Neuroimmunomodulation

Neuroimmunomodulation , DOI: 10.1159/000538927
Received: November 30, 2023
Accepted: April 9, 2024
Published online: April 17, 2024

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ISSN: 1021-7401 (Print), eISSN: 1423-0216 (Online)
https://www.karger.com/NIM
Neuroimmunomodulation

Review Article

STEERING THE MICROBIOTA-GUT-BRAIN AXIS BY ANTIBIOTICS TO MODEL NEURO-IMMUNE-ENDOCRINE DISORDERS

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Short Title: Animal models and use of antibiotics.

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Number of Tables: 3.
Number of Figures: 1.
Word count: Abstract: 187; body text: 3, 341; total: 3, 528

Keywords: antibiotics, animal models, brain, disease, gut microbiota

Abstract

Background: Over the last century, animal models have been employed to study the gut-brain axis and its relationship with physiological processes, including those necessary for survival, such as food intake regulation and

thermoregulation; those involved in diseases, ranging from inflammation to obesity; and those concerning the development of neurodegenerative diseases and neuropsychiatric disorders, such as Alzheimer's disease and autism spectrum disorder, respectively. ***Summary:*** The gut microbiota has been recognized in the last decade as an essential functional component of this axis. Many reports demonstrate that the gut microbiota influences the development of a vast array of physiological processes. Experiments that use animal models to assess the effect of the gut microbiota on the brain and behavior may involve the acute or chronic administration of broad-spectrum antibiotics. ***Key Messages:*** This narrative review summarizes the beneficial or detrimental effects of antibiotics administered prenatally or postnatally to rodents during acute or chronic periods in a wide range of protocols. These include animal models of disease and behavioral paradigms of learning and memory, anxiety, obsessive-compulsive disorder, and autism spectrum disorder. Biomarkers and behavioral assays associated with antibiotic exposure are also included in this review.

1. Introduction

Penicillin has been described as the first modern broad-spectrum antibiotic. However, before penicillin, a red dye showed activity against diverse *Streptococcus* strains. At the time, chemists knew that dye molecules and aniline dyes had the potential to bind to bacteria with varying degrees of specificity [1]. By the late 19th century, Paul Ehrlich published a paper speculating that if dye molecules could selectively bind to microorganisms, they could also selectively kill bacteria [2]. This red dye was sulfamidochrysoidine, and it was sold to the public for therapeutic use under the brand name Protonsil in the 1930s. Its mechanism of action as an antibiotic is based on the compound sulfanilamide, which prevents folic acid synthesis and is the metabolized product of sulfamidochrysoidine in the human body [3]. Although folic acid is an essential nutrient in the human diet, humans do not produce it and thus are not affected by sulfanilamide; consequently, sulfanilamide is potentially therapeutic for humans. The formulation of the pharmaceutical company that sold it in the United States as Elixir Sulfanilamide included diethylene glycol, which is poisonous to humans, and its use killed 100 people [2]. Though Protonsil, sulfanilamide without diethylene glycol, saved the life of Franklin Delano Roosevelt Jr., son of the United States President at the time, who was affected by a septic streptococcal throat infection [4], according to Zaffiri et al. [5].

Although this story is not directly related to the effect of an antibiotic *per se*, it points to the potentially harmful effect of antibiotic use in humans. Antibiotics have been widely employed and have saved millions of lives for almost a century; however, in the last few decades researchers have begun to understand how they kill both pathogenic and commensal microbes, and how their use as a first-line treatment for infections has modified the microbial ecology of humans [6]. As an example of their current extended use, antibiotics are even being used to assess their effects on depression, social anxiety, and obsessive-compulsive disorder (OCD) in teenagers with acne [7]. Consequently, pathogens (i.e., detrimental bacteria) have developed resistance to antibiotics, including methicillin-resistant Staphylococcus aureus [8], vancomycin-resistant enterococci [9], and drug-resistant tuberculosis [10], to the extent that one approval of antibiotics in healthcare occurs for every two withdrawals [11]. Likewise, it has been suggested that without policies to stop the alarming spread of antimicrobial resistance, which is associated with the indiscriminate use of antibiotics, the 70,000 deaths per year caused by this practice could escalate to 10 million deaths by 2050 [12]. Besides their side effects, including toxicity [13], antibiotics have also been associated with encephalopathy, aphasia, seizures, and coma [14].

Despite these negative data, antibiotics have not only helped to treat infections for nearly 80 years but also revealed the importance of microbiota. Although the effects of antibiotics have been linked to gut, oral, respiratory, skin, and vaginal microbiota [15], in this review we focus only on gut microbiota.

In recent years, researchers using diverse experimental approaches, such as high-throughput DNA sequencing and advanced computational tools, have described how a healthy gut microbiota influences physiological functions. These functions include essential processes for sustaining life (i.e., food intake [16, 17]) and thermoregulation [18]). Researchers have also found how changes in microbial ecology, associated with the indiscriminate use of broad-spectrum antibiotics, affect the immune system [19] and influence the incidence of obesity [20], neurological diseases such as Alzheimer's disease (AD) [21], and neuropsychiatric disorders like autism spectrum disorder (ASD) [22, 23]. Growing evidence suggests that even short-term antibiotic treatment shifts healthy microbiota to long-term alternative dysbiotic states [25], that is, compositional and functional alterations of gut microbiota diversity. In this review, we summarized the association between the use of antibiotics and their beneficial or detrimental

consequences for short and long-term periods of administration in animal models of disease. Behavioral paradigms of learning and memory [26, 27, 28], anxiety [29], depression [30], OCD [31], and ASD [32], along with biomarkers associated with these paradigms due to antibiotic exposure, are also covered.

1.1. Antibiotics as a tool for the study of health and disease

Several authors have described the impact of gut microbiota on the brain and behavior [33]. In this context, it has also been suggested that gut microbiota influences host physiology and disease. Thus, the prevalence of pathogenic bacteria over beneficial bacteria, termed "gut dysbiosis", has been linked to disease [34]. In the search for this relationship, scientists have utilized antibiotics as a tool to modify or deplete gut microbiota in animal models to assess their influence on inflammatory bowel diseases [35, 36], obesity [37], arterial dysfunction [38], multiple sclerosis [39] and mood disorders [40, 41]. Germ-free (GF) animals, mice raised without any exposure to microorganisms, are another approach to studying the role of microbiota in the brain and behavior. Although these animals provide the opportunity to introduce complete microbiota or defined consortia at various developmental stages [42], GF research has revealed that the microbiota is necessary for normal development and the maturation of immune, metabolic, digestive, gastrointestinal tract, and nervous system functions [43]. Also, GF breeding may induce permanent neurodevelopmental deficits that can make the model unsuitable for investigating specific neurological or neuropsychiatric diseases that require normal early-life microbiota [42]. Antibiotic treatment is an alternative to GF conditions for modeling microbiota-deficient animals at different developmental stages across the lifespan. In this review, we included detailed information based on the scientific literature that reports the use of two widely documented treatments involving a cocktail of broad-spectrum antibiotics. Animals were given these antibiotics, mixed in tap water, through *ad libitum* oral administration.

1.2. Antibiotics

1.2.1. Cocktails of antibiotics

We collected information about the widespread use of antibiotics to manipulate microbiota, in microbiota-gut-brain-axis studies in animal models. Between November 2021 and July 2023, we conducted searches on PubMed for research papers reporting the use of antibiotics in animal models of disease and changes in gut microbiota, with the terms "antibiotics", "animal models of disease" and "gut microbiota." Also, as inclusion criterion the papers had to be available to download via the Metropolitan Autonomous University (UAM) digital library (BIDI). The initial search yielded 1,091 results of papers published between 1986 and 2023, including "books and documents", "clinical trial", "meta-analysis", "randomized controlled trial", "review" and "systematic review". To delimit our search, based on key papers from our research group, we focused on documents published after 2006 that cited basic research studies using antibiotic cocktails in animal models of disease. With these criteria, we found a couple of cocktails frequently cited in the literature: one published by Rakoff-Nahoum et al. (2004), which we will refer to as the "Rakoff-Nahoum antibiotic cocktail", and another one published by Reikvam et al. (2011), which we will refer to as the "Reikvam antibiotic cocktail".

The Rakoff-Nahoum antibiotic cocktail comprises ampicillin, neomycin sulfate, metronidazole, and vancomycin [44; cited by 3,417 papers in Scopus on November 30th, 2023], and it induces a substantial depletion of commensal microbes and modulates diverse parameters of obesity, cardiovascular disease, colitis, and multiple sclerosis. This antibiotic treatment reduced the expression of antimicrobial factors to a level similar to that of GF mice and reduced the fecal bacterial DNA load 400-fold while ensuring the health of 6-to-10-week-old BALB/c mice when administered for 17 days [45]. A summary of original research papers using this cocktail of antibiotics [46, 38, 47, 40, 48, 49, 37, 50] is shown in Table 1.

The Reikvam antibiotic cocktail consists of ampicillin, metronidazole, vancomycin, ciprofloxacin, and imipenem [45; cited by 265 papers in Scopus on November 30th, 2023], and it has been utilized to assess neurobehavioral changes, including anhedonia and anxiety-like behaviors, associated with fecal microbiota transplantation from depressed patients into microbiota-depleted rats after 28 days of antibiotic treatment [41]. A summary of original research papers using this cocktail of antibiotics [29, 51, 30] is presented in Table 2.

Though we mainly focused on these cocktails of antibiotics to document their impact on gut microbiota and the beneficial or detrimental effects they exert on diverse animal models of disease, it is important to mention that the studies we included in this review vary in several factors: a) period of administration (i.e., from 17 days to 6 months),

b) duration of treatment (e.g., 2 weeks vs 7 weeks), c) species (i. e., rat or mouse), d) age and sex of the animal studied, and e) animal model (i. e., a disease-specific model or a healthy, conventional model). Within these considerations, we also included other antibiotic treatments commonly used in animal research. A summary of original research papers utilizing these antibiotic cocktails [52, 53, 54, 55, 56, 57, 58, 50] is shown in Table 3.

2. Gut microbiota

The normal human gut microbiota is composed of 29 phyla, with Bacteroidetes and Firmicutes [59] dominating the gut of healthy adult humans [60]. In rodents, diverse studies have accounted for the relationship between the manipulation of these bacterial phyla and the parameters of specific diseases. In this way, we describe the changes in microbiota following the administration of the Rakoff-Nahoum and Reikvam antibiotic cocktails.

2.1. Changes in gut microbiota

Rakoff-Nahoum antibiotic cocktail. This cocktail has been employed in animal models of obesity-related vascular dysfunction [46], stress-mediated vascular dysfunction [38], non-alcoholic fatty liver disease [49], and multiple sclerosis [47]. According to some research papers, it induces an incomplete depletion of cultivable bacteria [35, 61]. Sequencing obtained from fresh fecal samples has demonstrated that its administration depletes all detectable commensal bacteria [38], abrogates gut microbiota [46], reduces the relative abundance of Bacteroidetes and Firmicutes [62, 37, 50], and increases the abundance of Proteobacteria and Cyanobacteria [40].

Reikvam antibiotic cocktail. Sequencing from fecal and cecal samples after administering this cocktail to 10-week-old male Sprague Dawley rats for 4 to 6 weeks revealed a significant decrease in the relative abundance of Bacteroidetes and Firmicutes, and an increase in Proteobacteria and Cyanobacteria [30]. According to one paper, the administration of this cocktail to C57/BL6 mice for 6 to 8 weeks prevented the development of ileitis following oral infection by the intracellular cosmopolitan protozoan *Toxoplasma gondii* [36], which is associated with the accumulation of the gram-negative bacteria *Escherichia coli* and *Bacteroides/Prevotella* spp. in inflamed ileum [63]. This antibiotic cocktail has also been used in a mouse model of colonization resistance against *Campylobacter jejuni* infection [55], as well as in models of subclinical intestinal inflammation [65, 66]. It has also been used to assess the role of gut microbiota in major depressive disorder [67, 68, 41].

3. Mechanisms of action of the antibiotics

Ampicillin is a member of the ß-lactam family of antibiotics. It attaches to specific penicillin-binding proteins located within the bacterial cell wall, thus interfering with bacterial cell wall synthesis [69, 70, 71]. Ciprofloxacin is a second-generation fluoroquinolone [72]. It acts on bacterial topoisomerase II, a DNA gyrase, and topoisomerase IV [73]. It also targets the alpha subunits of DNA gyrase and impedes it from supercoiling bacterial DNA, thus preventing DNA replication [74]. The antimicrobial action of pencarbapenems like imipenem is mediated by binding to specific penicillin-binding proteins that catalyze peptidoglycan formation in the bacterial cell wall. Hence, pencarbapenems interrupt bacterial cell wall synthesis [75]. Metronidazole acts as an effective antimicrobial agent for the treatment of infections with anaerobic bacteria and protozoa [76]. It inhibits protein synthesis by interacting with host cell DNA, destabilizing of helical DNA structure and DNA strand breakage [77]. Neomycin is an aminoglycoside antibiotic agent that binds to the 30S ribosomal subunit of susceptible bacteria and disrupts the translational machinery of bacterial protein synthesis [78]. The bactericidal action of the glycopeptide antibiotic vancomycin is mediated by the inhibition of cell wall biosynthesis, which is associated with the binding of vancomycin to the acyl D-ala-D-ala portion of the growing peptidoglycan cell wall [79]. Vancomycin also alters cell membrane permeability and inhibits RNA synthesis [80]. Additional information on the mechanisms of action of the antibiotics mentioned above can be reviewed in Eyler and Shvets [81].

4. Effects of Rakoff-Nahoum and Reikvam antibiotic cocktails upon animal models of disease, behavioral paradigms, and associated biomarkers

4.1. Beneficial effects

We consider an effect to be beneficial when the administration of antibiotics: a) exerts a protective effect against a specific disease; b) reduces a detrimental condition in a specific animal model of disease; and c) reverses gut dysbiosis induced by a specific experimental approach.

4.1.1. Rakoff-Nahoum antibiotic cocktail: ampicillin, neomycin sulfate, metronidazole, and vancomycin
When administered to normal-weight Swiss Webster mice for 4 weeks, the Rakoff-Nahoum antibiotic cocktail improves glucose tolerance [37]; when administered to C57BL/6J mice for 8 weeks, along with a high-fat diet for the same period, it improves insulin tolerance, decreases both tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) levels, and reduces toll-like receptor 4 (TLR4) activation [62]. When this antibiotic cocktail is administered for 2 to 5 months to C57BL/6J mice fed a Western diet (WD), it abrogates the 7-month WD-induced gut dysbiosis, by increasing Firmicutes and decreasing Bacteroidetes, and it reverses WD-induced arterial stiffness and endothelial dysfunction [46]. In a model of stress-mediated arterial dysfunction, the administration of this cocktail for 4 weeks to 20-24 months old C57BL/6N mice reverses endothelial dysfunction and arterial stiffening, and it suppresses plasma levels of the adverse gut-derived metabolite trimethylamine N-oxide [38], which is an age-related sign of cardiovascular disease [82].
In an experimental autoimmune encephalomyelitis (EAE) multiple sclerosis animal model, female SJL/J or C57BL/6 mice were challenged s. c. with proteolipid protein (PLP)139-151 or myelin-oligodendrocyte glycoprotein (MOG)35-55. Oral administration of the antibiotic cocktail for seven days protected mice against EAE, as it induced a decrease in gut commensal bacteria and proinflammatory cytokines, and an increase in IL-10 and IL-13 [39].

4.1.2. Reikvam antibiotic cocktail: ampicillin, metronidazole, vancomycin, ciprofloxacin, and imipenem
This cocktail of antibiotics has been used to deplete gut microbiota in mice, to disrupt commensal host-bacterial relationships [53] and to explore the signaling pathways of diverse disease processes in rodent models of disease [83, 84, 85]. Thus far, no beneficial effects have been reported in association with its use.

4.2. Detrimental effects
We consider an effect to be detrimental when: a) the antibiotic cocktail provokes severe mortality or morbidity; b) the administration of antibiotics induces or exacerbates deficits in a specific behavioral paradigm; and c) the administration of antibiotics reduces the expression of biomarkers associated with improvement in a specific behavioral paradigm.

4.2.1. Rakoff-Nahoum cocktail: ampicillin, neomycin sulfate, metronidazole, and vancomycin
According to Reikvam et al. [45], the protocol for this cocktail is difficult to reproduce. Researchers who have successfully administered the antibiotics *ad libitum* to mice report a high rate of mortality and morbidity in certain strains and genotypes. For instance, a seven-day oral administration of dextran sulfate sodium (DSS), a heparin-like polysaccharide involved in the development of colitis with ulceration, induced intestinal and injury inflammation resembling that of human ulcerative colitis [86] in MyD88-deficient mice. MyD88 is an adaptor molecule essential for TLR-mediated induction of inflammatory cytokines [87]. However, administering this cocktail for 4 weeks led to depletion of all detectable commensals and resulted in severe mortality and morbidity in these mice [44].

4.2.2. Reikvam antibiotic cocktail: ampicillin, metronidazole, vancomycin, ciprofloxacin, and imipenem
This cocktail of antibiotics has been administered for 28 days to assess neurobehavioral changes, including anhedonia and anxiety-like behaviors, associated with fecal microbiota transplantation from depressed patients to microbiota-depleted adult rats [41]. When administered to 10-week-old Sprague-Dawley male rats for 6 weeks, it induces spatial memory deficits in the Morris water maze test and increases depressive-like behaviors in the forced swim test by decreasing swimming scores and increasing immobility scores [30]. These changes are associated with reduced gene expression of the glucocorticoid receptor (Nr3c1) and corticotropin-releasing hormone receptor 1 (Crhr1) in the hippocampus and amygdala [30]. Also, when administered to male C57BL mice for 3 weeks, from postnatal days 28 to 49 (i.e., adolescence), this cocktail of antibiotics depletes microbiota, induces body weight loss, increases anxiety-like behaviors in the elevated plus maze, increases freezing behavior in a fear-conditioning paradigm, and increases the expression of genes related to immune markers, neurotransmission, and neuroplasticity, such as TLR4, gamma-

aminobutyric acid type A receptor subunit alpha-2 (Gabra2) and synaptophysin (Syp), respectively, in the amygdala [29]. When administered for 7 weeks to female C57BL/6 mice, it reduces neurogenesis, as evaluated by hippocampal staining of mature neurons and transient proliferating mitotic neuronal progenitor cells, with antibodies against NeuN and doublecortin, respectively. It also induces memory retention impairment in the novel object recognition test [51].

4.3. Beneficial and detrimental effects

4.3.1. Rakoff-Nahoum antibiotic cocktail: ampicillin, neomycin sulfate, metronidazole, and vancomycin

An amplicon 16S rRNA gene sequencing analysis conducted in a murine model of chronic secondary-progressive multiple sclerosis, which is characterized by the development of a biphasic pattern of the disease that is more closely related to the human condition [88], revealed a significant difference between the gut microbiome of EAE-induced mice and healthy control mice, as well as a reduction in species in EAE-induced mice. This study evaluated the relative abundances of taxonomical groups and taxonomical levels, including phylum, class, order, family, genus, and species. A two-week treatment with this antibiotic cocktail, administered orally in drinking water, reduced disease progression in animals with mild EAE and exacerbated the disease in animals with severe EAE, according to their clinical scores [47]. This treatment has been shown to affect the balance of proinflammatory and regulatory responses with EAE, and it is speculated that T regulatory cells are partially responsible for protecting the gut microbiota. A report [39] showed that mice who develop severe EAE exhibit a dysbiotic gut microbiome compared to healthy control mice.

4.3.2. Reikvam antibiotic cocktail: ampicillin, metronidazole, vancomycin, ciprofloxacin, and imipenem

Unlike the Rakoff-Nahoum antibiotic cocktail research, studies employing the Reikvam antibiotic cocktail have not yet documented the detrimental and beneficial effects of this cocktail.

5. Conclusions

Although some studies suggest that successful colonization of the intestinal microbiome involves microbial selection and competition beginning in the first hours of life [89], and that this colonization occurs rapidly in mammals during and after birth [90, 6, 91], microbial composition continuously changes throughout life [25, 20, 92] due to the interaction with and exposure to a vast array of factors, including nutritional status, environmental temperature, water resources, lifestyle, diet, and age [93]. The use of antibiotics is also one of these factors.

At the beginning of the 20th century, antibiotics decreased the number of human deaths caused by infectious diseases from almost half of all deaths to nearly 10% of deaths. However, towards the end of the 20th century and the beginning of the 21st century, there was a surge in the indiscriminate use of antibiotics, which significantly increased microbial resistance to antibiotics in humans [3]. Similarly, abuse of antibiotics also seems to play a major role in the pathogenesis of mental disorders associated with gut microbiota dysbiosis. Therefore, antibiotics are now considered potentially harmful drugs. Clinical research has revealed that gut dysbiosis is associated with major depressive disorder, bipolar disorder [94], ASD [22, 23, 32], Parkinson's disease [95, 96], and AD [21]. However, these relationships can reflect that microbiota impairment is either preceded by or caused by the diseases, or both. To elucidate the relationship between gut microbiota and neurodegenerative or neuropsychiatric disorders, basic research has explored the impact of antibiotics on the brain and behavior. Researchers utilize antibiotics to make the host vulnerable to infection and then studying the molecular pathways associated with a specific disease. GF animals are an important model for studying the impact of gut microbes on the development and function of the nervous system [42]. However, GF mice exhibit alterations in the blood-brain barrier and brain ultrastructure [54]. In comparison to conventional specific pathogen-free animals, GF mice display increased plasma corticosterone levels, reduced anxiety-like behavior, decreased N-methyl-D-aspartate receptor subunit NR2B mRNA expression in the central amygdala, decreased serotonin receptor 1A mRNA expression in the hippocampus and increased brain-derived neurotrophic factor mRNA expression in the hippocampus [97]. These basal differences between GF mice and conventional mice make it difficult to interpret the behavioral and neurochemical outcomes associated with antibiotic administration. They also underscore the importance of the presence or absence of conventional intestinal microbiota in behavioral development and the associated neurochemical changes in the brain [97 98]. In another approach, antibiotics are administered to conventional animals for short or long periods, ranging from days to months, to modify or deplete gut microbiota and assess the relationship between gut microbiota and disease. In this approach, broad-

spectrum antibiotics can induce beneficial or detrimental effects, or both, depending on the stage of development of the disease, among other factors [47]. Researchers have also used multiple methodologies and antibiotics in their protocols, which can be difficult to replicate [45]. At times, broad-spectrum antibiotic cocktails reverse negative parameters of disease [46] or exacerbate several deficits in behavioral paradigms that evaluate memory, depression, and anxiety [30]. Despite these issues, and even though antibiotics *per se* could be toxic to the brain [54], the latter approach has become popular and has dominated the research field in recent years. The pharmacokinetics of the antibiotics (i. e., route of administration, absorption, distribution, metabolism, and clearance), should also be considered to determine the relevance of these issues. For instance, as compared to systemic antibiotics, host poorly absorbed antibiotics, such as rifaximin [95], may deliver high concentrations of antibiotics to the site of an enteric infection with minimal risk of systemic adverse effects, toxicity, and drug interactions, and can be associated with high bioavailability in the gastrointestinal tract and low concentrations in the bloodstream and the brain [100]. However, some poorly absorbed antibiotics, such as minocycline [101], may cross the blood-brain barrier [102]. The impact of this type of antibiotics on the brain could be masked by their pharmacokinetics. In acute bacterial meningitis, a disease with rapid onset and epidemiological potential, and high rates of mortality and morbidity [103, 104], bacteria evade the mucosa and immune responses, and invade the brain [105]. Therefore, research on the effects of antibiotics in animal models and their relationship with the microbiota-gut-brain axis should explore how specific host-pathogen interactions cause inflammation and brain damage. Nonetheless, this approach has also accounted for the molecular effects of these drugs in the brain and various peripheral tissues, and it has also allowed researchers to gain a deeper understanding of the effects of antibiotics on both gut microbiota and behavioral paradigms that evaluate aspects of neuropsychiatric disorders and neurodegenerative diseases, such as schizophrenia and AD, respectively.

Advances in gut-brain axis research and antibiotic-induced gut microbiota impairment are groundbreaking and provide the opportunity to prevent or control diseases, design nonpharmaceutical therapeutic strategies for the diseases, and have predictive microbiota-based biomarkers for the early stages of diseases. In this way, some clinical studies [106] and meta-analyses of clinical trials [107] have shown that single antibiotic treatment regimens, including penicillin and quinolones, may increase the risk of depression and anxiety [106]. Additionally, minocycline, when used as an adjunctive therapy in combination with anti-inflammatory drugs, has been found to alleviate symptoms of depression [107]. In rodents, decreased microbial diversity is associated with depressive-like behaviors [41] and alterations in tryptophan metabolism [108]. Because of these advances, we know, for instance, that the antidepressant isoniazid has antibacterial activity. As a result, it has been developed to treat tuberculosis [109]. Also, we know that minocycline, which enters the brain and interacts with microglia, exerts antidepressant activity [110, 111]. At another level, we know that antibiotic treatment and duration may alter microbiota compositions [25], and that there are critical developmental windows in which antibiotics may exert long-lasting effects [112] upon anxiety, depression [29], and cognition [51].

The antibiotic cocktails analyzed in this review reduce the relative abundance of Bacteroidetes and Firmicutes and increase the abundance of Proteobacteria and Cyanobacteria [62, 37, 50, 30]. To our knowledge, no papers have been published exploring the details of their toxicity in animal models. However, since antibiotics may act directly in the brain, this issue must be considered in research on the gut-microbiota-brain axis. Both antibiotic cocktails have been employed in a wide range of animal models and regimens. However, the Rakoff-Nahoum antibiotic cocktail (ampicillin, neomycin sulfate, metronidazole, and vancomycin) seems to be associated with high rates of morbidity and mortality, in MyD88 deficient mice [44]. Nonetheless, it is also related to both beneficial and detrimental effects [47]. The Reikvam antibiotic cocktail (ampicillin, metronidazole, vancomycin, ciprofloxacin, and imipenem) has been associated with detrimental effects, including reduced hippocampal neurogenesis and impaired memory [51], and increased anxiety-like behaviors and anhedonia [41].

Still, researchers and clinicians must be cautious because the methodological heterogeneity makes it difficult to compare basic studies and translate knowledge from preclinical research to the clinical field. However, the negative consequences of the indiscriminate use of antibiotics might encourage researchers to conduct a thorough review of the literature, the antibiotic administration protocols to be used, and the animal models of disease to be studied. The main conclusions of this review are summarized in Figure 1.

Statements

Acknowledgments

The authors wish to thank M. Sc. Jesús Andrade-Guerrero for figure editing and Jessica González Norris for proofreading the manuscript.
Neuro-Psycho-Biota Consortium:
Gustavo Pacheco-López, Sofía Díaz-Cintra, Jaime García-Mena, Marcel Pérez-Morales, Alejandra Vargas-Caraveo, Astrid Gómez-García, Paola Bello-Medina , Alexander González-Franco, Carla Alejandra Márquez-Muñoz, Azucena Aguilar-Vázquez, Fernando Hernández-Quiroz, Karina Corona-Cervantes, Norma Gabriela Zavala-Torres.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Funding Sources
This research was supported by the Mexican Council for Science and Technology (CONACYT) grants CB-2015 255399 to G.P.-L., 163235, INFR-2011-01 to J.G.-M., Postdoctoral Fellowship to P.C.B-M (CONACYT 24649). It was also supported by the Secretariat of Public Education (SEP)-PRODEP postdoctoral fellowship to D.A.G.-F. (511-6/2019-15935), the State of Mexico Science and Research Council (COMECYT) grant FICDTEM-126 to G.P.-L., and the Autonomous Metropolitan University (UAM) institutional fund to G.P.-L. The following are National System of Researchers Fellows (SNI-CONACYT, Mexico): G. P-L, S. D.-C., J. G.-M., M. P.-M., P. C. B.-M., and D. A. G.-F.

Author Contributions
M. P.-M, G. P.-L., S. D.-C. and J. G.-M., conceptualization; M. P.-M. and P. C. B.-M., literature research and acquisition of information; M. P.-M., analysis, organization of information and methodology; M. P.-M, G. P.-L., S. D.-C., and J. G.-M., writing—original draft; M.P.-M., G. P.-L., S. D.-C., J. G.-M., P. C. B.-M., and D. A. G.-F., writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Figure 1. Microbiota is an essential component of the gut-brain axis. Clinical evidence has suggested that gut dysbiosis is associated with the incidence of neurodegenerative and neuropsychiatric diseases. Acute or chronic administration of antibiotics has been employed to deplete or modify gut microbiota in animal models, to study the impact of gut microbiota on brain and behavior. In animal models, the Rakoff-Nahoum and Reikvam antibiotic cocktails reduce the relative abundance of Firmicutes and Bacteroidetes and increase the abundance of Proteobacteria and Cyanobacteria. These cocktails also induce neurobehavioral changes in animal models of disease. Abbreviations: WD, Western Diet (consisting of 42.0% fat –61.8% saturated, 27.3% monounsaturated, 4.7% polyunsaturated–, 42.7% carbohydrate –80% sucrose–, and 15.2% protein calories, for 7 months).

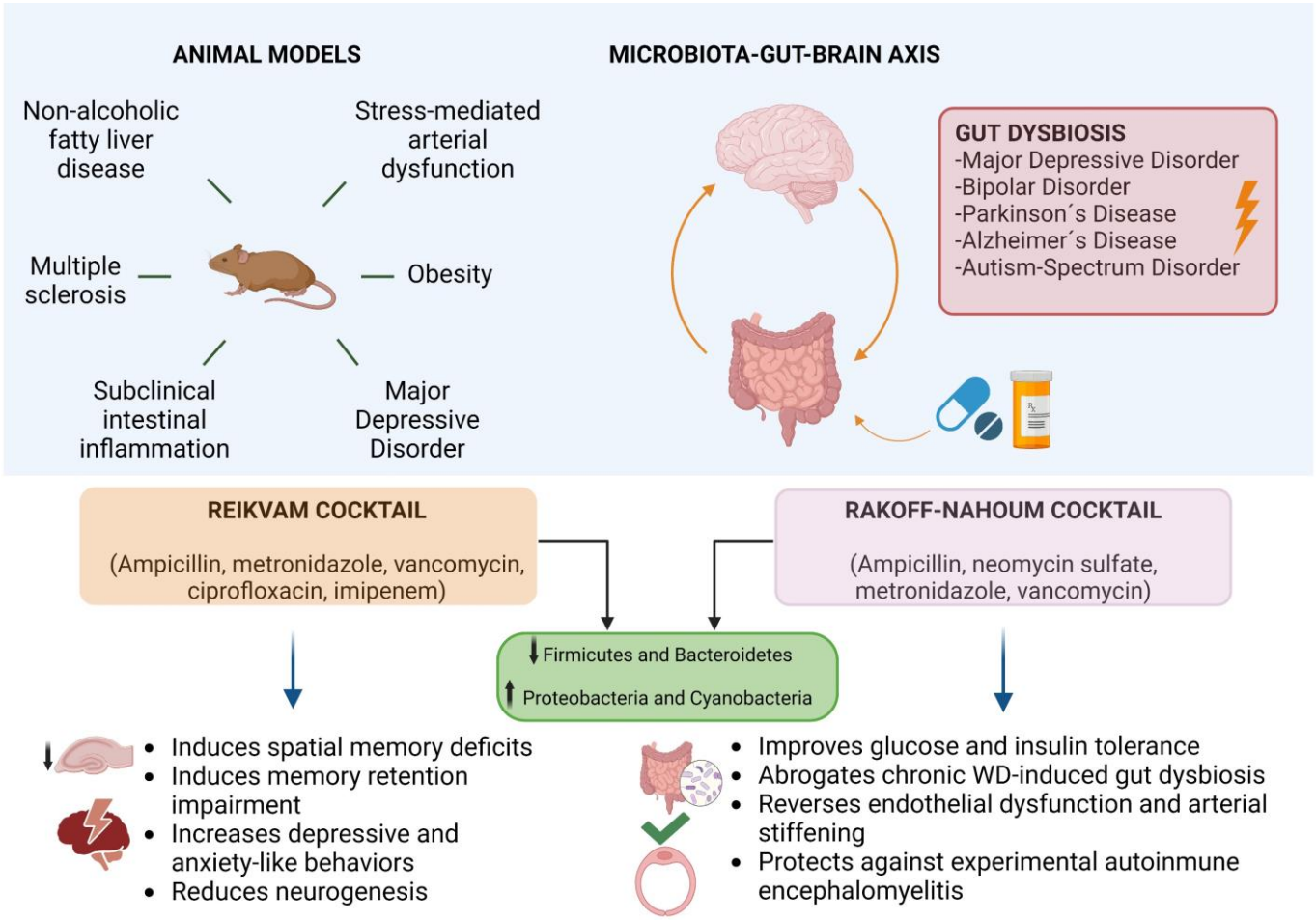

Table 1. Summary of antibiotics employed in diverse animal models of disease: antibiotic cocktail one: ampicillin, neomycin sulfate, metronidazole, and vancomycin (first published in Rakoff-Nahoum et al., 2004).

Reference	Animal model/species/sex/age	Duration of antibiotic treatment	Main effects
Battson et al., 2017 [46].	Male C57BL/J6 mice (7-9 mo)	2 mo	Reversed Western diet-induced vascular dysfunction.
Brunt et al., 2019 [38].	Young (8-10 wk) and old (20-24 mo) male C57BL/6N mice	3-4 wk	Reversed endothelial dysfunction and arterial stiffening. Suppression of T-MAO, an adverse gut-derived metabolite associated with ageing.
Colpitts et al., 2017 [47].	Model of SP-MS, female NOD mice (10 wk)	2 wk	Reduced mortality and clinical disease severity; prevention or exacerbation of experimental autoimmune encephalomyelitis clinical scores.
Desbonnet et al., 2015 [40].	Swiss male mice (PND 21 onwards)	2 mo	Object recognition memory impairment and increased anxiety. Reduced BDNF hippocampal expression. Increased tryptophan and reduced kynurenine serum levels.
Flannigan et al., 2018 [48].	SPF male C57BL/6 and BALB/c mice (7-10 wk)	2 wk	Prevented and reversed MMF- induced weight loss and colonic inflammation.
Janssen et al., 2017 [49].	Non-alcoholic fatty liver disease mouse model, male C57Bl/6 mice (10 wk)	22 wk	Suppressed colon bacteria, i.e., reduced expression of short-chain fatty acids in the cecum; decreased portal secondary acid levels; attenuated hepatic inflammation and fibrosis associated with non-alcoholic fatty liver disease development.
Rakoff-Nahoum et al., 2004 [44].	MyD88 deficient mice, a mouse model of inflammatory bowel diseases (no age provided)	4 wk	Worsening of the severity of dextran sodium sulfate-induced colitis.
Rodrigues et al., 2017 [37].	Male Swiss Webster mice (8 wk)	4 wk	Reduced fasting-glucose levels and improved glucose tolerance.
Xu et al., 2021 [50].	SPF male Balb/c mice (5 wk)	4 wk	Low-grade colonic inflammation; renal and liver dysfunction; increased serum levels of creatinine and urea, decreased serum levels of albumin.

Reference	Animal model/species/sex/age	Duration of antibiotic treatment	Main effects
Lach et al., 2020 [29].	Adolescent (PND 28-49) and adult (PND 76-97), male C57Bl/6OlaHsd mice	3 wk	Increased anxiety-like behavior at both ages; stronger freezing behavior and gene expression in the AMG during adolescence.
Möhle et al., 2016 [51].*	Female C57BL/6 mice (6-8 wk)	7 wk	Reduced neurogenesis in HP and memory retention impairment.
Heimesaat et al., 2006 [36].	C57BL/6 mice (2-4 mo)	6-8 wk	Prevented development of ileitis following *Toxoplasma gondii* oral infection.
Hoban et al. 2016 [30].	Adult male Sprague-Dawley rats (10 wk onwards)	6-10 wk	Increased depressive-like behavior in the FST, increased levels of tryptophan in plasma, and reduced levels of 5-HT in HP.
Kelly et al., 2016 [41].	Adult male Sprague-Dawley rats	28 days	Induced behavioral and physiological features of depression after fecal microbiota transplantation from depressed patients.

Table 2. Summary of antibiotics employed in diverse animal models of disease. Antibiotic cocktail two: ampicillin, metronidazole, vancomycin, ciprofloxacin, and imipenem (first published in Heimesaat et al., 2006).

Abbreviations: mo, months; wk, weeks; PND, postnatal day; AMG, amygdala; HP, hippocampus; FST, forced swimming test; *according to Hoban et al. (2016), this protocol is a "chronic" administration.

Table 3. Summary of antibiotics employed in diverse animal models of disease: other cocktails of antibiotics.

References	Antibiotics	Animal model/species/sex/age	Duration of antibiotic treatment	Main effects
Fagarasan et al., 2002 [52].	Ampicillin, imipenem, neomycin, and metronidazole	$AID^{+/+}$, $AID^{+/-}$ and $AID^{-/-}$ mice bred on a BALB/c or a C57BL/6 background (3 wk)	2 wk	Abolished hyperplasia of small intestinal lymphoid follicles associated with deficiency of AID.
Fan et al., 2022 [53].	Bacitracin, neomycin, and natamycin	Male C57BL/6J mice (2 mo)	11 days	Reduced sucrose preference rate, longer immobility time in FST and TST; increased serum concentrations of ACTH and CORT; lower levels of BDNF; lower concentrations of 5-HT and NE in PFC and HP.
Frölich et al., 2016 [54].	Ampicillin, bacitracin, meropenem, neomycin, and vancomycin	Male C57BL/6N mice (8-11 wk)	11 days	Object recognition memory impairment; reduced expression of CLDN5 in HP; increased expression of TJP1 in AMG.
Guida et al., 2018 [55].*	Ampicillin streptomycin and clindamycin	Male C57/bl6 mice (6 wk)	2 wk	Increased immobility time in TST and FST; reduced BDNF in HP.
Kang et al., 2017 [56].	Streptomycin, neomycin, vancomycin, and metronidazole	Adult male Swiss Webster mice	10 days (each 12 h, per day)	Prevented the development of antinociceptive tolerance to chronic morphine.
Saunders et al., 2020 [57].	Streptomycin	CD1 male mice (15-20 wk) born to influenza virus-infected mothers	One single dose at PND28	Reversed novel object recognition influenza virus-induced impairment.
Verdú et al., 2006 [58].	Bacitracin, neomycin, and primaricin	Female Swiss mice (6-8 wk)	10 days	Visceral hypersensitivity increased myeloperoxidase activity and increased substance P immunolabelling in the colon.
Xu et al., 2021 [50].	Ampicillin, vancomycin, metronidazole, neomycin, and streptomycin	SPF male Balb/c mice (5 wk)	4 wk	Renal and liver dysfunction; increased serum urea and total cholesterol concentrations.