that of competitive gut bacteria. Diminished microbial diversity may be associated with unhealthy eating patterns that can lead to the debilitating weight loss associated with AN (Alcock *et al.*, 2014). Alterations to gut microbial profiles in AN patients persist after weight gain, although some increase in microbial richness, including elevated numbers of operational taxonomic units and increased diversity of population, is observed (Mack *et al.*, 2016). Additionally, the presence of specific strains of gut bacteria have been linked to ED-related clinical features, e.g. *Escherichia coli* K12 and Body Mass Index (Million *et al.*, 2013). Bulimia nervosa (BN) and binge-eating disorder (BED) diagnoses on the other hand have been correlated to antimicrobial medication use prior to ED treatment (Raevuori *et al.*, 2016).

Central controls of satiety and food intake takes place through the integration of information from sensory, behavioral, physiological and neurohormonal signals (Morton *et al.*, 2006). Orexigenic hormones like ghrelin are released in the fasting state and cross the blood–brain barrier to promote food intake by activating neurons that produce neuropeptides like neuropeptide Y and agouti-related peptide (AgRP) in the arcuate nucleus (Pimentel *et al.*, 2012) . Anorexigenic hormones like leptin are released after food ingestion and, via similar mechanisms, activate neuropeptides such as glucagon-like peptide 1 and α-melanocyte-stimulating hormone (α-MSH) to promote satiety (Pimentel *et al.*, 2012). When bound to melanocortin receptor 4 (MC4R), the final pathway in satiety signaling (Cone, 2005), α-MSH is involved in the regulation of mood and emotion as well (Kokare *et al.*, 2010).

Immunoglobulins (Igs) can cross the blood–brain barrier and access the arcuate nucleus (Fetissov and Dechelotte, 2008). EDs may stem from the production of Igs, or auto-antibodies (auto-Abs),
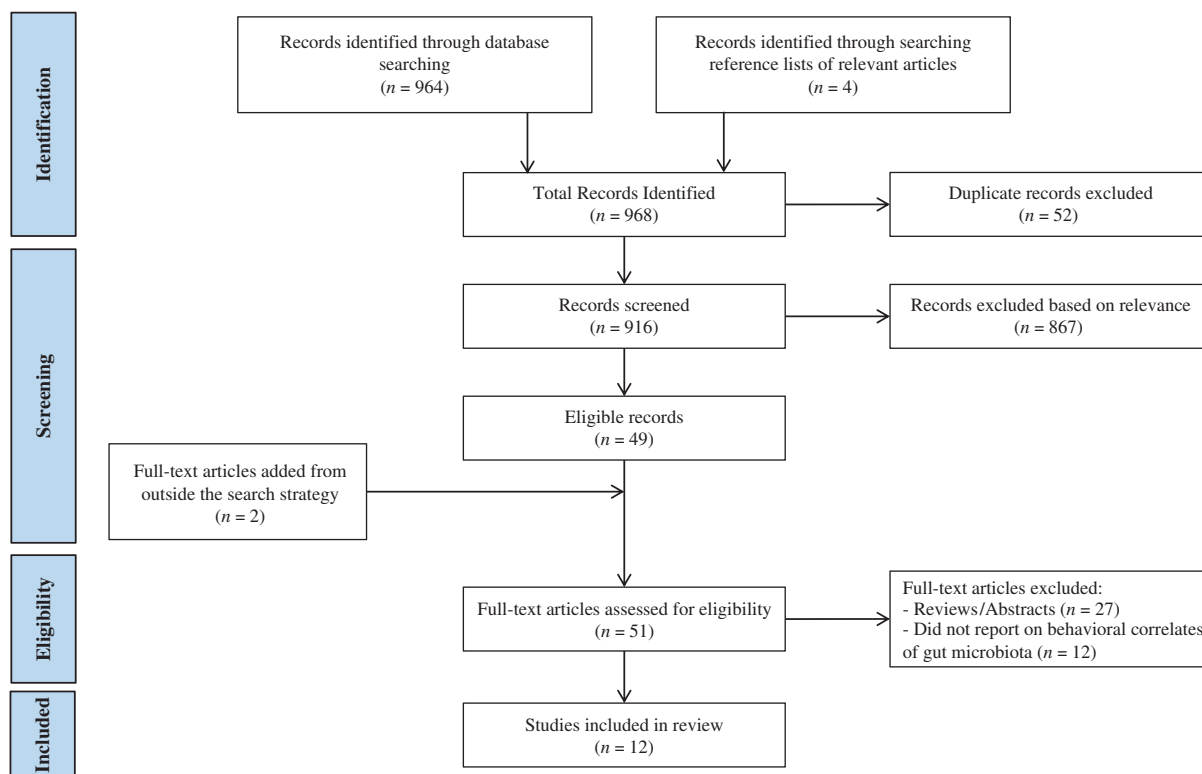
Fig. 1. Flow diagram of the systematic review.

that modulate biological responses to appetite-regulating neuropeptides by binding to hormone receptors (Inui *et al.*, 2015), and affect appetite and emotion. Rodent studies show a role of α-MSH-reactive auto-Abs, stemming from altered immune functioning and food-restriction-induced stress, in the regulation of appetite and emotion (Sinno *et al.*, 2009).

Though causation has not been established, mechanisms explaining the link between gut microbiome and EDs have been proposed, including signaling via vagal tone (Kollai *et al.*, 1994) and changes in immune functions (Kleiman *et al.*, 2015a). EDs have also been linked to the gut microbiota through a process called 'molecular mimicry'. Whereby, microbial proteins—with amino-acid sequence homology to appetite-regulating neuropeptides—are made available as a result of altered intestinal permeability and cross-react with auto-Abs, of which production have also been linked to intestinal inflammation (Coquerel *et al.*, 2012). Indeed, increased levels of α-MSH auto-Abs were observed after food restriction (mild stressor). A subsequent significant increase in food intake and anxiety levels was observed in food-restricted rats when compared to a non-stressed control group, who exhibited acute bulimic and anxiolytic responses when exposed to α-MSH auto-Abs (Coquerel *et al.*, 2012). Thus, evidence suggests that auto-Abs are dynamic, influenced by stress, and likely involved in neuroendocrine control of eating (Fetissov *et al.*, 2008b). Because appetite-regulating neuropeptides are important signaling molecules in hunger and satiety, the ability of colonic microbiota to impact appetite and emotion via molecular mimicry may be involved in the development of EDs.

This review led to eight papers investigating the potential link between the gut microbiota and ED-related behaviors, as outline in Table 3 and discussed next.

Preclinical studies

Serum levels of auto-Abs against appetite-regulating neuropeptides were tested in both germ-free (GF; sterile of bacterial colonization) and specific-pathogen free (SPF; lacking only particular bacteria) rats to assess the dependence of auto-Ab production on the gut microbiota (Fetissov *et al.*, 2008a). Serum auto-Abs against 14 appetite-regulating peptides were detected in both GF and SPF rats. However, observed levels differed, e.g. significantly lower levels of auto-Abs against AgRP were found in GF than SPF rats, whereas ghrelin auto-Abs were significantly higher in GF than SPF rats (Fetissov *et al.*, 2008a).

A proteomics mouse study was conducted to validate the role of the gut microbiota and molecular mimicry in the pathophysiology of EDs (Tennoune *et al.*, 2014). *E. coli* K12 was selected for its sequence homology with α-MSH. Specifically, a Caseinolytic protease B (ClpB) heat-shock disaggregation chaperone protein from *E. coli* K12 can mimic α-MSH. ClpB-immunized mice produced anti-ClpB auto-Abs that also demonstrated cross-reactivity with α-MSH, and demonstrated lower MC4R signaling, which is involved in energy balance regulation (Tennoune *et al.*, 2014). ClpB-immunization of mice did not contribute to a significant change in locomotor behavior. However, immunized mice demonstrated reduced time spent, and shorter distance traveled, in closed arms of the O-maze test, suggesting decreased anxiety. Mean daily food intake was higher in immunized mice operating via interference with satiation mechanisms (i.e. constant number of meals, but increased meal size) rather than operating through hunger. Thus, anti-ClpB auto-Abs influence anxiety behavior and food intake by reduced sensitivity to the anorexigenic effects of α-MSH (Tennoune *et al.*, 2014).

As an immediate follow-up study, the role of *E. coli*-related auto-Abs against α-MSH was further investigated as a possible

Table 3. Summary of findings on eating disorders

Sample studied	Main findings	Sample size	Reference
Germ-free and Specific-pathogen free rats	Presence of auto-Abs against appetite-regulating neuropeptides found in both groups Levels differed (lower levels observed in Germ-free rats) *Conclusion*: Gut microbiota modulates auto-Ab levels against appetite-regulating neuropeptides, but not necessary for auto-Ab production	$n = 6$/group	Fetissov *et al.* (2008a)
Healthy female participants	Auto-Abs against appetite-regulating neuropeptides and sequence homology confirmed in healthy subject sera *Conclusion*: Possible general role of auto-Abs in feeding behavior and appetite	$n = 15$	
Male C57Bl/6 mice	Anti-ClpB auto-Abs cross-reactive with α-MSH lead to reduced MC4R signaling and influenced food intake, body weight, and anxiety behavior *Conclusion*: Anti-ClpB auto-Abs influence ED-related behaviors via reduced sensitivity to anorexigenic effects of α-MSH	$n = 8$/group	Tennoune *et al.* (2014)
Female patients with AN, BN, or BED	Anti-ClpB auto-Abs against α-MSH elevated in ED patients and correlated with ED-related behaviors *Conclusion*: ClpB protein can alter auto-Ab production and impact feeding and emotion in humans with EDs	AN $n = 27$ BN $n = 32$ BED $n = 14$ Control $n = 65$	
Adult Wistar rats	Sex-related differences in male and female rat food and water intake in response to *E. coli* K12 gavage *Conclusion*: Sex-related differences in gut microbiota contribute to differences in satiety, feeding, and emotion and may represent a risk factor for ED development	$n = 48$	Tennoune *et al.* (2015)
Female patients with AN or BN	Auto-Abs present in both ED and control participants; elevated levels in EDs; differences in auto-Ab levels between AN and BN Auto-Abs correlate with of ED-related behaviors *Conclusion*: ED-related psychobehavioral outcomes correlate with auto-Ab levels against α-MSH	AN $n = 12$ BN $n = 42$ Control $n = 41$	Fetissov *et al.* (2005)
Female patients with AN or BN and male Sprague-Dawley rats	Human sera (from ED and control participants) applied to rat brains and adsorbed with appetite-regulating neuropeptides; reduced staining observed for α-MSH and ACTH *Conclusion*: Plasma auto-Abs from ED participants have the potential to cross-react with administered peptides	AN $n = 12$ BN $n = 42$ Control $n = 41$	Fetissov *et al.* (2005)
Female patients with AN, BN or BED	ClpB present in all groups, but higher in EDs ClpB positively correlated with α-MSH auto-Abs and ED-related behaviors *Conclusion*: ED-related psychobehavioral outcomes are correlated with gut microbial factors	AN $n = 24$ BN $n = 29$ BED $n = 13$ Control $n = 29$	Breton *et al.* (2016a)
Female patients with AN	AN patients demonstrated lower microbial diversity than healthy controls; diversity correlated with ED-related clinical assessments Slight improvements in diversity occurred during AN treatment *Conclusion*: Intestinal dysbiosis is associated with altered mood and ED symptoms in patients with AN	T1 $n = 16$ T2 $n = 19$ Control $n = 12$	Kleiman *et al.* (2015b)
Female patients with AN and/or BN and Sprague-Dawley rats	Human sera applied to rat brains and adsorbed with melanotropes and/or corticotropes; reduced staining observed for α-MSH and ACTH *Conclusion*: A significant subpopulation of ED patients has auto-Abs with the potential to cross react with administered peptides	AN $n = 28$ BN $n = 22$ AN/BN $n = 7$ Control $n = 13$	Fetissov *et al.* (2002)

explanation for sex-related differences in ED prevalence (Tennoune *et al.*, 2015). *E. coli* K12 was more abundant in female than male rat feces. When administered by gavage over 3 weeks, the strain led to higher food and water intake, and weight gain, in females (Tennoune *et al.*, 2015). Male rats trended towards increased anti-ClpB IgM, whereas plasma levels of α-MSH-reactive IgG were elevated in females. This study suggests that at least a part of sex-differences in food intake, immune response, kinetics and energy balance are related to differences in the prevalence of *E. coli* K12 in male and female rats, which contributes to differences in satiety, feeding, and emotional behavior in rats (Tennoune *et al.*, 2015).

Clinical studies

Fetissov *et al.* (2005) investigated correlations of auto-Ab levels [against α-MSH, adrenocorticotropic hormone (ACTH), oxytocin (OT) and arginine vasopressin (AVP)] with ED-related psychological traits in both control and ED participants. Although auto-Abs were found in both groups, suggesting possible homeostatic functions, their levels differed. AN patients had significantly increased auto-Abs against α-MSH, OT and AVP, compared to BN and controls. These changes correlated with differences in ED-related cognitive and behavioral traits [assessed by Eating Disorder Inventory-Two (EDI-2)] among the three groups; AN and BN patients had similar

total EDI-2 scores, which were higher than that of the control group. Auto-Abs against the peptides of interest showed correlations with EDI-2 scores in the ED groups; α-MSH demonstrated the highest correlation. Furthermore, auto-Abs against adrenocorticotropic hormone (ACTH) correlated positively (in AN) and negatively (in BN patients) with maturity fears, a wish for the security of pre-adolescent years in response to the demands of adulthood (Fetissov *et al.*, 2005). A translational component of this study is discussed below.

To better understand if the presence of auto-Abs was involved in the etiology of ED pathology, or if auto-Ab dysregulation was a consequence of pathology, the presence of auto-Abs against 14 appetite-regulating peptides in the sera of healthy women was tested (Fetissov *et al.*, 2008a). Auto-Abs against all peptides were identified, suggesting a general role of auto-Abs in normal physiology, not just in EDs. Sequence homology of at least 5 consecutive amino-acids between microorganisms and peptides of interest was confirmed, a criterion for molecular mimicry (Fetissov *et al.*, 2008a).

To extend preclinical findings, Tennoune and colleagues (2014) took plasma samples from participants with EDs who also completed the EDI-2. Patients demonstrated elevated α-MSH cross-reactive anti-ClpB antibodies when compared to healthy female controls. Auto-Ab levels positively correlated to EDI-2 scores, demonstrating a potential link between the gut microbiota and ED behaviors.

Using similar methods, Breton and colleagues (Breton *et al.*, 2016a) tested for plasma ClpB-levels in both ED and control participants, and administered the EDI-2 and the Montgomery-Åsberg Depression Rating Scale (MADRS), confirming previous findings (Tennoune *et al.*, 2014). The plasma ClpB protein was present in all groups, with higher levels in the ED groups, that were not different from each other (Breton *et al.*, 2016a). In ED patients, plasma ClpB positively correlated with α-MSH auto-Abs and significantly correlated with EDI-2 subscales: interpersonal distrust, social insecurity, and bulimia in AN patients; bulimia, maturity fears, interceptive awareness, and EDI-2 total score in BN patients; and ineffectiveness, maturity fears, and drive for thinness in BED patients. Significant correlations between ClpB and MADRS total scores were found only in AN patients (Breton *et al.*, 2016a).

In an observational study of patients undergoing treatment for AN, stool samples were collected at admission and discharge (Kleiman *et al.*, 2015b). Lower microbial diversity was observed in AN patients compared to healthy controls ($n = 12$). Microbial diversity had a significant negative association with self-reported depression, anxiety and ED psychopathology, and was negatively correlated with more severe ED symptoms. Slight improvements in diversity were made throughout the course of weight-restoration treatment (Kleiman *et al.*, 2015a).

Translational studies

Human sera were applied to sections of rat brains (Fetissov *et al.*, 2002). Approximately 74% of human sera bound to rat pituitary melanotropes and corticotropes, and approximately 20% selectively bound to α-MSH projections. When adsorbed with α-MSH and ACTH peptides, reduced staining was observed for both peptides, demonstrating that plasma auto-Abs from ED individuals and administered peptides share similar binding sites and can cross-react (Fetissov *et al.*, 2002, 2005).

In summary, the ability of auto-Abs to interfere in appetite-related peptide signaling, and the influence of gut microbial proteins in modulating this signaling, demonstrates the potential impact of the microbiome-gut-brain axis on behaviors related to EDs.

Gut microbiota, dysbiosis and alcohol and substance use disorders

Broad overview of the field

Until recently, little was known about the specific effects of alcohol on the gut microbiome. Dysbiosis was reported in only a subset of patients with alcohol use disorder (AUD) who showed lower and higher median abundances of *Bacteroidetes* and *Proteobacteria*, respectively, such that microbial alterations correlated with elevated serum endotoxin levels (Mutlu *et al.*, 2012). Alterations in microbial composition were not correlated to the duration of sobriety, suggesting alcohol-related dysbiosis is long-lasting and persists despite abstinent periods (Mutlu *et al.*, 2012), though abstinence was measured primarily via self-report assessments. Chronic alcohol consumption can also induce bacterial overgrowth in the small intestine, mucosal damage in the large intestine, and subsequent elevations in intestinal permeability (Keshavarzian *et al.*, 2009). Intestinal permeability increases with chronic stress, causing immunomodulatory bacterial cell wall components, such as lipopolysaccharides (LPS), to escape into the circulation and impact the host. For example, by altering the immune system (Cryan and Dinan, 2012) or lowering pain sensitivity thresholds (Benson *et al.*, 2012). Although gut microbial alterations related to chronic alcohol use are supported, the mechanism of action is still unclear (Vassallo *et al.*, 2015). A recent preclinical study evaluating the effect of vaporized ethanol administration in mice demonstrated elevated alpha diversity, significantly increased *Alistipes* genus, and significantly diminished genra *Clostridium IV* and *XIVb*, *Dorea*, and *Coprococcus* between mice exposed to ethanol vapor and controls (Peterson *et al.*, 2017). Additionally, current understandings of the effect of drugs abuse on the gut microbiota are sparse, although differences have been demonstrated between the commensal gut microbiota of cocaine users and control subjects (Volpe *et al.*, 2014).

This review led to the identification of three clinical papers that investigated the potential link between gut microbiome and AUD-related behaviors (Table 4). No preclinical studies were identified in the timeframe of the literature review. However, one preclinical paper was published investigating cocaine-related behaviors, and was included (Table 4).

Preclinical study

A recent study by Kiraly and colleagues (2016) represents the first published investigation, to our knowledge, of the direct effects of gut microbiota manipulation on cocaine-related behaviors. Mice treated with antibiotics to diminish their gut microbiota demonstrated enhanced cocaine-reward and locomotive sensitivity. Antibiotic-treated mice exhibited conditioned place preference at lower doses than control mice, revealing a shifted dose-response curve in mice with reduced gut bacteria. Effects were unrelated to drug metabolism, the experimental methods, or increased intestinal permeability. The alterations were correlated to changes in the encoding of proteins important in reward circuitry transcription, and the repletion of bacterial products reversed behavioral outcomes.

Clinical studies

A study by Leclercq and colleagues (2012) evaluated the relationships between abstinence and intestinal permeability, inflammation, and symptoms relevant to alcohol relapse (i.e. alcohol craving, depression, anxiety, selective attention, or reaction time measured through a response inhibition task). Patients were tested the day after admission to a detoxification ward (T1) and at discharge (T2) for those who remained abstinent over the 18–19 days stay. Though significantly

Table 4. Summary of findings on alcohol and substance use disorders

Sample studied	Main finding	Sample size	Reference
Alcohol Dependent (AD) patients undergoing 3-week abstinence	Intestinal permeability, LPS, and cytokine levels elevated at T1 (admission); full recovery of intestinal permeability and LPS and slight recovery of cytokines at T2 (after 3 weeks of abstinence) AD subjects showed elevated depression, anxiety, and craving, with slight decrease over short-term abstinence *Conclusion:* Inflammation and intestinal permeability associated with AD diagnosis and AD-related behavioral measures, suggesting role of gut-brain axis in AD pathogenesis	T1 *n* = 52 T2 *n* = 40 Control *n* = 16	Leclercq *et al.* (2012)
Actively drinking AD patients undergoing alcohol detoxification	LPS and PGN, and associated TLR4 and TLR2 Complexes elevated in AD patients Plasma levels of pro-inflammatory cytokines elevated and positively correlated with consumption of, and craving for, alcohol at T1 Short-term withdrawal associated with recovery of LPS-dependent receptors *Conclusion:* LPS and PGN from gut microbiota stimulate inflammatory pathways that are correlated with behavioral measures of AD	T1 *n* = 63 T2 *n* = 41 Control *n* = 14	Leclercq *et al.* (2014a)
AD patients undergoing 3-week abstinence	*Preliminary Study:* A subset of AD patients demonstrated IP; this group had higher scores on depression, anxiety, and craving after 3 weeks of abstinence *Main Study:* High intestinal permeability associated with altered gut microbiota composition and activity *Conclusion:* Evidence for involvement of gut-brain axis in AD with a role of IP and inflammation in behavioral correlates of AD	*Preliminary:* T1 *n* = 60 T2 *n* = 44 Control *n* = 15 *Main:* AD *n* = 13 Control *n* = 15	Leclercq *et al.* (2014b)
Male C57Bl/6 Mice	Diminished gut bacteria associated with enhanced cocaine-induced conditioned place preference Reduced gut bacteria associated with increased sensitivity to locomotor-sensitizing effects of repeated cocaine dosing Altered behavioral outcomes correlated to altered transcription encoding for reward circuitry proteins *Conclusion:* Alterations to the gut microbiota impact behavioral responses to cocaine in mice	*n* = 5–22/group	Kiraly *et al.* (2016)

elevated at T1, alcohol dependent patients intestinal permeability and LPS concentrations recovered completely by T2. Alcohol dependent individuals were characterized by low-grade inflammation and greater psychological symptomology, with significantly elevated pro-inflammatory cytokines and higher depression, anxiety, and craving. A slight recovery in cytokine levels and a significant decrease in psychobehavioral outcomes was observed throughout short-term abstinence, though levels were still higher than controls. Additionally, alcohol dependent individuals performed worse than controls on the selective attention task, but demonstrated improvements in reaction time at T2 compared to T1. Importantly, cytokine levels were significantly positively associated with craving; selective attention was correlated with intestinal permeability; and cytokines such as interleukin-6 (IL-6) and IL-10 were positively correlated with anxiety, and negatively correlated with depression.

In another study, the same research team investigated whether gut-derived bacterial products influence peripheral blood mononuclear cells (PBMCs), important components of the immune system (Leclercq *et al.*, 2014a). By comparing alcohol dependent patients to healthy controls at T1 and T2, links between immune, inflammatory, and psychobehavioral changes were assessed (Leclercq *et al.*, 2014a). Gut bacteria-derived LPS and peptidoglycan (PGN) were elevated in alcohol dependent patients, and were accompanied by enhanced expression and activation of Toll-like receptor 4 (TLR4) and TLR2 complexes in PBMCs, which bind LPS and PGN, respectively (Leclercq *et al.*, 2014a). Additionally, plasma levels of pro-inflammatory cytokines were elevated in alcohol dependent versus control patients, and were positively correlated with alcohol consumption and craving scores at T1. Changes in craving scores, which decreased over the course of

detoxification, correlated significantly with the changes in cytokine levels. IL-8 levels best predicted alcohol craving in alcohol dependent patients (Leclercq *et al.*, 2014a).

The effects of short-term abstinence on inflammatory pathways were further investigated (Leclercq *et al.*, 2014a). Pro-inflammatory cytokine messenger ribonucleic acid (mRNA) levels were reduced while TLR4, but not TLR2, mRNA levels decreased significantly towards levels similar to controls. Thus, short-term withdrawal was associated with recovery of LPS-dependent receptors, but not PGN-dependent receptors (Leclercq *et al.*, 2014a).

In a follow-up study, the relationships between altered intestinal permeability, gut microbial composition and activity, and AD-related clinical symptoms were evaluated using similar methods as described above (Alcock *et al.*, 2014). Only 43% of patients had elevated intestinal permeability at T1. The remaining 57% had intestinal permeability levels similar to controls. The patients were split into high and low intestinal permeability groups. Differences in the family and genus levels of bacteria were observed between high intestinal permeability, low intestinal permeability, and control participants. Some degree of microbiota composition recovery was observed following abstinence. Specifically, increases in *Ruminococcaceae* in high intestinal permeability individuals. Additional assessments revealed elevated cytokine and pro-inflammatory levels dependent on intestinal permeability status, such that significantly high IL-8 levels were observed when intestinal permeability was high. Positive correlations between psychological symptoms and intestinal permeability suggest a role for the gut-barrier in the clinical presentation of alcohol dependence. High intestinal permeability was associated with a more severe clinical alcohol dependence profile, with persistent symptoms despite slight recovery

of intestinal permeability observed at T2. In contrast, psychological symptoms of those with low intestinal permeability recovered at T2 (Leclercq *et al.*, 2014a).

DISCUSSION

Critical view of the studies reported

Though preliminary and limited, existing literature suggests a role of the gut microbiota in behaviors associated with EDs and alcohol and substance use disorder. Evidence of molecular mimicry and auto-Abs against appetite-regulating neuropeptides highlight a potential mechanism by which gut level activity impacts neural signaling and ED-related host behavior. The gut microbiota is not necessary for the production of auto-Abs, but can modulate their levels and may serve as a potential target for ED interventions (Fetissov *et al.*, 2008a). Auto-abs against central, appetite-regulating peptides like α-MSH alter melanocortin system functioning, which can contribute to non-metabolic alterations in eating behavior and food craving, and may explain the clinical correlations between auto-Abs and self-reported ED symptoms (Fetissov *et al.*, 2005; Tennoune *et al.*, 2014; Kleiman *et al.*, 2015b). Though the presence of auto-Abs is insufficient to cause EDs (Fetissov *et al.*, 2005), high blood–brain-barrier permeability, which can result from stress, may be involved in triggering pathogenesis. The research presented identifies auto-Abs against key peptides as not only treatment targets, but also as markers for the function of the gut-brain axis and for response to microbiota-related ED treatments (Inui *et al.*, 2015) and as potential means of identifying ED risk factors (Tennoune *et al.*, 2015). By checking auto-Ab levels and sequence homology comparisons between ED and healthy participants, the studies reviewed support the possibility that gut microbiota-driven dysregulation of neuroendocrine control of appetite leads to behavioral profiles associated with food intake in patients with EDs.

With respect to AUD, chronic alcohol use induces dysbiosis and increased intestinal permeability in only a subset of alcohol dependent patients (Bode and Bode, 2003; Mutlu *et al.*, 2012), allowing microbial metabolites to enter peripheral and central circulation and impact behavioral correlates such as alcohol craving (Leclercq *et al.*, 2014b). Thus, chronic excessive alcohol use is necessary, but not sufficient to cause gut dysfunction in alcohol dependent patients (Mutlu *et al.*, 2012; Leclercq *et al.*, 2014b). Still, gut microbial and peripheral metabolite level alterations were linked to alcohol craving, anxiety, and depression, which are important factors associated with relapse (de Timary *et al.*, 2015). Interestingly, the level of intestinal permeability was differentially associated with behaviors, with recovery in depression, anxiety, and craving levels observed in individuals with low intestinal permeability, but not in those with high intestinal permeability. This further suggests a role of the microbiome-gut-brain axis in AUD. The low intestinal permeability group demonstrated reduced severity and duration of psychological symptoms, while the high intestinal permeability group showed persistent AD-related behavioral changes throughout short-term abstinence, despite intestinal permeability recovery (Leclercq *et al.*, 2014b). Two possible conclusions regarding the persistence of symptoms in the high intestinal permeability group were proposed (de Timary *et al.*, 2015). On one hand, perhaps the symptomatology is not related to the amount of alcohol consumed, as both groups demonstrated comparable use, but is instead related to the composition and function of the gut microbiota, which differed between high and low intestinal permeability patients. On the other hand, perhaps the observed levels of anxiety

and depression in the low intestinal permeability group are due to ethanol rather than direct influence of the gut microbiota, as these symptoms decrease during abstinence. Still, craving persists in both the intestinal permeability groups despite abstinence, suggesting alcohol alone is an insufficient explanation. Altered microbial measures associated with altered peripheral signaling and low-grade systemic inflammation that were observed at T2 suggest that alcohol use is likely the primary effector of how the inflammatory response affects alcohol craving (Leclercq *et al.*, 2014a). Nonetheless, the influence of a possible interaction between the gut microbiota and ethanol in impacting the psychological profiles of AUD patients must be considered. Finally, a promising preliminary preclinical investigation demonstrating altered behavioral responses to repeated cocaine administration in mice with diminished gut microbiota profiles provides further validation for this area of research and must be replicated, as well as tested in other animal models and with additional drugs of abuse (Kiraly *et al.* 2016).

Both EDs and AUD seem to be linked with some form of barrier imbalance: elevated blood–brain barrier in the case of ED and elevated intestinal barrier permeability in the case of AUD. This opens the door for gut microbial products to reach the central and peripheral circulations to contribute to pathogenesis in both disorders. Moreover, binge eating and binge drinking are associated with forms of negative affect, such as anxiety and depression (Ferriter and Ray, 2011). The studies reviewed above demonstrate that alterations to the gut microbiota in clinical presentations of both ED and AUD have been correlated with altered anxiety and depressive behavior, potentially indicating a shared mechanism of influence exerted by the gut microbiota on these behavioral disorders.

Although this review facilitates the identification of promising directions for future research, the paucity of existing research on the topic of behavioral outcomes associated with EDs and alcohol and substance use disorders is a limitation of this assessment. Moreover, the majority of relevant studies produced by our search are concentrated in specific labs, and the results need additional validation.

Future directions

Limitations from the studies reviewed here call for additional research. Only one of the above studies involved inpatient admission for ED treatment, the duration of which was not mentioned (Kleiman *et al.*, 2015b). Additionally, preclinical work found that the prevalence of *E. coli*-related auto-Ab is different between female and male rats, while most of the clinical investigations were conducted with female participants.

Investigating the potential causal relationship between gut microbiota and the pathological behaviors here reviewed is another important future direction. Though establishing causality in human models may prove challenging, translational models transplanting human gut microbiota samples to rodent models could be utilized to clarify the directionality of the microbiota-behavior relationship. Moreover, given the relative independence of the amount of ethanol consumed from the presence or absence of dysbiosis and intestinal permeability (de Timary *et al.*, 2015), it will be important to determine whether altered gut microbial profiles can be viewed as a precursor to AUD. Greater awareness of the causal relationship between dysbiosis and alcohol intake may shed light on the mechanisms of communication between the gut microbiota and the brain. Understanding whether the effects are carried out via metabolites released into peripheral circulation (Mutlu *et al.*, 2012), altered vagal nerve signaling (Forsythe *et al.*, 2012), changes in addiction-related

neurotransmitter systems (Cryan and Dinan, 2012), and/or inflammatory mechanisms (Leclercq *et al.*, 2014a) will better inform potential intervention strategies. Given existing evidence, it is possible these mechanisms occur simultaneously and complement each other, but this must be confirmed in targeted studies. Treatments to improve intestinal integrity and modify serum metabolites are possible intervention strategies that should be investigated, as has been proposed for treatment of autistic behavior and related gut microbial dysregulation (Vuong and Hsiao, 2016). Finally, recent evidence suggests that the gut microbiota may influence host appetite via both short-term, local effects, and long-term changes (Breton *et al.*, 2016b). Further investigation into the role of molecular mimicry in the longevity and mechanism of appetite regulation is needed.

Both EDs and AUDs affect nutritional intake or exert caloric effects. Given the known role of diet and caloric intake in driving gut microbiota composition, it must be noted that none of the studies included here controlled for the diet-microbiota interactions. Such experimental controls would be valuable areas of future research. Moreover, consumption of different types of alcohol was found to lead to different permeability profiles. For example, high intestinal permeability individuals tended to consume less wine and more spirits than low intestinal permeability patients (Leclercq *et al.*, 2014b). Additionally, red wine consumption has been associated with higher microbial diversity and enhancement of bacterial species that convey anti-inflammatory properties (Zhernakova *et al.*, 2016). Thus, perhaps types of alcoholic beverages, or specific components of certain alcohols, have differential effects on behaviors associated with AUD and must therefore be controlled for in future investigations.

Finally, molecular mimicry has been implicated in a number of disease states, including autoimmune disorders (Hornig, 2013), Alzheimer's disease, and Parkinson's disease (Friedland, 2015), and it is associated with some health-promoting effects (Friedland, 2015). Given the shared reward mechanisms associated with EDs and alcohol and substance use disorders, and the proven relevance of hormonal signaling in alcohol and substance use disorders (Kenna *et al.*, 2012; Engel and Jerlhag, 2014), perhaps molecular mimicry plays a role in the pathology of behavioral disorders other than EDs. Additionally, molecular mimicry involving auto-Abs against other neuronal peptides relevant to alcohol and substance use disorders and ED may be involved through interference with reward pathways (Morris *et al.*, 2016).

A better understanding of the factors contributing to, or causing, persistent craving for alcohol, cocaine and/or other drugs of abuse, ED-related behavior, depression, and anxiety symptoms can better inform clinicians and scientists to develop targeted treatment strategies. As a recent review suggests, perhaps the gut microbiota can be targeted to mitigate alcohol relapse behavior (Gorky and Schwaber, 2016). More generally, psychobiotics, organisms that beneficially impact the health of patients with psychiatric illnesses, have been demonstrated to impact depression and anxiety (Dinan *et al.*, 2013; Fondo *et al.*, 2015) and could be tested in future studies on ED and alcohol and substance use disorders.

CONCLUSION

This systematic review supports preliminary evidence for the role of the gut microbiota in EDs and alcohol and substance use disorders and suggests that additional investigation is needed to determine what is causative versus epiphenomenological. Should the promising findings outlined here be replicated and expanded towards causal and mechanistic relationships, manipulations of the microbiome-gut-brain axis may represent novel approaches for the treatment of EDs and/or alcohol and substance use disorders.

AUTHORS' CONTRIBUTIONS

L.L. thought of the scientific basis and rationale for this systematic review. J.E.T. and S.B. performed the literature search and screened titles and abstracts for their relevance to the objective of this work. J.E.T. wrote the first draft of the manuscript. L.L. supervised the manuscript preparation. S.B., M.F., M.R.L. and J.F.C. contributed to the interpretation of the results and manuscript preparation. All authors critically *reviewed* the contents and approved the final version of the manuscript.

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