Drug-Gut Microbiota Interactions: Implications for Neuropharmacology

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List of abbreviations

BBR, berberine; (B-GOS), Bimuno^{TM} galacto-oligosaccharides; CDI, *Clostridium difficile* infection; CNS, central nervous system; CYP, cytochrome P450; DMARDS, disease modifying anti-rheumatic drugs; FMT, faecal microbiota transplantation; GF, germ-free; GI, gastrointestinal; HFD, high fat diet; MAOI, monoamine oxidase inhibitor; MDD, major depressive disorder; NAPQI, n-acetyl-p-benzoquinone imine; NSAID, non-steroidal anti-inflammatory drug; PKPD, pharmacokinetics/pharmacodynamics; PEG, polyethylene glycol; PPI, proton pump inhibitor; SCFA, short chain fatty acids; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme.

Abstract

The fate and activity of drugs are frequently dictated not only by the host *per se* but also by the microorganisms present in the gastrointestinal tract. The gut microbiome is known to, both directly and indirectly, affect drug metabolism. More evidence now hints at the impact that drugs can have on the function and composition of the gut microbiome. Both microbiota-mediated alterations in drug metabolism and drug-mediated alterations in the gut microbiome can have beneficial or detrimental effects on the host. Greater insights into the mechanisms driving these reciprocal drug-gut microbiota interactions are needed, to guide the development of microbiome-targeted dietary or pharmacological interventions, with the potential to enhance drug efficacy or reduce drug side-effects. In this review, we explore the relationship between drugs and the gut microbiome, with a specific focus on potential mechanisms underpinning the drug-mediated alterations on the gut microbiome and the potential implications for psychoactive drugs.

Key words: drug metabolism; neuropharmacology; microbial enzymes; drug; microbiome

Introduction

Although not prominently featured in the classical teaching of pharmacokinetics and pharmacodynamics (PKPD), the fate and activity of drugs are frequently decided not only by the host *per se* but also by the collection of microorganisms present in the gastrointestinal (GI) tract. These microorganisms represent the gut microbiota; the trillions of microbes with more than 700-1000 different bacterial species which reside in the gut (Qin *et al.* 2010). The terms microbiota and microbiome are often used interchangeably in the literature but can be differentiated based on their more recent definitions. The term "microbiota" refers to the collection of microorganisms whereas the term "microbiome" refers to the collective genomes of these microorganisms (Jandhyala *et al.* 2015; Marchesi *et al.* 2015). It's estimated that ten different phyla are thought to contribute to the functional role of the gut microbiome; Firmicutes and Bacteroidetes are the most dominant phyla. Indeed, the ratio of Firmicutes to Bacteroidetes (F/B ratio) is often used as a surrogate indicator of bacterial shifts although it may be an overly simplistic descriptor of variations in human gut microbiota composition (Mariat *et al.* 2009). Many factors have been identified that influence the biogeography and composition (both abundance and diversity) of bacteria along the GI tract including pH variation, diet, mucus, host immunity and environmental factors (Thursby and Juge 2017). For example, the acidic environment of the stomach has a sparse microbiota (10^1 bacteria/g) compared to the estimated 10^3 bacteria/g found in the less acidic small intestine, which is the main site of drug absorption. The neutral pH or weakly basic environment found in the large intestine is the most densely colonised area of the GI tract, with an estimated 10^{12} bacteria/g (O'Hara and Shanahan 2006).

The gut microbiome is predominantly implicated in the maturation of the immune system, nutrient absorption, energy homeostasis and protection against pathogens (Antunes *et al.* 2011; Jandhyala *et al.* 2015). It also plays a significant role in health, both in homeostasis and, likely, in the pathogenesis and treatment of disease. The initial composition and assembly of the gut microbiome are considered unique amongst individuals and research has identified many factors which impact its' composition including age, ethnicity, gender, environmental factors, diet, and lifestyle (Quigley 2017). For example, the composition of the gut microbiota in the newborn is dictated by the method of delivery, gestational age, infant antibiotic exposure, and method of feeding (formula versus breastfeeding) (Penders *et al.* 2006; Salazar *et al.* 2014). Though considered highly stable and resilient to change during adulthood, instability and variability in the gut microbiome become more prevalent during disease and at the extremes of life (e.g. early infancy and senescence) (Salazar *et al.* 2014). The young and the old are also considered to be the most vulnerable patient groups regarding the occurrence of adverse drug reactions and display the most variation in drug pharmacokinetic responses (Turnheim 2003).

The implication of the gut microbiome in drug metabolism is gaining more traction and has led to the emergence of a new term, Pharmacomicrobiomics (Rizkallah *et al.* 2010). Pharmacomicrobiomics investigates the effect of variations of the gut microbiome on drugs, through the lens of PKPD. In this review, we explore the relationship between drugs and the gut microbiota, with a specific focus on the potential mechanisms underpinning the drug-mediated alterations on the gut microbiota and the potential implications for the pharmacokinetic and pharmacodynamic profile of drugs. With the increasing development of extended-release drug formulations, especially in the field of neuropharmacology, which may increase the proportions of the drug reaching the bacterially-dense large intestine, a higher number of psychoactive drugs, which are subject to microbiota-mediated degradation, may be identified. While the gut microbiome may affect all classes of drugs, the purpose of this review is to focus specifically on psychoactive drugs given that most of these drugs display

high lipophilicity to ensure central nervous system (CNS) penetration (Alavijeh *et al.* 2005). In general, highly lipophilic drugs are at greater risk of poor drug absorption from the intestine hence any microbial-mediated changes in intestinal absorption or metabolism merit further consideration. Developing a greater understanding of these important, but underappreciated, drug-microbiota dynamics could help to identify drug-drug interactions and perhaps explain inter-individual variation in drug efficacy and adverse effects.

1 The Effects of Drug Therapy on the Gut Microbiome

1.1 Drug effects on the gut microbiome:

Recently, two large-scale human observational studies highlighted correlations between the gut microbiota and the intake of different classes of medication. Falony *et al.* compared the composition of the gut microbiota against the use of β-lactam antibiotics, nitrofuran antibiotics (nitrofurantoin), an osmotic laxative, biologics (TNF-α inhibitor), disease modifying anti-rheumatic drugs (DMARDS; mesalazine and azathioprine), estrogen and progesterone hormones, benzodiazepines (clonazepam), anti-depressants (venlafaxine), and anti-histamines (Falony *et al.* 2016). While 95% of samples had a similar core microbiome, medication exposure was identified as the factor causing greatest variability in the study cohort, with 63% of the detected covariate interactions driven by medication. A positive correlation, for example, was identified between the abundance of a species from the *Eggerthella* and *Coprabacillus* genera and medication use. In another metagenomic-based study, Zhernakova *et al.* identified extrinsic and intrinsic factors correlating with microbial shifts in a Dutch-population study. Forty-four categories of drugs were tested in the analysis and antibiotics, gastric-acid suppressants (proton pump inhibitors, PPIs), lipid-lowering medication (3-hydroxy-3-methylglutaryl coenzyme reductase inhibitors or "statins"), anti-hyperglycaemic (metformin), and laxatives were found to have a distinct impact on the gut microbiome. PPIs were, for example, associated with profound changes in 33 bacterial pathways and metformin-use positively correlated with *Escherichia coli* abundance (Zhernakova *et al.*, 2016). While a wide range of medication has been identified to interact with the gut microbiota, certain drug classes including antibiotics, anti-inflammatories, acid-suppressants, anti-hyperglycaemics and psychotropics, have repeatedly been shown to have distinct gut microbiota signatures and will be discussed further in the review (Section 3.2 and 4.5).

1.2 Combination of drugs and polypharmacy:

The composition of the microbiota can vary depending on both the number and combination of medication ingested. A significant difference in microbiota composition was observed in patients taking a single drug on a long-term basis in comparison to non-drug taking patients (Rogers and Aronoff 2016). Moreover, the combined use of non-steroidal anti-inflammatory drugs (NSAIDs) and PPIs differentially influenced the relative abundance of *Bacteroides* spp. and *Erysipelotrichaceae* spp. compared to NSAIDs alone. Similarly, *Bacteroides* spp. and a species belonging to the Ruminococcaceae family differentiated individuals who were concomitantly taking NSAIDs, antidepressants, and laxatives from NSAID-only users. Therefore, the composition of the gut microbiota may depend on an individual's drug use. In this study, the diversity of the gut microbiota was not significantly influenced by the number of medications taken, even though it was significantly influenced by the specific type of medication ingested by study participants. Of note, the median number of medications taken

by the community-dwelling subjects was four, which falls below the threshold for polypharmacy. Once this threshold is reached (i.e. ≥five concomitant taken medication), an impact is frequently observed. A significant decrease in species richness, a measurement of the number of species present in an area, and significant alterations in the relative abundance of 15 bacterial taxa have been reported in patients taking more than five concomitant medications (Ticinesi *et al.* 2017). Specifically, the relative abundance of the *Helicobacter* genus was significantly associated with polypharmacy and, an inverse relationship between polypharmacy and the abundance of the Lachnospiraceae and Succinivibrionaceae families was also found. The co-administration of drugs may precipitate shifts in the composition of the microbiota to favour the abundance of microbial taxa that have metabolising capacity for those drugs (Ticinesi *et al.* 2017). Not only is it important, therefore, to evaluate specific microbiota alterations induced by a single drug but it's also imperative to account for differences when multiple drugs are co-administered. Further study of the impact of multiple co-administered drugs on the gut microbiota is very relevant due to the increasing prevalence of polypharmacy in the ageing population (Dagli and Sharma 2014). In a European study across eight countries, the prevalence of polypharmacy in 4023 nursing home residents, the mean age of whom was 83.5 (SD 9.3) years, was found to be 49.7% whilst excessive polypharmacy (defined as ≥10 medication) was evident in an additional 24.3% of residents, which serves to highlight the potential for drug-associated effects on the microbiota in a vulnerable population in which the gut microbiome is already compromised (Claesson *et al.* 2012; Onder *et al.* 2012).

1.3 Duration of drug treatment and recovery:

There is some *in vitro* evidence to suggest that even single antibiotic dose or short-term antibiotic courses, albeit at high doses, can significantly impact the microbiome (Maurice *et al.* 2013). Specifically, eight different antibiotics altered the faecal microbiota after a four-hour incubation, and a significantly increased proportion of cells associated with loss of membrane integrity and altered polarity was observed. Most antibiotic-induced alterations to the microbiome are reversed upon cessation of treatment. Some modifications can, however, persist after treatment (Jakobsson *et al.* 2010). For example, the combined treatment of the antibiotics clarithromycin and metronidazole with a proton pump inhibitor, omeprazole, induced alterations to macrolide resistance gene, *ermB*, which persisted in patients for up to four years after treatment.

Additionally, the antipsychotic, risperidone is associated with a reduction in the F/B ratio which was evident at the 2-3 month study follow-up of treatment but a further reduction in Bacteroidetes, and increased weight gain, was observed after prolonged use (Bahr *et al.* 2015a). The continued use of drugs may, therefore, strengthen microbiota alterations, which may be evident from initiation of treatment but further comparisons between short-term and long-term users of medication are required.

1.4 Excipient effects:

An excipient is a pharmacologically inert substance or non-active ingredient that is added to a formulation to stabilise the active substance or enhance the function of the dosage form (Debotton and Dahan 2017). Most excipients are not absorbed from the gut lumen. Moreover,

polysaccharide-based formulations are often utilised in the delivery of probiotics and colonic-targeted drug dosage forms, for example, targeted local delivery of the anti-inflammatory sulfasalazine (Kosaraju 2005; Prudhviraj *et al.* 2015). Recent research hints at the possibility that the excipients themselves in the drug formulation might mediate microbiota changes, independent of changes induced by the drug. Recently, it was hypothesised that the presence of fermentable polysaccharides as drug formulation excipients may act as metabolic substrates for the gut microbiota thereby promoting their growth (Ticinesi *et al.* 2017). Additionally, polyethylene glycol (PEG), a polymer used in drug delivery, is linked to changes in GI transit time and changes in the gut microbiota (Kashyap *et al.* 2013). Humanised mice (ex-germ-free mice colonised with human faecal microbiota), fed a standard diet supplemented with 15% PEG 3350 for 10 days, had significantly reduced abundance of the Peptococcaceae, Eubacteriaceae bacterial families and the Anaeroplasmataceae order. As the dosage used in this animal study is detailed as %w/w of the animal chow, it is, however, difficult to extrapolate this dose to that used in pharmaceutical formulations. PEGs are commonly included in dosage forms, with diverse excipient functions including as solubilising agents, tablet binders, plasticisers in film coating, tablet lubricants and vehicles (D'souza and Shegokar 2016). Additional studies are warranted to elucidate the potential effect of the different categories of PEG polymers used in drug formulations at pharmaceutically-relevant concentrations. Excipient-induced changes in the composition or functionality of the microbiome could potentially complicate the interpretation of drug-microbiota based observational studies as it may be challenging to differentiate drug-induced changes from excipient-induced changes in the gut microbiome. Greater research is needed to evaluate the independent effects of different formulation excipients, and the possible unintended consequences their inclusion in drug formulations could have on the gut microbiome.

1.5 Gut microbiome drug-drug interactions:

Drug-induced changes in the gut microbiome may alter the pharmacokinetics of concomitant taken medication. An increased plasma level of the antiplatelet drug, aspirin, was observed in rats who were treated with a β-lactam antibiotic, ampicillin, three days previous (Kim *et al.* 2016). This enhanced bioavailability was attributed to the antibiotic-induced suppression of the metabolic activity of the gut microbiome. Moreover, ampicillin treatment significantly prolonged bleeding time in aspirin-treated rats, suggesting antibiotic treatment may potentiate its anti-thrombotic effect. Similar microbiome-mediated drug interactions have been demonstrated for both the lipid-lowering drug, lovastatin and the antihypertensive, amlodipine (Yoo *et al.* 2014; Yoo *et al.* 2016).

2 Gut Microbiota-Drug Interactions

The gut microbiome can both directly metabolise drugs and indirectly influence host metabolising capacity of drugs (Haiser and Turnbaugh 2013; Wilson and Nicholson 2017). While many drugs undergo microbial biotransformation, the specific microorganisms involved are often unknown. In this review, we will mainly refer to bacterial drug-

metabolising enzymes and the impact of the gut microbiome on the metabolism of drugs by the liver, as the primary focus of the review is the reciprocal impact drugs can have on the gut microbiome, a relatively unexplored and unappreciated area in the literature. Microbial drug metabolism has been extensively reviewed recently (Koppel *et al.* 2017; Wilson and Nicholson 2017).

2.1 Drug metabolism by microbial enzymes:

Microbial-derived drug metabolising enzymes have been implicated both in the activation and inactivation of drugs. For example, the activation of the prodrug sulfasalazine into its anti-inflammatory active moiety mesalazine (5aminosalicyclic acid or 5-ASA) is mediated by bacterial azoreductase (Peppercorn and Goldman 1972). The direct metabolism of drugs by microbial-derived enzymes has been shown for a variety of different drugs, examples of which are detailed in Table 1. In contrast to the oxidation and conjugation reactions characteristic of hepatic metabolism, reduction and hydrolysis reactions dominate gut microbiota-mediated metabolic reactions (Haiser and Turnbaugh 2013). β-glucuronidase is amongst the most studied bacterial drug-metabolising enzymes due to its role in the deconjugation of hepatically-glucuronidated metabolites and the resultant enterohepatic recycling of drugs (Takasuna *et al.* 1998; Klaassen and Cui 2015). Multiple different bacterial genera, including *Clostridium*, *Streptococcus*, *Lactobacillus*, *Ruminococcus* and *Bifidobacterium*, express β-glucuronidase (Gloux *et al.* 2011).

The development of a chemically-guided functional strategy to identify, quantify and assign functionality of enzymes associated with the gut microbiome is amongst the recent advances in this area. This strategy unravelled the discovery of trans-4-hydroxy-L-proline dehydratase microbial enzyme implicated in the metabolism of a non-proteinogenic amino acid, trans-4-hydroxy-L-proline (Levin *et al.* 2017). Application of this strategy for the identification and characterisation of microbial drug-metabolising enzymes holds much potential and could uncover previously unknown metabolic activity of the gut microbiome.

2.2 Indirect effects of the gut microbiome on host drug metabolism:

It was traditionally assumed that only drugs reaching the colon are subject to microbiota-associated alterations with relevance to drug PKPD. The concerted role of the gut bacteria and the liver in the enterohepatic recirculation of drugs is not a new concept (Takasuna *et al.* 1998). However, more recently it was highlighted that the gut microbiome could also influence hepatic function which may, in turn, precipitate changes in patient response to drugs.

Microbial-derived metabolites can mimic and compete with drug intermediates of hepatic metabolic reactions and thereby interfere with host detoxification pathways. For example, P-cresol is a microbial metabolite of tyrosine or phenylalanine which competes with paracetamol for the hepatic enzyme, sulfotransferase family 1A member 1 (SULT1A1) (Clayton *et al.* 2009). This competition for SULT1A1 induces a shift in the metabolism of paracetamol towards alternative host metabolic reactions leading to increased production of the paracetamol metabolite, N-acetyl-4-benzoquinoneimine (NAPQI); the accumulation of which has been associated with hepatotoxicity (Mitchell *et al.* 1973).

Moreover, the gut microbiome can alter the expression of hepatic enzymes or genes responsible for host metabolism. Claus *et al*. detected significantly reduced expression of hepatic cytochrome P450 (CYP) 2c29, CYP3a11 and CYP8b1 in germ-free (GF) mice in

comparison to conventionally-raised control mice (Claus *et al.* 2011). Increased expression of hepatic drug-sensing transcription factors has also been observed in GF mice including, for example, the aryl hydrocarbon receptor, a regulator of downstream CYP enzyme expression (Selwyn *et al.* 2015). However, the mechanisms responsible for the microbiome-induced changes in the expression of hepatic enzymes requires further exploration. The accumulation of bilirubin, bile acids and steroid hormones in GF mice has been suggested as a potential mechanism mediating the altered activation of the constitutive androstane receptor, a "xenosensor" nuclear receptor, and further implicates an indirect role for the microbiome in drug metabolism (Björkholm *et al.* 2009).

2.3 Effect of diet-induced changes on the gut microbiome and drug pharmacokinetics:

Diet is a factor that can shape the composition and function of the gut microbiome (Sandhu *et al.* 2016; Shanahan *et al.* 2017). It is plausible that in the future dietary interventions may be utilised to augment drug efficacy or decrease toxicity. The amino acid arginine has been shown to inhibit the *Eggerthella lenta*-encoding *cgr* operon, previously identified as essential for the microbiota-mediated reduction of the cardiac glycoside, digoxin (Haiser *et al.* 2013). Following confirmation of high levels of *cgr* operon expression, *E. lenta* colonised GF mice were fed a high-protein diet and significantly increased serum and urine levels of digoxin (following a single 0.2 mg/ml intra-gastric digoxin dose) were identified. Interestingly, high protein diet conferred no effect on digoxin pharmacokinetics in GF mice colonised with a non-digoxin reducing strain, FAA1-3-56, ruling out any indirect effect of host diet. Increased dietary protein, leading to increased arginine, could thus constitute a potential dietary intervention to improve digoxin efficacy. Recent data has also elucidated the role of diet-induced changes in the gut microbiota on the oral bioavailability of the herbal supplement, berberine (BBR), used for the treatment of hyperlipidaemia and type 2 diabetes (Wang *et al.* 2017). BBR bioavailability was significantly increased in high-fat diet (HFD) fed hamsters in comparison to hamsters fed a normal diet. The HFD significantly induced bacterial nitro-reductase activity in hamster faeces. The authors also observed higher blood BBR concentrations in individuals with a higher faecal nitro-reductase activity which further corroborated the findings in their animal model.

2.4 Effect of probiotics on drug pharmacokinetics:

Microbiota-targeted interventions, including probiotics, prebiotics, and antibiotics, may alter drug pharmacokinetics. There are no studies thus far, to our knowledge, exploring the impact of probiotics on psychoactive drug absorption and metabolism. The pharmacokinetics of an oral hypoglycaemic agent, gliclazide (administered as a single dose at 20 mg/kg) was significantly altered in diabetic rats pre-treated with a cocktail of three probiotics (*Lactobacillus acidophilus, Lactobacillus rhamnosus* and, *Bifidobacterium lactis*) for three days (Al-Salami *et al.* 2008). Similarly, probiotic treatment significantly altered the absorption of the anti-arrhythmic agent amiodarone (Matuskova *et al.* 2014). In this study, the probiotic *E. coli* strain Nissile (EcN) 1917 was administered to rats for seven days, prior to a single oral dose of amiodarone hydrochloride (50 mg/kg). EcN significantly increased amiodarone bioavailability by 43% in comparison to saline-treated control rats. No significant effect was observed with the treatment of a non-probiotic *E. coli* strain, further indicating the bacterial-mediated changes in drug pharmacokinetics may be strain specific.

Probiotics may also affect drug pharmacokinetics by modulating the composition or metabolic activity of the gut microbiota. Recent data showed probiotic treatment significantly increased the microbiota-mediated degradation of the antipyretic and analgesic, paracetamol; an effect suggested to be mediated by probiotic-induced modulation of gut microbial enzyme activity (Kim *et al.* 2018). In this study, the probiotic, *Lactobacillus reuteri*, significantly increased both sulfatase and arylsulfate transferase, and significantly decreased β-glucuronidase activity during treatment, which are the bacterial enzymes implicated in paracetamol metabolism. Following a 24-hour washout period after pre-treatment with *L. reuteri*, a single dose of paracetamol (by IV (0.5 mg/kg) or oral gavage (10 mg/kg)) was administered to mice. *L. reuteri* significantly reduced the paracetamol plasma concentration to 68.4% in comparison to control mice. Similarly, administration of a probiotic cocktail consisting of *L. acidophilus*, *B. lactis*, and *Streptococcus salivarius* to rats for three days significantly increased azoreductase activity in *ex vivo* colon contents (Lee *et al.* 2012). The *ex vivo* incubation of sulfasalazine with colon contents significantly increased the metabolism of the drug. No clinical significance was observed, however, in the *in vivo* setting when a single dose of sulfasalazine (100 mg/kg) was administered to rats following pre-treatment with the probiotic cocktail for three days.

3 Drug-Microbiome Interactions

In this section, the mechanisms underpinning the drug-mediated changes to the composition and function of the gut microbiome will be explored.

3.1 Antibacterial effects of drugs on the gut microbiota:

Direct disruption of the gut microbiota, through bacteriostatic or bactericidal activity, can alter the metabolic capability of the microbiota and have long-term effects on host functions and health. Such 'antibiotic' induced changes have been well studied. Antibiotic therapy in neonates can disrupt the bacterial colonisation of the intestine and can have long-term health implications with links to the development of eczema, allergic rhinitis, and inflammatory bowel disease in later life (Rodríguez *et al.* 2015). Antimicrobial activity, linked to the depletion of "good" commensal gut microbiota, provides an impetus for the overgrowth of hazardous commensal bacteria (Antunes *et al.* 2011). For example, antibiotics can decrease hosts resistance against the growth of the opportunistic pathogen, *Clostridium difficile* (Theriot *et al.* 2016).

Non-antibiotic drugs have also been shown to exert antimicrobial activity. *In vitro* studies have detected antimicrobial activity, against at least one bacterial strain, for a wide range of non-antibiotic drug classes including NSAIDs, mucolytic agents, bisphosphonates, PPIs, antihistamines, statins, cytostatic agents and psychotropics (Kruszewska *et al.* 2000; Kruszewska *et al.* 2002). Sulfasalazine and the bacterial metabolite, N-acetyl 5ASA, inhibited the *in vitro* growth of faecal anaerobic strains including *C. difficile* (Sandberg-Gertzen *et al.* 1985; Deloménie *et al.* 2001). The anti-neoplastic drug, 5-fluorouracil (5-FU) has also shown bactericidal effects against clinical isolates of *Staphylococcus aureus* even at lower concentrations than would what be expected after i.v. administration of 5-FU (Bodet *et al.* 1985). Similarly, the antimetabolite and antifolate drug, methotrexate, displayed strong *in*

vitro antibacterial activity only against *S. aureus* following a surveillance study of the drug against a variety of microbial strains including *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Kruszewska *et al.* 2000). Non-antibiotic drugs, chiefly psychotropics, can also affect the gut microbiome by acting synergistically with a co-administered antibacterial or by modulating the activity or pathogenicity of microbes, which will be discussed later in the review (Section 4.5) (Martins *et al.* 2008).

Recently, Maier *et al.* further elucidated the extensive impact of non-antibiotic drugs on the gut microbiota (Maier *et al.* 2018). The authors explored the antibacterial effects of 362 anti-infective and 835 host-targeted drugs (all drugs screened at 20μM; a concentration deemed representative of the predicted concentration of the drugs reaching the bacterial-dense ileum and colon) *in vitro* against 40 bacteria found to colonise the GI tract. 24% of host-targeted drugs had antibacterial activity against at least one bacterial strain with 40 drugs inhibiting the growth of up to 10 different bacteria. Antipsychotics, chemotherapeutic agents and antihypertensives were amongst the host-targeted drugs exhibiting the greatest antibacterial activity. Certain bacteria, previously identified as being highly abundant in healthy individuals such as *Prevotella copri* and *Eubacterium rectale*, were found to be most susceptible to the host-targeted drugs. Using available data from previously published metagenomic based human studies, the authors validated the antibacterial effects of the drugs identified in their high-throughput drug screens and suggested the use of some host-targeted drug may increase patient susceptibility to antibiotic resistance. This study reaffirms the importance of accounting for drug-induced changes in the gut microbiota as a potential confounder in assessing compositional changes in microbiota-related studies.

3.2 Secondary effects of drugs on the gut microbiome:

Drugs can directly alter the GI tract environment (e.g. pH and transit time), mucosa integrity, host and bacterial metabolic activity and the production of microbial-metabolites. These drug-induced changes could, in turn, have secondary effects on the microbiome with the potential to cause drug-drug interactions.

As the gradient of pH along the GI tract is known to influence bacterial abundance and diversity, drug-induced changes in gastric and intestinal pH may shape the gut microbiome (Walker *et al.* 2005; Krajmalnik-Brown *et al.* 2012). Imhann *et al*. reported changes in 20% of bacterial taxa and a significant decrease in alpha diversity in PPI users (211 participants) compared with non-users (1604 participants) (Imhann *et al.* 2017). Alpha diversity refers to the within habitat or sample diversity (a single sample value for average species diversity at an individual site). It usually includes species richness but also accounts for the abundance of the species present in the sample (Gotelli and Colwell 2001). PPI-mediated increase in the pH of the stomach and upper small intestine precipitated the growth of specific taxa (*Enterococcaceae* and *Streptococcaceae*) and increased the susceptibility of enteric mucosa to NSAID-induced damage (Freedberg *et al.* 2015). Increased abundance of gastric and faecal *Streptococcus* in PPI users has been linked to the suppression of gastric acid production and associated with increased risk of *C. difficile* infection (CDI). Moreover, PPI-use has also been linked to increases in expression of bacterial invasion genes, which may also mediate the predisposition to CDI in PPI users (Freedberg *et al.* 2015).

Additionally, GI transit time can dictate the length of exposure of the microbiota to the gut environment. The anti-diarrhoeal, loperamide, administered to humanised GF mice via the drinking water (0.1%) for 10 days, significantly increased GI transit time and altered the composition of the distal gut microbiota with an increased F/B ratio and a significant reduction in abundance of the Lachnospiraceae family (Kashyap *et al.* 2013). These changes were reversible upon cessation of loperamide administration and normalised gut transit time.

Drugs may also challenge both the integrity and permeability of the intestinal mucosa. The antihyperglycaemic drug, metformin, has been linked with mucosal modifications that influenced the bacterial growth of *Akkermansia* spp (Forslund *et al.* 2015). An enrichment of virulence factors and gas metabolism genes, mediated by the associated increase in Escherichia, are thought to contribute to the GI disturbances, bloating and increased flatulence, associated with metformin use.

The liver is continually exposed to gut microbiota-derived metabolites, including secondary bile acids and short chain fatty acids (SCFAs), as it receives an estimated 70% of its blood supply from the intestine (Marchesi *et al.* 2015). Recent data proved bile acids are implicated in the solubilisation and absorption of lipophilic drugs (Enright *et al.* 2017). Drug treatment can, however, alter gut-microbiota production of bile salts which may subsequently impact the absorption and metabolism of co-administered medication. Cefoperazone, vancomycin, and clindamycin were identified as antibiotics associated with changes in the gut microbiota composition and caused decreased levels of secondary bile acids precipitating the growth and spore germination of *C. difficile* (Theriot *et al.* 2016). Studies have found a correlation between the reduced levels of SCFAs in the proximal to distal colon with the corresponding increase in pH from the cecum to rectum (den Besten *et al.* 2013). Drug-induced changes in the production of SCFAs could thus indirectly impact gastrointestinal pH, which as mentioned previously may precipitate changes in the microbiome. For example, increased levels of SCFAs were linked with metformin, which could instigate the microbiota modifications associated with this drug (Zhernakova *et al.* 2016).

Secondary effects of drugs on the gut microbiome could also arise from drug-induced modification of genes or enzymes involved in drug metabolism or drug transport. Metabolomic studies have been employed to investigate the impact of antibiotics on the gut microbiome before and after treatment (Antunes *et al.* 2011). High dose treatment of mice with an aminoglycoside antibiotic (20 mg streptomycin via oral gavage) altered 87% of all detected intestinal metabolite features and affected many host metabolic pathways involving the metabolism of bile acids, sugars, amino acids, fatty acids with the most significant impact on the steroidal metabolic pathway. Additionally, a marginally increased level of CYP metabolising enzymes was observed after antibiotic treatment. Metatranscriptomic approaches have also been employed to analyse gut microbiome samples exposed to various drugs and have identified multiple candidate genes for their microbial metabolism (Maurice *et al.* 2013). This *ex vivo* study investigated the impact of short-term exposure of human faeces to various non-antibiotic drugs (10 mg/ml concentration used for all the following drugs) including cardiac glycosides (digoxin and digitoxin), an anthelmintic (levamisole), gastric-acid suppressant (nizatidine), an analgesic (phenacetin), and sulfasalazine. Even though these drugs did not directly alter microbial physiology (i.e. membrane integrity and polarity), even at very high concentrations, they all significantly changed the expression of microbial genes linked to drug import and metabolism. For example, sulfasalazine was

demonstrated to induce expression of thioredoxins and nitrate reductases while nizatidine, subject to bacterially-mediated N-oxide bond cleavage, upregulated the expression of drug enzymes and transporters acting on nitrogen bonds (Maurice *et al.* 2013). This finding supports the earlier hypothesis that drugs may shift the microbiota to favour the abundance of taxa involved in its metabolism. Furthermore, this altered metabolic capacity of the microbiota could consequently affect not only the pharmacokinetics of subsequent doses of the drug itself (a phenomenon referred to as autoinduction) but also the pharmacokinetics of co-administered medication that are substrates of the same metabolic pathway or transporter.

4 Implications of the Drug-Gut Microbiome Relationship for Neuropharmacology

4.1 Effect of antibiotics on inflammation and brain-gut axis:

Recent studies have explored the impact of the antibiotics on the inflammasome-gut microbiota regulation of brain function and behaviour. Wong *et al.* investigated whether the caspase-1 antagonist and tetracycline antibiotic, minocycline, has a protective effect on the stress response by modulating the microbiota-gut-brain axis (Wong *et al.* 2016). Minocycline-treatment (5 mg/kg via i.p. injection for 21 days) increased the abundance of *Akkermansia* spp., which is consistent with attenuation of inflammation, and ameliorated the stress-induced depressive-like behaviour in wild-type mice. This suggests that the behavioural effects of minocycline may be mediated via the microbiota and not just via its effects on the central nervous system microglial activation (Inta *et al.* 2017).

4.2 Psychoactive drugs and the gut-brain axis: Impact on intestinal barrier function

Several psychoactive drugs have marked effects on the intestinal barrier (for example permeability and mucus secretion) and alter GI transit. Serotonergic psychoactive drugs have been utilised as a treatment in functional GI diseases; the selective serotonin reuptake inhibitor (SSRI), paroxetine, increases the motility rate of the small intestine whilst tricyclic antidepressants are known to delay gastric emptying (Grover and Camilleri 2013). Concurrently, the gut microbiota modulates intestinal permeability with consequential effects on the absorption and metabolism of psychoactive drugs. Maintenance of intestinal mucosal integrity is important for appropriate absorption of psychoactive drugs in the small intestine. The gut microbiota challenges the intestinal barrier function via induced alterations in intestinal pH and effects on intestinal epithelial integrity (Cani *et al.* 2008; Carvalho *et al.* 2012). The presence of the bacterium *H. pylori* decreases the absorption of the anti-Parkinson's drug and dopamine precursor, levodopa. It was hypothesised *H. pylori* might alter gastric motility, disrupt the duodenal mucosa or produce reactive oxygen species which may inactivate levodopa (Hamlet *et al.* 1999; Miyaji *et al.* 1999). More recently, eradication of *H. pylori* has been shown to significantly improve clinical symptoms of patients with Parkinson's disease and improve levodopa efficacy (Hashim *et al.* 2014). Additionally, several neuropsychiatric disorders have been associated with a dysfunctional intestinal barrier; acute stress is associated with expression of the tight junction proteins, zonula occludens-1 and occludin, in the duodenal mucosa of rats subjected to water immersion

restraint stress (Lee *et al.* 2013). Moreover, corticotrophin-releasing hormone receptors mediate the colonic barrier dysfunction observed in response to maternal separation-induced mild stress (Söderholm *et al.* 2002). Hence, both the gut microbiota and psychiatric disorders modulate intestinal permeability and barrier function which in turn may precipitate changes in the absorption of psychoactive drugs (Kelly *et al.* 2015).

4.3 Impact of CNS-related disorders on the composition of the gut microbiota:

In addition to drug-induced changes in the gut microbiome, alterations in the composition of the gut microbiota have also been associated with neurological dysfunction and psychiatric disorders (Cenit *et al.* 2017). Kelly *et al*. observed decreased richness and diversity of the gut microbiota in depressed patients and numerous studies have identified an altered microbiota composition in children with autism spectrum disorder (ASD); increased abundance of *Lactobacillus* and *Desulfovibrio* spp. has correlated with autism severity (Finegold 2011; Tomova *et al.* 2015; Kelly *et al.* 2016). The gut microbiota has also been identified as a potential regulator of other neuropsychiatric disorders, for example, schizophrenia (Dinan *et al.* 2014; Shen *et al.* 2018). It is yet to be established whether these disease-associated changes to the gut microbiota are involved in the initiation of disease pathogenesis (altered microbiota state precipitating changes in brain development or function) or occur because of the disease (i.e. whether changes in gut microbiota arise following alterations in brain development or function). Alternatively, the microbiota changes could also occur as a result of dietary patterns or medication (Mayer *et al.* 2015). As both the CNS-related disease itself and the pharmacological treatment of the disease can alter the composition of the microbiota, it is thus essential to account for independent effects of the disease or drug treatment in both animal and human studies.

4.4 Microbial metabolism of psychoactive drugs:

Few studies have offered mechanistic insight into the microbiota-mediated metabolism of psychoactive drugs however those that have are summarised in Table 2. In most cases, the specific microbial species, or microbial-derived enzymes, responsible for the metabolism of psychoactive drugs by the gut microbiome are unknown.

There is evidence to suggest benzodiazepines are subject to microbiota-mediated drug metabolism. Older studies have demonstrated the microbiota-mediated nitro-reduction of the hypnotics, clonazepam and nitrazepam, to their corresponding 7-amino metabolite derivatives. Elmer *et al.* administered radiolabelled clonazepam and quantified the production of the nitro-reduced clonazepam metabolites in GF rats before and after colonisation with faecal microbiota. The production of these metabolites increased significantly from 15% in GF mice to 77% after microbial colonisation (Elmer and Remmel 1984). Similarly, the reduction of the hypnotic, nitrazepam, to 7-aminonitrazepam and 7-acetylaminonitrazepam was shown to be mediated by several anaerobic bacteria isolated from human gut isolates including species from the *Clostridium, Bacteroides* and *Eubacterium* genera. The authors identified the bacterium, *Clostridium leptum,* as having highly specific nitroreductase activity (Rafii *et al.* 1997). A previous study highlighted antibiotics can deplete the activity of this microbial enzyme (Takeno and Sakai 1991). The production of nitro-reduced nitrazepam metabolites in pregnant rats, following an oral dose of nitrazepam 300 mg/kg, was quantified before and after antibiotic treatment, which was given to diminish nitro-reductase enzymatic

activity. The levels of 7aminonitrazepam and 7acetylaminonitrazepam decreased from 30% pre-treatment to 2% after antibiotic treatment.

4.5 Impact of psychoactive drugs on the composition of the gut microbiota:

Antidepressants and antipsychotics are associated with distinct gut microbiota signatures. Gastric acid suppressants, antipsychotics, and antidepressants have repeatedly been confirmed as the three non-antibiotic drug classes most associated with the abundance of single taxa. Antipsychotics, for example, were associated with the abundance of *Prevotella*, an unclassified member of the Desulfovibrionaceae family and *Victivallis* genus in an observation-based study in an elderly (≥ 65 years) hospitalised cohort (Ticinesi *et al.* 2017). On the other hand, antidepressant-use in this cohort significantly correlated with the increased abundance of five specific bacterial taxa belonging to the *Helicobacter*, *Asteroleplasma*, *Marinilactibacillus* genera and unclassified members of both the Bacillus class and Succinivibrionaceae family. In an observation based study in community-dwelling patients (mean age 52 years), the SSRI antidepressant, citalopram, was associated with a significantly increased relative abundance of the Enterobacteriaceae family in comparison to patients not taking any medication (Rogers and Aronoff 2016). The specific use of the antipsychotics, olanzapine, and risperidone, causes a shift in the gut microbiota composition to a state previously shown to be associated with obesity in both rat and human (12.2 (SD 2.5) years) *in vivo* studies (Davey *et al.* 2012; Bahr *et al.* 2015a). Chronic olanzapine treatment of rats (2-4 mg/kg/day) and mice (50 mg/kg HFD) was associated with an increase in Firmicutes, a decrease in Bacteroidetes and an overall reduction in biodiversity (Davey *et al.* 2012; Morgan *et al.* 2014). Similarly, chronic risperidone treatment (approximately 80 µg risperidone/day for 58 days) resulted in a 22.4% decrease in Bacteroidetes and a reciprocal 32.6% increase in Firmicutes in the risperidone-treated mice relative to control mice (Bahr *et al.* 2015b).

The mechanisms underpinning these changes in bacterial abundance or diversity induced by psychoactive drugs are not fully understood, but both these drug classes have shown *in vitro* antimicrobial activity. There is preliminary evidence to suggest that olanzapine has antimicrobial activity. Morgan *et al.* found olanzapine inhibited the *in vitro* growth of two commensal strains, *E. coli* and *Enterococcus faecalis*. The dose investigated, however, was above the recommended dosage range of 5-20 mg/day and therefore whether clinically-relevant doses of olanzapine would still possess antimicrobial activity remains to be tested (Morgan *et al.* 2014). Similarly, the phenothiazine group of antipsychotics exerted antimicrobial effects at higher than clinically-relevant drug doses (Amaral *et al.* 2004). The phenothiazine antipsychotic, chlorpromazine was the first psychoactive drug associated with antibacterial properties. *S. aureus*, *Mycobacterium*, and some gram-negative rods such as *Shigella* spp. have been identified as the bacteria most susceptible to the antibacterial activity of phenothiazine antipsychotics (Amaral *et al.* 2004). Evidence suggests phenothiazine antipsychotics may mediate their effects on microbial growth through the alteration of bacterial morphology (phenothiazines cause filamentation of *E. coli*) or inhibition of bacterial adherence to epithelial cells (phenothiazines reduce *E. coli* adherence to urinary epithelium) (Amaral *et al.* 2004).

Similarly, antidepressant-induced changes in gut microbiota may be mediated by direct antimicrobial activity. SSRIs (including paroxetine, sertraline, and fluoxetine) are associated

with a broad-spectrum of antibacterial activity, including activity against strains of *Staphylococcus*, *Enterococcus*, *Clostridium*, *Pseudomonas* and *Citrobacter* (Munoz-Bellido *et al.* 2000). Sertraline can also influence microbial growth by acting synergistically with other antibiotics. Sertraline increased the efficacy of co-administered tetracycline and fluoroquinolone antibiotics against, a pathogenic strain associated with urinary tract infections, *Corynebacterium urealyticum* (Munoz-Bellido *et al.* 1996). The inhibition of efflux pumps in bacterial cells by sertraline is hypothesised as one causative mechanism. SSRIs may also interfere with the biosynthesis of the slime layer on bacteria (Munoz-Bellido *et al.* 2000); the disruption of the slime layer may also act to increase bacterial susceptibility to co-administered antibiotics. Apart from SSRIs, other antidepressants have antimicrobial effects, including monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs). The inhibition of cell wall synthesis and anti-plasmid activity have been proposed as mechanisms underlying the antimicrobial activity of the MAOI, iproniazid, and the TCA drug class respectively (Molnar 1988; Macedo *et al.* 2017).

Psychotropic drugs can also modulate the activity and pathogenicity of microbes which can precipitate changes in the gut microbiome. Sertraline can alter the in-vitro pathogenicity of fungi, for example, by significantly affecting the virulence properties associated with *Candida* including decreased hyphal elongation and reduced secretion of aspartyl proteinases, thereby preventing fungal adherence and tissue invasion of *Candida* spp. (Lass-Florl *et al.* 2003).

4.6 The Impact of psychoactive drug-microbiota interactions: Implications for drug response and toxicity

The growing evidence of the complex relationship between drugs and the gut microbiome, illustrated in Figure 1, underscores the importance of considering the gut microbiota as an additional factor contributing to the inter-individual variation observed in drug metabolism and response.

The interaction of psychoactive drugs with the gut microbiota has implications for drug absorption and bioavailability. As mentioned previously, levodopa is one of the most studied psychoactive drugs that interact with the gut microbiome. Research illustrating the metabolism of this drug by the gut microbiota-mediated dehydroxylation dates back to the 1970s (Goldin *et al.* 1973). Follow-on studies found decreased plasma levels of the drug with the presence of *H. pylori*, attributed to levodopa-mediated interaction with the bacterial surface adhesions (Niehues and Hensel 2009).

Antidepressants are associated with considerable inter-individual variation in drug response and a lack of efficacy; it's estimated that antidepressants are only 20-30% more effective than placebo (Arroll *et al.* 2005). Pharmacogenetics, although still in its relative infancy, has thus far proven unsuccessful in identifying and optimising factors that may hinder antidepressant efficacy. The pathophysiology of major depression disorder (MDD) has, however, been linked to alterations in the gut microbiota composition; MDD is associated with increased levels of Bacteroidetes and Enterobacteriaceae and decreased levels of Firmicutes. This altered microbiota state has been linked to increased gut permeability, a factor that can dictate intestinal drug transport and absorption (Jiang *et al.* 2015). Continued metabolomic and metagenomic analysis of the microbiome and further pharmacomicrobiomic based-studies may thus offer some additional insight.

The gut microbiota has also been implicated in the propagation of the side effects and toxicity of psychoactive drugs. With patient adherence to psychotropic therapy estimated to be only 30%, poor compliance has been linked to the unfavourable side-effect profile associated with these drugs (Weich *et al.* 2007). As previously highlighted, antibiotic treatment of rats attenuated both the microbial metabolism and the teratogenicity-associated adverse effect of nitrazepam and its metabolites (Elmer and Remmel 1984). More recently, antipsychotic-induced metabolic dysfunction has been linked to shifts in the composition of the gut microbiota. The role of the gut microbiome in the development of olanzapine-induced weight gain was elucidated when olanzapine-treated GF mice did not gain weight but did so upon colonisation with caecal microbiota (Morgan *et al.* 2014). Furthermore, an antibiotic cocktail co-administrated with olanzapine, to chemically deplete the gut microbiota, attenuated the metabolic side effects associated with the drug treatment alone in rats; increased weight gain, increased uterine fat deposition and increased plasma free fatty acid levels were side-effects attenuated in microbiota-depleted rats (Davey *et al.* 2013).

A recent follow-on study by Kao *et al.* investigated whether treatment with a prebiotic, BimunoTM galacto-oligosaccharides (B-GOS), modified the olanzapine-induced weight gain (Kao *et al.* 2018). Female rats were treated with B-GOS (0.5 g/kg/day) for 21 days, in conjunction with administration of olanzapine (10 mg/kg once daily via i.p. injection) or saline on days 8-21. B-GOS treatment significantly attenuated the drug-induced weight gain without altering the antagonism of central serotoninergic receptors necessary for drug efficacy. B-GOS treatment alone altered the microbiota composition; differences in the abundance of Bifidobacterium spp. and Firmicutes spp. were evident in B-GOS only treated rats in comparison to both water-only and B-GOS-olanzapine-treated rats. Contrary to the previous findings by Davey *et al.* and Morgan *et al.* discussed above, administration of olanzapine, however, did not significantly alter the composition of the faecal microbiota, albeit different dosage regimens were employed in the respective studies (Davey et al. 2013; Morgan et al. 2014). Furthermore, the authors deduced, contrary to their hypothesis, that elevated levels of the SCFA, acetate, may mediate the olanzapine-induced weight gain. Considering the risks associated with antibiotic resistance, prebiotic treatment may constitute a more suitable adjunctive therapy in comparison to long-term antibiotic use to improve patient tolerance to olanzapine.

Both olanzapine-induced and risperidone-induced weight gain correlated with an altered gut microbiota composition (Davey *et al.* 2012; Bahr *et al.* 2015b). Suppressed energy expenditure, induced by the gut microbiota, was found to cause the observed weight gain in risperidone-treated mice. Furthermore, faecal microbiota transplantation (FMT) from risperidone-treated mice to treatment-naive mice induced weight gain and suppressed energy expenditure in the FMT-recipient mice (Bahr *et al.* 2015b). On the premise that some antidepressants (e.g. SSRIs and TCAs) are associated with similar effects to these antipsychotics, i.e. changes in the gut microbiota and drug-induced weight gain, it may be timely to extend these studies to antidepressants.

Conclusion/Future Directions

Research has identified many clinically relevant drugs, including psychotropics, which are metabolised by the microbiome, often via as yet unknown mechanisms. There remains a need to establish the microorganism responsible for metabolism and identify the molecular

mechanisms involved. While medication-use can precipitate changes in the abundance and diversity of a wide array of different bacterial phyla, the resultant impact of these compositional changes on drug response and patient outcomes requires further study. Matching specific microbial genes or enzymes, which may dictate drug response, to specific bacterial phyla could lead to greater understanding of the consequential impact of disturbances in the microbiome on patient outcomes and provide additional impetus to explore the contribution of these important, but underappreciated, drug-microbiome interactions to the inter-individual variability observed in drug response.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

Reference List

Alexander S.PH, Fabbro D, Kelly E, Marrion N.V, Peters J.A, Faccenda E*, et al.* (2017). THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Enzymes. British Journal of Pharmacology 174**:** S272-S359.

Al-Salami, H., Butt, G., Fawcett, J.P., Tucker, I.G., Golocorbin-Kon, S. and Mikov, M. (2008) 'Probiotic treatment reduces blood glucose levels and increases systemic absorption of gliclazide in diabetic rats', *Eur J Drug Metab Pharmacokinet*, 33(2), 101-6.

Alavijeh, M.S., Chishty, M., Qaiser, M.Z. and Palmer, A.M. (2005) 'Drug Metabolism and Pharmacokinetics, the Blood-Brain Barrier, and Central Nervous System Drug Discovery', *NeuroRx*, 2(4), 554-571.

Amaral, L., Viveiros, M. and Molnar, J. (2004) 'Antimicrobial activity of phenothiazines', *In Vivo*, 18(6), 725-31.

Antunes, L.C.M., Han, J., Ferreira, R.B.R., Lolić, P., Borchers, C.H. and Finlay, B.B. (2011) 'Effect of Antibiotic Treatment on the Intestinal Metabolome', *Antimicrobial agents and chemotherapy*, available: http://dx.doi.org/10.1128/AAC.01664-10.

Arroll B, Macgillivray S, Ogston S, Reid I, Sullivan F, Williams B*, et al.* (2005) 'Efficacy and Tolerability of Tricyclic Antidepressants and SSRIs Compared With Placebo for Treatment of Depression in Primary Care: A Meta-Analysis', *Annals of Family Medicine*, 3(5), 449-456, available: http://dx.doi.org/10.1370/afm.349.

Bahr SM, Tyler BC, Wooldridge N, Butcher BD, Burns TL, Teesch LM*, et al.* (2015a) 'Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children', *Translational Psychiatry*, 5, e652, available: http://dx.doi.org/10.1038/tp.2015.135

Bahr SM, Weidemann BJ, Castro AN, Walsh JW, deLeon O, Burnett CML*, et al.* (2015b) 'Risperidone-induced weight gain is mediated through shifts in the gut microbiome and suppression of energy expenditure', *EBioMedicine*, 2(11), 1725-1734, available: http://dx.doi.org/10.1016/j.ebiom.2015.10.018.

Björkholm, B., Bok, C.M., Lundin, A., Rafter, J., Hibberd, M.L. and Pettersson, S. (2009) 'Intestinal Microbiota Regulate Xenobiotic Metabolism in the Liver', *PLoS ONE*, 4(9), e6958, available: http://dx.doi.org/10.1371/journal.pone.0006958.

Bodet, C.A., Jorgensen, J.H. and Drutz, D.J. (1985) 'Antibacterial activities of antineoplastic agents', *Antimicrobial Agents and Chemotherapy*, 28(3), 437-439.

Caldwell, J. and Hawksworth, G.M. (1973) 'The demethylation of methamphetamine by intestinal microflora', *Journal of Pharmacy and Pharmacology*, 25(5), 422-424, available: http://dx.doi.org/10.1111/j.2042-7158.1973.tb10043.x.

Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM*, et al.* (2008) 'Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice', *Diabetes*, 57(6), 1470-81, available: http://dx.doi.org/10.2337/db07-1403.

Carvalho BM, Guadagnini D, Tsukumo DML, Schenka AA, Latuf-Filho P, Vassallo J*, et al.* (2012) 'Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice', *Diabetologia*, 55(10), 2823-2834, available: http://dx.doi.org/10.1007/s00125-012-2648-4.

Cenit, M.C., Sanz, Y. and Codoñer-Franch, P. (2017) 'Influence of gut microbiota on neuropsychiatric disorders', *World Journal of Gastroenterology*, 23(30), 5486-5498, available: http://dx.doi.org/10.3748/wjg.v23.i30.5486.

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S*, et al.* (2012) 'Gut microbiota composition correlates with diet and health in the elderly', *Nature*, 488(7410), 178-184, available: http://dx.doi.org/http://www.nature.com/nature/journal/v488/n7410/abs/nature11319.html#supplementary-information.

Claus SP, Ellero SL, Berger B, Krause L, Bruttin A, Molina J*, et al.* (2011) 'Colonization-Induced Host-Gut Microbial Metabolic Interaction', *Mbio*, 2(2), available: http://dx.doi.org/10.1128/mBio.00271-.

Clayton, T.A., Baker, D., Lindon, J.C., Everett, J.R. and Nicholson, J.K. (2009) 'Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism', *Proc Natl Acad Sci U S A*, 106(34), 14728-33, available: http://dx.doi.org/10.1073/pnas.0904489106.

Dagli, R.J. and Sharma, A. (2014) 'Polypharmacy: A Global Risk Factor for Elderly People', *Journal of International Oral Health : JIOH*, 6(6), i-ii.

Davey KJ, Cotter PD, O'Sullivan O, Crispie F, Dinan TG, Cryan JF*, et al.* (2013) 'Antipsychotics and the gut microbiome: olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat', *Transl Psychiatry*, 3, e309, available: http://dx.doi.org/10.1038/tp.2013.83.

Davey KJ, O'Mahony SM, Schellekens H, O'Sullivan O, Bienenstock J, Cotter PD*, et al.*(2012) 'Gender-dependent consequences of chronic olanzapine in the rat: effects on body weight, inflammatory, metabolic and microbiota parameters', *Psychopharmacology*, 221(1), 155-169, available: http://dx.doi.org/10.1007/s00213-011-2555-2.

Debotton, N. and Dahan, A. (2017) 'Applications of Polymers as Pharmaceutical Excipients in Solid Oral Dosage Forms', *Medicinal Research Reviews*, 37(1), 52-97, available: http://dx.doi.org/10.1002/med.21403.

Deloménie C, Fouix S, Longuemaux S, Brahimi N, Bizet C, Picard B*, et al.* (2001) 'Identification and Functional Characterization of Arylamine N-Acetyltransferases in Eubacteria: Evidence for Highly Selective Acetylation of 5-Aminosalicylic Acid', *Journal of Bacteriology*, 183(11), 3417-3427, available: http://dx.doi.org/10.1128/JB.183.11.3417-3427.2001.

den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.-J. and Bakker, B.M. (2013) 'The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism', *Journal of Lipid Research*, 54(9), 2325-2340, available: http://dx.doi.org/10.1194/jlr.R036012.

Dinan, T.G., Borre, Y.E. and Cryan, J.F. (2014) 'Genomics of schizophrenia: time to consider the gut microbiome?', *Molecular Psychiatry*, 19, 1252, available: http://dx.doi.org/10.1038/mp.2014.93.

D'souza, A.A. and Shegokar, R. (2016) 'Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications', *Expert Opinion on Drug Delivery*, 13(9), 1257-1275, available: http://dx.doi.org/10.1080/17425247.2016.1182485.

Elmer, G.W. and Remmel, R.P. (1984) 'Role of the intestinal microflora in clonazepam metabolism in the rat', *Xenobiotica*, 14(11), 829-40, available: http://dx.doi.org/10.3109/00498258409151481.

Enright, E.F., Joyce, S.A., Gahan, C.G.M. and Griffin, B.T. (2017) 'Impact of Gut Microbiota-Mediated Bile Acid Metabolism on the Solubilization Capacity of Bile Salt Micelles and Drug Solubility', *Molecular Pharmaceutics*, 14(4), 1251-1263, available: http://dx.doi.org/10.1021/acs.molpharmaceut.6b01155.

Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K*, et al.* (2016) 'Population-level analysis of gut microbiome variation', *Science*, 352(6285), 560.

Finegold, S.M. (2011) 'Desulfovibrio species are potentially important in regressive autism', *Medical Hypotheses*, 77(2), 270-274, available: http://dx.doi.org/10.1016/j.mehy.2011.04.032.

Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S*, et al.* (2015) 'Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota', *Nature*, 528(7581), 262-6, available: http://dx.doi.org/10.1038/nature15766.

Freedberg DE, Toussaint NC, Chen SP, Ratner AJ, Whittier S, Wang TC*, et al.* (2015) 'Proton Pump Inhibitors Alter Specific Taxa in the Human Gastrointestinal Microbiome: A Crossover Trial', *Gastroenterology*, 149(4), 883-885.e9, available: http://dx.doi.org/10.1053/j.gastro.2015.06.043.

Gloux, K., Berteau, O., El oumami, H., Béguet, F., Leclerc, M. and Doré, J. (2011) 'A metagenomic β-glucuronidase uncovers a core adaptive function of the human intestinal microbiome', *Proceedings of the National Academy of Sciences of the United States of America*, 108(Suppl 1), 4539-4546, available: http://dx.doi.org/10.1073/pnas.1000066107.

Goldin, B.R., Peppercorn, M.A. and Goldman, P. (1973) 'Contributions of host and intestinal microflora in the metabolism of L-dopa by the rat', *J Pharmacol Exp Ther*, 186(1), 160-6.

Gotelli, N.J. and Colwell, R.K. (2001) 'Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness', *Ecology Letters*, 4(4), 379-391, available: http://dx.doi.org/10.1046/j.1461-0248.2001.00230.x.

Grover, M. and Camilleri, M. (2013) 'Effects on gastrointestinal functions and symptoms of serotonergic psychoactive agents used in functional gastrointestinal diseases', *Journal of Gastroenterology*, 48(2), 177-181, available: http://dx.doi.org/10.1007/s00535-012-0726-5.

Haiser, H.J., Gootenberg, D.B., Chatman, K., Sirasani, G., Balskus, E.P. and Turnbaugh, P.J. (2013) 'Predicting and manipulating cardiac drug inactivation by the human gut bacterium Eggerthella lenta()', *Science (New York, N.Y.)*, 341(6143), 295-298, available: http://dx.doi.org/10.1126/science.1235872.

Haiser, H.J. and Turnbaugh, P.J. (2013) 'Developing a metagenomic view of xenobiotic metabolism', *Pharmacological research : the official journal of the Italian Pharmacological Society*, 69(1), 21-31, available: http://dx.doi.org/10.1016/j.phrs.2012.07.009.

Hamlet, A., Thoreson, A.C., Nilsson, O., Svennerholm, A.M. and Olbe, L. (1999) 'Duodenal Helicobacter pylori infection differs in cagA genotype between asymptomatic subjects and patients with duodenal ulcers', *Gastroenterology*, 116(2), 259-268, available: http://dx.doi.org/10.1016/S0016-5085(99)70121-6.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 46: D1091-D1106.

Hashim H, Azmin S, Razlan H, Yahya NW, Tan HJ, Manaf MRA*, et al.* (2014) 'Eradication of Helicobacter pylori Infection Improves Levodopa Action, Clinical Symptoms and Quality of Life in Patients with Parkinson's Disease', *PLoS ONE*, 9(11), e112330, available: http://dx.doi.org/10.1371/journal.pone.0112330.

Imai, T. and Ohura, K. (2010) 'The role of intestinal carboxylesterase in the oral absorption of prodrugs', *Curr Drug Metab*, 11(9), 793-805.

Imhann F, Vich Vila A, Bonder MJ, Lopez Manosalva AG, Koonen DPY, Fu J*, et al.* (2017) 'The influence of proton pump inhibitors and other commonly used medication on the gut microbiota', *Gut Microbes*, 8(4), 351-358, available: http://dx.doi.org/10.1080/19490976.2017.1284732.

Inta, D., Lang, U.E., Borgwardt, S., Meyer-Lindenberg, A. and Gass, P. (2017) 'Microglia Activation and Schizophrenia: Lessons From the Effects of Minocycline on Postnatal Neurogenesis, Neuronal Survival and Synaptic Pruning', *Schizophrenia Bulletin*, 43(3), 493-496, available: http://dx.doi.org/10.1093/schbul/sbw088.

Jakobsson, H.E., Jernberg, C., Andersson, A.F., Sjölund-Karlsson, M., Jansson, J.K. and Engstrand, L. (2010) 'Short-Term Antibiotic Treatment Has Differing Long-Term Impacts on the Human Throat and Gut Microbiome', *PLOS ONE*, 5(3), e9836, available: http://dx.doi.org/10.1371/journal.pone.0009836.

Jandhyala, S.M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M. and Reddy, D.N. (2015) 'Role of the normal gut microbiota', *World Journal of Gastroenterology : WJG*, 21(29), 8787-8803, available: http://dx.doi.org/10.3748/wjg.v21.i29.8787.

Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y*, et al.* (2015) 'Altered fecal microbiota composition in patients with major depressive disorder', *Brain, Behavior, and Immunity*, 48(Supplement C), 186-194, available: http://dx.doi.org/https://doi.org/10.1016/j.bbi.2015.03.016.

Kao, A.C.-C., Spitzer, S., Anthony, D.C., Lennox, B. and Burnet, P.W.J. (2018) 'Prebiotic attenuation of olanzapine-induced weight gain in rats: analysis of central and peripheral biomarkers and gut microbiota', Translational Psychiatry, 8(1), 66, available: **http://dx.doi.org/10.1038/s41398-018-0116-8.**

Kashyap PC, Marcobal A, Ursell LK, Larauche M, Duboc H, Earle KA*, et al.* (2013) 'Complex Interactions Among Diet, Gastrointestinal Transit, and Gut Microbiota in Humanized Mice', *Gastroenterology*, 144(5), 967-977, available: http://dx.doi.org/10.1053/j.gastro.2013.01.047.

Kelly JR, Borre Y, C OB, Patterson E, El Aidy S, Deane J*, et al.* (2016) 'Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat', *J Psychiatr Res*, 82, 109-18, available: http://dx.doi.org/10.1016/j.jpsychires.2016.07.019.

Kelly, J.R., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G. and Hyland, N.P. (2015) 'Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders', *Frontiers in Cellular Neuroscience*, 9, 392, available: http://dx.doi.org/10.3389/fncel.2015.00392.

Kim IS, Yoo DH, Jung IH, Lim S, Jeong JJ, Kim KA*, et al.* (2016) 'Reduced metabolic activity of gut microbiota by antibiotics can potentiate the antithrombotic effect of aspirin', *Biochem Pharmacol*, 122, 72-79, available: http://dx.doi.org/10.1016/j.bcp.2016.09.023.

Kim J-K, Choi MS, Jeong J-J, Lim S-M, Kim IS, Yoo HH*, et al.* (2018) 'Effect of Probiotics on Pharmacokinetics of Orally Administered Acetaminophen in Mice', *Drug Metabolism and Disposition*, 46(2), 122.

Klaassen, C.D. and Cui, J.Y. (2015) 'Review: Mechanisms of How the Intestinal Microbiota Alters the Effects of Drugs and Bile Acids', *Drug Metabolism and Disposition*, 43(10), 1505-1521, available: http://dx.doi.org/10.1124/dmd.115.065698.

Koch, R.L., Chrystal, E.J.T., Beaulieu, B.B. and Goldman, P. (1979) 'Acetamide—a metabolite of metronidazole formed by the intestinal flora', *Biochemical Pharmacology*, 28(24), 3611-3615, available: http://dx.doi.org/https://doi.org/10.1016/0006-2952(79)90407-6.

Koppel, N., Maini Rekdal, V. and Balskus, E.P. (2017) 'Chemical transformation of xenobiotics by the human gut microbiota', *Science*, 356(6344).

Kosaraju, S.L. (2005) 'Colon Targeted Delivery Systems: Review of Polysaccharides for Encapsulation and Delivery', *Critical Reviews in Food Science and Nutrition*, 45(4), 251-258, available: http://dx.doi.org/10.1080/10408690490478091.

Krajmalnik-Brown, R., Ilhan, Z.-E., Kang, D.-W. and DiBaise, J.K. (2012) 'Effects of Gut Microbes on Nutrient Absorption and Energy Regulation', *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition*, 27(2), 201-214, available: http://dx.doi.org/10.1177/0884533611436116.

Kruszewska, H., Zareba, T. and Tyski, S. (2000) 'Antimicrobial activity of selected non-antibiotics--activity of methotrexate against Staphylococcus aureus strains', *Acta poloniae pharmaceutica*, 57 Suppl, 117-119.

Kruszewska, H., Zareba, T. and Tyski, S. (2002) 'Search of antimicrobial activity of selected non-antibiotic drugs', *Acta Pol Pharm*, 59(6), 436-9.

Laizure, S.C., Herring, V., Hu, Z., Witbrodt, K. and Parker, R.B. (2013) 'The role of human carboxylesterases in drug metabolism: have we overlooked their importance?', *Pharmacotherapy*, 33(2), 210-222, available: http://dx.doi.org/10.1002/phar.1194.

Lass-Florl C, Ledochowski M, Fuchs D, Speth C, Kacani L, Dierich MP*, et al.* (2003) 'Interaction of sertraline with Candida species selectively attenuates fungal virulence in vitro', *FEMS Immunol Med Microbiol*, 35(1), 11-5.

Lee, H.J., Zhang, H., Orlovich, D.A. and Fawcett, J.P. (2012) 'The influence of probiotic treatment on sulfasalazine metabolism in rat', *Xenobiotica*, 42(8), 791-797, available: http://dx.doi.org/10.3109/00498254.2012.660508.

Lee, H.S., Kim, D.-K., Kim, Y.B. and Lee, K.J. (2013) 'Effect of Acute Stress on Immune Cell Counts and the Expression of Tight Junction Proteins in the Duodenal Mucosa of Rats', *Gut and Liver*, 7(2), 190-196, available: http://dx.doi.org/10.5009/gnl.2013.7.2.190.

Levin BJ, Huang YY, Peck SC, Wei Y, Campo AM-d, Marks JA*, et al.* (2017) 'A prominent glycyl radical enzyme in human gut microbiomes metabolizes trans-4-hydroxy-l-proline', *Science*, 355(6325), available: http://dx.doi.org/10.1126/science.aai8386.

Macedo D, Filho AJMC, Soares de Sousa CN, Quevedo J, Barichello T, Júnior HVN*, et al.* (2017) 'Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness', *Journal of Affective Disorders*, 208(Supplement C), 22-32, available: http://dx.doi.org/https://doi.org/10.1016/j.jad.2016.09.012.

Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E.E., *et al.* (2018) 'Extensive impact of non-antibiotic drugs on human gut bacteria', Nature, 555, 623, available: http://dx.doi.org/10.1038/nature25979

Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold G*, et al.* (2015) 'The gut microbiota and host health: a new clinical frontier', *Gut*.

Mariat D, Firmesse O, Levenez F, Guimarăes VD, Sokol H, Doré J*, et al.* (2009) 'The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age', *BMC Microbiology*, 9(1), 123, available: http://dx.doi.org/10.1186/1471-2180-9-123.

Martins M, Dastidar SG, Fanning S, Kristiansen JE, Molnar J, Pagès J-M*, et al.* (2008) 'Potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant Gram-negative infections: mechanisms for their direct and indirect activities', *International Journal of Antimicrobial Agents*, 31(3), 198-208, available: http://dx.doi.org/10.1016/j.ijantimicag.2007.10.025.

Matuskova, Z., Anzenbacherova, E., Vecera, R., Tlaskalova-Hogenova, H., Kolar, M. and Anzenbacher, P. (2014) 'Administration of a Probiotic Can Change Drug Pharmacokinetics: Effect of E. coli Nissle 1917 on Amidarone Absorption in Rats', *PLoS ONE*, 9(2), e87150, available: http://dx.doi.org/10.1371/journal.pone.0087150.

Maurice, C.F., Haiser, H.J. and Turnbaugh, P.J. (2013) 'Xenobiotics shape the physiology and gene expression of the active human gut microbiome', *Cell*, 152(1-2), 39-50, available: http://dx.doi.org/10.1016/j.cell.2012.10.052.

Mayer, E.A., Tillisch, K. and Gupta, A. (2015) 'Gut/brain axis and the microbiota', *The Journal of Clinical Investigation*, 125(3), 926-938, available: http://dx.doi.org/10.1172/JCI76304.

Meuldermans W, Hendrickx J, Mannens G, Lavrijsen K, Janssen C, Bracke J*, et al.* (1994) 'The metabolism and excretion of risperidone after oral administration in rats and dogs', *Drug Metabolism and Disposition*, 22(1), 129.

Mikov, M. (1994) 'The metabolism of drugs by the gut flora', *Eur J Drug Metab Pharmacokinet*, 19(3), 201-7.

Mitchell, J.R., Jollow, D.J., Potter, W.Z., Davis, D.C., Gillette, J.R. and Brodie, B.B. (1973) 'Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism', *J Pharmacol Exp Ther*, 187(1), 185-94.

Miyaji, Azuma, Ito, Abe, Ono, Suto*, et al.* (1999) 'The effect of Helicobacter pylori eradication therapy on gastric antral myoelectrical activity and gastric emptying in patients with non-ulcer dyspepsia', *Alimentary Pharmacology & Therapeutics*, 13(11), 1473-1480, available: http://dx.doi.org/10.1046/j.1365-2036.1999.00634.x.

Molnar, J. (1988) 'Antiplasmid activity of tricyclic compounds', *Methods Find Exp Clin Pharmacol*, 10(7), 467-74.

Morgan AP, Crowley JJ, Nonneman RJ, Quackenbush CR, Miller CN, Ryan AK*, et al.* (2014) 'The Antipsychotic Olanzapine Interacts with the Gut Microbiome to Cause Weight Gain in Mouse', *PLoS ONE*, 9(12), e115225, available: http://dx.doi.org/10.1371/journal.pone.0115225.

Munoz-Bellido, J.L., Munoz-Criado, S. and Garcia-Rodriguez, J.A. (1996) 'In-vitro activity of psychiatric drugs against Corynebacterium urealyticum (Corynebacterium group D2)', *J Antimicrob Chemother*, 37(5), 1005-9.

Munoz-Bellido, J.L., Munoz-Criado, S. and Garcia-Rodriguez, J.A. (2000) 'Antimicrobial activity of psychotropic drugs: selective serotonin reuptake inhibitors', *Int J Antimicrob Agents*, 14(3), 177-80.

Niehues, M. and Hensel, A. (2009) 'In-vitro interaction of L-dopa with bacterial adhesins of Helicobacter pylori: an explanation for clinicial differences in bioavailability?', *J Pharm Pharmacol*, 61(10), 1303-7, available: http://dx.doi.org/10.1211/jpp/61.10.0005.

O'Hara, A.M. and Shanahan, F. (2006) 'The gut flora as a forgotten organ', *EMBO Rep*, 7(7), 688-93, available: http://dx.doi.org/10.1038/sj.embor.7400731.

Onder G, Liperoti R, Fialova D, Topinkova E, Tosato M, Danese P*, et al.* (2012) 'Polypharmacy in Nursing Home in Europe: Results From the SHELTER Study', *The Journals of Gerontology: Series A*, 67A(6), 698-704, available: http://dx.doi.org/10.1093/gerona/glr233.

Penders, J., Thijs, C., Vink, C., Stelma, F.F., Snijders, B., Kummeling, I., van den Brandt, P.A. and Stobberingh, E.E. (2006) 'Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy', *Pediatrics*, 118(2), 511.

Peppercorn, M.A. and Goldman, P. (1972) 'THE ROLE OF INTESTINAL BACTERIA IN THE METABOLISM OF SALICYLAZOSULFAPYRIDINE', *Journal of Pharmacology and Experimental Therapeutics*, 181(3), 555.

Prudhviraj G, Vaidya Y, Singh SK, Yadav AK, Kaur P, Gulati M*, et al.* (2015) 'Effect of co-administration of probiotics with polysaccharide based colon targeted delivery systems to optimize site specific drug release', *European Journal of Pharmaceutics and Biopharmaceutics*, 97, 164-172, available: http://dx.doi.org/https://doi.org/10.1016/j.ejpb.2015.09.012.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C*, et al.* (2010) 'A human gut microbial gene catalogue established by metagenomic sequencing', *Nature*, 464(7285), 59-65, available: http://dx.doi.org/10.1038/nature08821.

Quigley, E.M.M. (2017) 'Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet?', *Nature Reviews Gastroenterology &Amp; Hepatology*, 14, 315, available: http://dx.doi.org/10.1038/nrgastro.2017.29.

Rafii, F., Sutherland, J.B., Hansen, J.E.B. and Cerniglia, C.E. (1997) 'Reduction of Nitrazepam by Clostridium leptum, a Nitroreductase-Producing Bacterium Isolated from the Human Intestinal Tract', *Clinical Infectious Diseases*, 25(Supplement_2), S121-S122, available: http://dx.doi.org/10.1086/516204.

Rizkallah, M.R., Saad, R. and Aziz, R.K. (2010) 'The Human Microbiome Project, Personalized Medicine and the Birth of Pharmacomicrobiomics', *Current PPharmacogenomics and Personalized Medicine*, 8(3), 182-193, available: http://dx.doi.org/info:doi/10.2174/187569210792246326.

Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N*, et al.* (2015) 'The composition of the gut microbiota throughout life, with an emphasis on early life', *Microbial Ecology in Health and Disease*, 26, 10.3402/mehd.v26.26050, available: http://dx.doi.org/10.3402/mehd.v26.26050.

Rogers, M.A.M. and Aronoff, D.M. (2016) 'The influence of non-steroidal anti-inflammatory drugs on the gut microbiome', *Clinical Microbiology and Infection*, 22(2), 178.e1-178.e9, available: http://dx.doi.org/https://doi.org/10.1016/j.cmi.2015.10.003.

Ryan, A. (2017) 'Azoreductases in drug metabolism', *Br J Pharmacol*, 174(14), 2161-2173, available: http://dx.doi.org/10.1111/bph.13571.

Saitta, K.S., Zhang, C., Lee, K.K., Fujimoto, K., Redinbo, M.R. and Boelsterli, U.A. (2014) 'Bacterial beta-glucuronidase inhibition protects mice against enteropathy induced by indomethacin, ketoprofen or diclofenac: mode of action and pharmacokinetics', *Xenobiotica*, 44(1), 28-35, available: http://dx.doi.org/10.3109/00498254.2013.811314.

Salazar N, Arboleya S, Valdés L, Stanton C, Ross P, Ruiz L*, et al.* (2014) 'The human intestinal microbiome at extreme ages of life. Dietary intervention as a way to counteract alterations', *Frontiers in Genetics*, 5, 406, available: http://dx.doi.org/10.3389/fgene.2014.00406.

Sandberg-Gertzen, H., Kjellander, J., Sundberg-Gilla, B. and Jarnerot, G. (1985) 'In vitro effects of sulphasalazine, azodisal sodium, and their metabolites on Clostridium difficile and some other faecal bacteria', *Scand J Gastroenterol*, 20(5), 607-12.

Sandhu, K.V., Sherwin, E., Schellekens, H., Stanton, C., Dinan, T.G. and Cryan, J.F. (2016) 'Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry', *Translational Research*, 179, 223-244, available: http://dx.doi.org/10.1016/j.trsl.2016.10.002.

Selwyn, F.P., Cui, J.Y. and Klaassen, C.D. (2015) 'RNA-Seq Quantification of Hepatic Drug Processing Genes in Germ-Free Mice', *Drug Metabolism and Disposition*, 43(10), 1572-1580, available: http://dx.doi.org/10.1124/dmd.115.063545.

Shanahan, F., van Sinderen, D., O'Toole, P.W. and Stanton, C. (2017) 'Feeding the microbiota: transducer of nutrient signals for the host', *Gut*, 66(9), 1709.

Shen Y, Xu J, Li Z, Huang Y, Yuan Y, Wang J*, et al.* (2018) 'Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: A cross-sectional study', *Schizophrenia Research*, available: http://dx.doi.org/https://doi.org/10.1016/j.schres.2018.01.002.

Söderholm, J.D., Yates, D.A., Gareau, M.G., Yang, P.-C., MacQueen, G. and Perdue, M.H. (2002) 'Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress', *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 283(6), G1257-G1263, available: http://dx.doi.org/10.1152/ajpgi.00314.2002.

Takasuna K, Hagiwara T, Hirohashi M, Kato M, Nomura M, Nagai E*, et al.*(1998) *Inhibition of intestinal microflora β-glucuronidase modifies the distribution of the active metabolite of the antitumor agent, irinotecan hydrochloride (CPT-11) in rats*.

Takeno, S., Nakagawa, M. and Sakai, T. (1990) 'Teratogenic effects of nitrazepam in rats', *Res Commun Chem Pathol Pharmacol*, 69(1), 59-70.

Takeno, S. and Sakai, T. (1991) 'Involvement of the intestinal microflora in nitrazepam-induced teratogenicity in rats and its relationship to nitroreduction', *Teratology*, 44(2), 209-214, available: http://dx.doi.org/10.1002/tera.1420440209.

Theriot, C.M., Bowman, A.A., Young, V.B. and Ellermeier, C.D. (2016) 'Antibiotic-Induced Alterations of the Gut Microbiota Alter Secondary Bile Acid Production and Allow for Clostridium difficile Spore Germination and Outgrowth in the Large Intestine', *mSPHERE*, available: http://dx.doi.org/10.1128/mSphere.00045-15.

Thursby, E. and Juge, N. (2017) 'Introduction to the human gut microbiota', *Biochemical Journal*, 474(11), 1823-1836, available: http://dx.doi.org/10.1042/BCJ20160510.

Ticinesi A, Milani C, Lauretani F, Nouvenne A, Mancabelli L, Lugli GA*, et al.* (2017) 'Gut microbiota composition is associated with polypharmacy in elderly hospitalized patients', *Scientific Reports*, 7(1), 11102, available: http://dx.doi.org/10.1038/s41598-017-10734-y.

Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K*, et al.* (2015) 'Gastrointestinal microbiota in children with autism in Slovakia', *Physiol Behav*, 138, 179-87, available: http://dx.doi.org/10.1016/j.physbeh.2014.10.033.

Turnheim, K. (2003) 'When drug therapy gets old: pharmacokinetics and pharmacodynamics in the elderly', *Exp Gerontol*, 38(8), 843-53.

Ulmer JE, Vilén EM, Namburi RB, Benjdia A, Beneteau J, Malleron A*, et al.* (2014) 'Characterization of Glycosaminoglycan (GAG) Sulfatases from the Human Gut Symbiont Bacteroides thetaiotaomicron Reveals the First GAG-specific Bacterial Endosulfatase', *The Journal of Biological Chemistry*, 289(35), 24289-24303, available: http://dx.doi.org/10.1074/jbc.M114.573303.

van Hogezand, R.A., Kennis, H.M., van Schaik, A., Koopman, J.P., van Hees, P.A. and van Tongeren, J.H. (1992) 'Bacterial acetylation of 5-aminosalicylic acid in faecal suspensions cultured under aerobic and anaerobic conditions', *Eur J Clin Pharmacol*, 43(2), 189-92.

Walker, A.W., Duncan, S.H., McWilliam Leitch, E.C., Child, M.W. and Flint, H.J. (2005) 'pH and Peptide Supply Can Radically Alter Bacterial Populations and Short-Chain Fatty Acid Ratios within Microbial Communities from the Human Colon', *Applied and Environmental Microbiology*, 71(7), 3692-3700, available: http://dx.doi.org/10.1128/AEM.71.7.3692-3700.2005.

Wang Y, Tong Q, Shou J-W, Zhao Z-X, Li X-Y, Zhang X-F*, et al.* (2017) 'Gut Microbiota-Mediated Personalized Treatment of Hyperlipidemia Using Berberine', *Theranostics*, 7(9), 2443-2451, available: http://dx.doi.org/10.7150/thno.18290.

Weich, S., Nazareth, I., Morgan, L. and King, M. (2007) 'Treatment of depression in primary care', *The British Journal of Psychiatry*, 191(2), 164.

Wilson, I.D. and Nicholson, J.K. (2017) 'Gut microbiome interactions with drug metabolism, efficacy, and toxicity', *Translational Research*, 179(Supplement C), 204-222, available: http://dx.doi.org/https://doi.org/10.1016/j.trsl.2016.08.002.

Wong ML, Inserra A, Lewis MD, Mastronardi CA, Leong L, Choo J*, et al.* (2016) 'Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition', *Molecular Psychiatry*, 21, 797, available: http://dx.doi.org/10.1038/mp.2016.46

Yamamoto M, Kurita A, Asahara T, Takakura A, Katono K, Iwasaki M*, et al.* (2008) 'Metabolism of irinotecan and its active metabolite SN-38 by intestinal microflora in rats', *Oncol Rep*, 20(4), 727-30.

Yoo, D.H., Kim, I.S., Van Le, T.K., Jung, I.H., Yoo, H.H. and Kim, D.H. (2014) 'Gut microbiota-mediated drug interactions between lovastatin and antibiotics', *Drug Metab Dispos*, 42(9), 1508-13, available: http://dx.doi.org/10.1124/dmd.114.058354.

Yoo, H.H., Kim, I.S., Yoo, D.H. and Kim, D.H. (2016) 'Effects of orally administered antibiotics on the bioavailability of amlodipine: gut microbiota-mediated drug interaction', *J Hypertens*, 34(1), 156-62, available: http://dx.doi.org/10.1097/hjh.0000000000000773.

Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T*, et al.* (2016) 'Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity', *Science*, 352(6285), 565.

Table 1: The Metabolism of Drugs by Bacterial Drug Metabolising Enzymes

Microbiota-Derived Enzyme:	**Hypothesised reaction mechanisms:**	**Drug (or Metabolite) Substrate:**	**Ref:**
β-glucuronidase	Remove glucuronic acid moiety from hepatic phase 2 metabolites	irinotecan (SN-38 glucuronide) NSAIDS e.g. indomethacin and diclofenac	(Yamamoto *et al.* 2008; Saitta *et al.* 2014)
Azoreductase	Reduction of azo or quinone bonds	Azo-containing drugs e.g. Olsalazine (5ASA prodrug) Nitrofuran antibiotics e.g., nitrofurazone and nitrofurantoin Ester containing prodrugs	(Ryan 2017)
Carboxylesterase	Hydrolyse ester, thioester, amide, or carbamate containing drugs to respective free acids Hydrolyse esters to carboxylic acids	Aspirin, Ester containing prodrugs	(Imai and Ohura 2010; Laizure *et al.* 2013; Kim *et al.* 2016)
Nitroreductase	Reduction of nitro group	Metronidazole Benzodiazepines	(Koch *et al.* 1979; Elmer and Remmel 1984; Takeno *et al.* 1990)
N-acetyltransferase	Transfer of acetyl group to nitrogen or oxygen atom of primary arylamines, hydrazines and N-hydroxylated metabolites	5-aminosalicylic acid	(van Hogezand *et al.* 1992; Deloménie *et al.* 2001)
β-Lyase	Cleavage of C-S bond in hepatic-production cysteine-S-conjugated metabolites	Cysteine-conjugated metabolites Bio-activation of sulfur- and selenocysteine derivatives	(Mikov 1994)
Sulfatases	Hydrolysis of sulfate esters utilising formylglycine	Sulfate ester hepatic metabolites	(Ulmer *et al.* 2014; Koppel *et al.* 2017)

Table 2: Psychoactive drugs are subject to direct metabolism by the gut microbiota.

Drug:	**Drug Class:**	**Nature of Study; Animal species/human:**	**Suggested Implicated Microbial Species:**	**Microbial-Mediated Mechanism:**	**Ref.:**
Risperidone	Anti-Psychotic	*In vivo*; rats & dogs	unknown	Isoxazole scission of benzisoxazole ring system	(Meuldermans *et al.* 1994)
Nitrazepam and Clonazepam	Hypnotic	*In vivo*; rats	unknown	Nitro-reductase mediated reduction	(Takeno *et al.* 1990)
Methamphetamine	Psychostimulant	*In vitro*	Lactobacilli, Enterococci, Clostridia.	N-demethylation	(Caldwell and Hawksworth 1973)
Levodopa	Dopamine precursor	*In vivo*; rats	unknown	Decarboxylation and p-dehydroxylation mediated conversion to m-tyramine and subsequent oxidation to m-hydroxyphenylacetic acid	(Goldin *et al.* 1973)

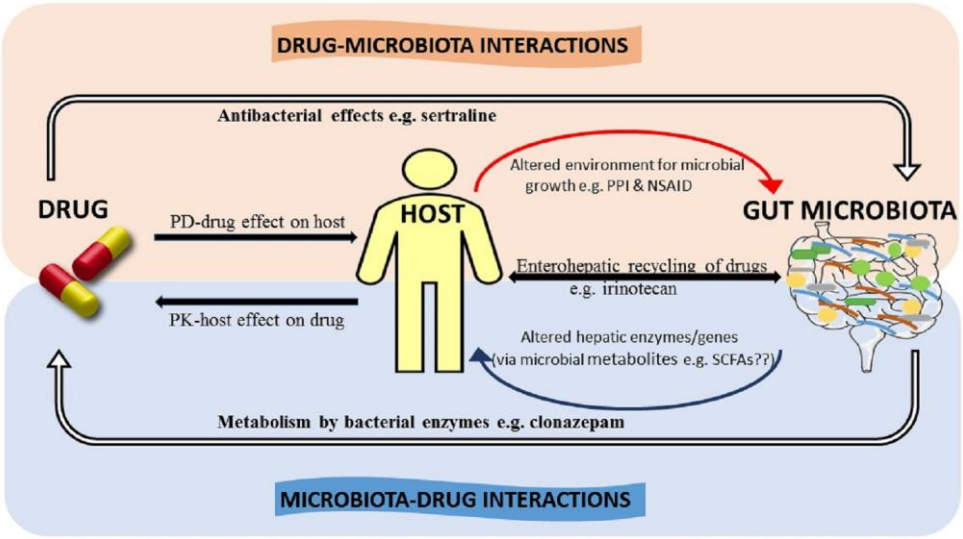

Fig. 1: ***The complex interplay between drugs and the gut microbiota.*** The reciprocal relationship between drugs and the gut microbiome is comprised of both microbiota-mediated alterations to drug pharmacokinetics and drug-mediated alterations to the function/composition of the gut microbiome. These interactions can occur by both direct (illustrated by solid white arrows with black outline) and indirect/secondary (illustrated by curved line arrows) mechanisms. "Microbiota-Drug Interactions": the microbiota can directly metabolise drugs through bacterial-derived enzymes (e.g. nitro-reductase mediated metabolism of clonazepam) but can also indirectly affect drug metabolism through the alteration of the hosts capacity to metabolise drugs (curved-up line arrow) i.e. (PK effect); microbial-derived metabolites (e.g. SCFAs and secondary bile acids) may be potential mediators of this effect. The interactions between the host and gut microbiome are responsible for the enterohepatic recirculation of drugs e.g. the hepatic-glucuronidated irinotecan metabolite is deconjugated by β-glucuronidase enzymes expressed by the gut microbiota. "Drug-Microbiota Interactions": The mechanisms underpinning the drug-mediated changes to the function and composition the gut microbiome are yet to be fully elucidated. Drugs can have antibacterial properties that directly affect the composition of the gut microbiota (e.g. sertraline). Drugs can also alter the physiological properties or functions of host organs (i.e. PD effect) (e.g. PPI-mediated alterations to gastric acid production and pH, NSAID-induced changes to mucosal integrity) which may, in turn, precipitate secondary effects to the composition of the gut microbiota (illustrated by the curved-down line arrow).

PK- Pharmacokinetic; PD-Pharmacodynamic

Ligand/Drug links for BJP Review Article:

The following linked words are in order of appearance of first mention in the manuscript:

TNF-α

mesalazine

azathioprine

clonazepam

venlafaxine

3-hydroxy-3-methylglutaryl coenzyme reductase

metformin

omeprazole

risperidone

sulfasalazine

aspirin

lovastatin

amlodipine

trans-4-hydroxy-L-proline

paracetamol

tyrosine

D-phenylalanine L-phenylalanine

N-acetyl-4-benzoquinoneimine

aryl hydrocarbon receptor

bilirubin

Constitutive androstane receptor

D-arginine L-arginine

digoxin

amiodarone

5-fluorouracil

methotrexate

loperamide

digitoxin

levamisole

nizatidine

phenacetin

paroxetine

levodopa

corticotrophin-releasing hormone

clonazepam

citalopram

olanzapine

risperidone

chlorpromazine

sertraline

fluoxetine

Drugs appearing in tables (not referenced in main text:

Irinotecan

Indomethacin

diclofenac

methamphetamine