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Gut microbiota and neurological effects of glyphosate

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ABSTRACT

There are currently various concerns regarding certain environmental toxins and the possible impact they can have on developmental diseases. Glyphosate (Gly) is the most utilised herbicide in agriculture, although its widespread use is generating controversy in the scientific world because of its probable carcinogenic effect on human cells. Gly performs as an inhibitor of 5-enolpyruvylshikimate-3-phospate synthase (EPSP synthase), not only in plants, but also in bacteria. An inhibiting effect on EPSP synthase from intestinal microbiota has been reported, affecting mainly beneficial bacteria. To the contrary, *Clostridium* spp. and *Salmonella* strains are shown to be resistant to Gly. Consequently, researchers have suggested that Gly can cause dysbiosis, a phenomenon which is characterised by an imbalance between beneficial and pathogenic microorganisms. The overgrowth of bacteria such as clostridia generates high levels of noxious metabolites in the brain, which can contribute to the development of neurological deviations. This work reviews the impact of Gly-induced intestinal dysbiosis on the central nervous system, focusing on emotional, neurological and neurodegenerative disorders. A wide variety of factors were investigated in relation to brain-related changes, including highlighting genetic abnormalities, pregnancy-associated problems, diet, infections, vaccines and heavy metals. However, more studies are required to determine the implication of the most internationally used herbicide, Gly, in behavioural disorders.

1. Introduction

The influence of microbiome on mood regulation, depression and cognitive function has been demonstrated and accepted for years (Mangiola et al., 2016; Naseribafrouei et al., 2014). Found inside the digestive system, the enteric nervous system consists of a network of neurons forming a nerve plexus, which is interconnected with the central nervous system (CNS) (Furness et al., 2014). Pathways linking between the microbiome and CNS are established by vagal afferences and the circulatory system (neurotransmitters, hormones, cytokines, and metabolites) (Forsythe et al., 2014). Among the microorganisms (bacteria, fungi, viruses) that make up the intestinal microbiome, the bacteria are the most important (10^{14}), mainly dominated by *Firmicutes* and *Bacteroidetes*, as well as *Actinobacteria* and *Proteobacteria* phyla (Thursby and Juge, 2017). Harmony between them has beneficial effects on host individuals including the production of vitamins, protection against pathogens and modulation of the immune system (Shreiner et al., 2015). An imbalance in intestinal microorganisms, with an increase in *Firmicutes* phylum and a decrease in *Bacteroidetes* phylum, causes an adverse host response, provoking a phenomenon known as dysbiosis (Shreiner et al., 2015). The gut microbiota dysbiosis has been related to an increased susceptibility to intestinal, cardiovascular and nervous pathologies. Thus, irritable bowel syndrome (IBS) is the consequence of an inappropriate inflammatory response to intestinal microbes (Bonaz, 2013). Likewise, IBS patients have less *Lactobacillus* and *Bifidobacterium* spp. and a 2- fold augmented ratio in the *Firmicutes*/*Bacteroidetes* relationship in comparison to healthy subjects (Icaza-Chávez, 2013; Kassinen et al., 2007; Rajilić-Stojanović et al., 2011). Metabolic endotoxemia, an occurrence generated by systemic exposure to certain compounds of bacterial membrane, has emerged as a cardiovascular risk factor (Manco et al., 2010). Compelling evidence supports a correlation between excessive levels of *Firmicutes* and neuropsychiatric disorders such as depression, bipolar disorder and dementia (Huang et al., 2018; Naseribafrouei et al., 2014). Currently, microbiome-based strategies, including prebiotics, probiotics and faecal transplants, aim to promote eubiosis to encourage metabolic and

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mental health (Jia et al., 2018; Vigvári et al., 2018).

Furthermore, dysbiosis may be caused by a wide variety of factors, including some environmental pollutants, fungicides, insecticides or herbicides such as glyphosate, which is the focus of our work. *N*-(phosphonomethyl) glycine, commonly known as glyphosate (Gly), is the active compound in several formulations with surfactants, referred to as Gly-based herbicides (GBH), e.g. Roundup®. Roundup® was developed in the 1970s as a broad-spectrum systemic herbicide to remove weeds and shrubs from agricultural crops. This organophosphorus compound penetrates the soil, posing a threat for soil microbial ecology (Helander et al., 2018; Liu et al., 2018). Moreover, it has been confirmed that Gly has the capacity to contaminate aquatic ecosystems (Peruzzo et al., 2008; Van Stempvoort et al., 2016). In addition, subsequent studies have demonstrated harmful effects on growth, range of movement and animals' behaviour on the water surface and ground water (Bridi et al., 2017; Frontera et al., 2011). It is argued that Gly is a low risk compound for human health due to the absence of the shikimic acid pathway in mammals, blocking the route for Gly. Nevertheless, there is certain ongoing controversy because it has recently been demonstrated that it may provoke cellular alterations (Gasnier et al., 2009; International Agency for Research on Cancer, 2015).

Human exposure might be possible through the ingestion of several Gly-contaminated foods of agricultural origin. Given that traces of herbicide have recently been found in formula milk, honey, cereal grains or soy, human health might be compromised following Gly exposure (Rubio et al., 2014). A current report has shown the presence of glyphosate (95%) in most beverages for human consumption, from agricultural crops, such as beer and wine, in concentrations ranging from 51 ppb to 3.5 ppb (Cook, 2019). The gastrointestinal tract can absorb a limited part of Gly, a minimal proportion ($< 0.7\%$) of the ingested dose. It is subsequently metabolised by hydrolysis to aminomethylphosphoric acid (AMPA), its main metabolite, and the rest is rapidly eliminated through urine and faeces (Brewster et al., 1991; Williams et al., 2000). Despite this, the risk of human consumption of Gly-containing foods is still being evaluated, especially in children and during pregnancy. In this work, we state a possible link between Gly-induced dysbiosis and cognitive and motor aggravations in neurodegenerative and neurodevelopmental pathologies, such as autism spectrum disorder (ASD). Hence, we review the negative impact that Gly-induced dysbiosis may have on depression/anxiety, autism, Alzheimer's and Parkinson's diseases

2. Gly mode of action

As mentioned previously, Gly, the main component of GBH, is an essential compound for the farming industry, since it can eliminate weeds in crops, thus improving performance and providing greater economic benefits, according to some authors (Kraehmer et al., 2014). In plants, all the primordial aromatic compounds that are involved in primary metabolism of aromatic amino acids are created by the shikimate pathway. The route of shikimic acid from glucose, discovered by Bernhard Davis and others in 1956 (Herrmann, 1995; Srinivasan et al., 1956), is responsible for the biosynthesis of fundamental aromatic amino acids such as phenylalanine, tyrosine and tryptophan (Herrmann and Weaver, 1999). Regarding Gly, the site of inhibiting action is in the sixth step of the pathway, where the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) catalyses the reaction between shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP), generating 5-enolpyruvylshikimate-3-phosphate (EPSP), an intermediary that leads to the formation of chorismate, precursor of the three aromatic amino acids (Maeda and Dudareva, 2012; Zhan et al., 2018) (Fig. 1). In addition, all three types of amino acids are precursors to a large variety of secondary plant metabolites and the intermediates of the main branch of the shikimate pathway also serve as starting points for biosynthesis of secondary products (Herrmann, 1995).

The enzyme EPSP synthase has a monomeric structure and a molecular mass close to 46 Kda. Furthermore, it is obtained by purification (Shuttleworth et al., 1992) and its 3D formation has been confirmed by X-ray crystallography in *Escherichia coli* (Schonbrunn et al., 2001).

EPSP synthase has two domains, whose intersection or interdomain is located at Gly's action site. The reaction is carried out through the cleavage of the C—O bond, with the PO— cleavage being normal in almost all enzymes that have PEP as substrate. Gly is structurally similar to PEP and competes with it to inhibit EPSP synthase in a slowly reversible reaction. This enzyme is folded into two almost equal domains, each of which comprises of three copies of a βαβαββ-folding unit (Ribeiro Marques et al., 2007).

The two domains of the EPSP synthase approach each other, producing a cavity in the interdomain. Gly binds near the S3P, almost without disturbing the cavity of the active site, very similar to the enzyme-S3P complex. There is an interaction of hydrogen bonds between the 5-hydroxyl group of S3P and the Gly nitrogen. In addition, there are two further interactions between the substrate and the inhibitor, through the side chains of Lys-22 and the water molecule W2. At the Gly binding site (in both enzymatic domains), there are predominately charged residues, such as Lys-22, Arg-124 and Lys-411, which are involved in the PEP bond. Asp-313 probably acts as a proton acceptor, whereas Lys-22 can protonate the oxygen of the scissile bond to support the formation of inorganic phosphate (Schonbrunn et al., 2001). The change of conformation between the open and closed state is thus due to the S3P being joined in a first step, producing an accumulation of positive charges in the cavity, having the capacity to attract negatively charged molecules to the active site of the enzyme (PEP or Gly). Consequently, Gly occupies the binding site of the PEP in a competitive manner, rather than binding to the enzyme allosterically (Herrmann and Weaver, 1999; Ribeiro Marques et al., 2007),

3. Glyphosate main toxic effects

A concern is emerging on the toxicity of Gly in humans, considering that the majority of Gly and GBH's effects have been observed in mammals. It was shown that there is a direct relationship between infertility and deformities in pigs, and exposure to Gly in high concentrations in the liver and kidney (Krüger et al., 2014b, 2014a). Sufficient evidence confirms a correlation between an increase in the use of Gly and a wide variety of human diseases. Investigations show harmful outcomes including kidney damage, various types of cancer and neurological and emotional diseases such as attention deficit hyperactivity disorder, autism, depression, anxiety, Alzheimer's and Parkinson's disease (Van Bruggen et al., 2018). The International Agency Research on Cancer (IARC), the specialised cancer organisation of the World Health Organization (WHO), reclassified Gly as a probable carcinogen to humans (Group 2A) based on sufficient evidence of its carcinogenic nature in experimental animals and limited evidence in humans (International Agency for Research on Cancer, 2015). This potential role as a carcinogenic molecule has generated great discussion amongst researchers. In fact, in 2016 the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) postulated that such a risk does not exist because its carcinogenic effect in mice is produced at such high doses that would be impossible to reach in humans (FAO/WHO Joint Meeting on Pesticide Residues, 2016). Nevertheless, several findings support and confirm that Gly and GBH induce DNA damage in rodents treated with 400 mg/kg, as well as human cell lines at acceptable daily intake doses of 0,5 μg/ml. For example, there is an increase in the amount of micronuclei cell and chromosomal aberrations in human lymphocytes and stimulation of cell proliferation in the presence of Gly (Guyton et al., 2015; Kašuba et al., 2017; Mañas et al., 2006; Santovito et al., 2018). The idea that Gly could generate alterations related to the development of tumours is reinforced by epidemiological studies which show that occupational exposure to Gly considerably increases the prevalence of Non-Hodgkin´s lymphoma and suggests a risk of multiple myeloma

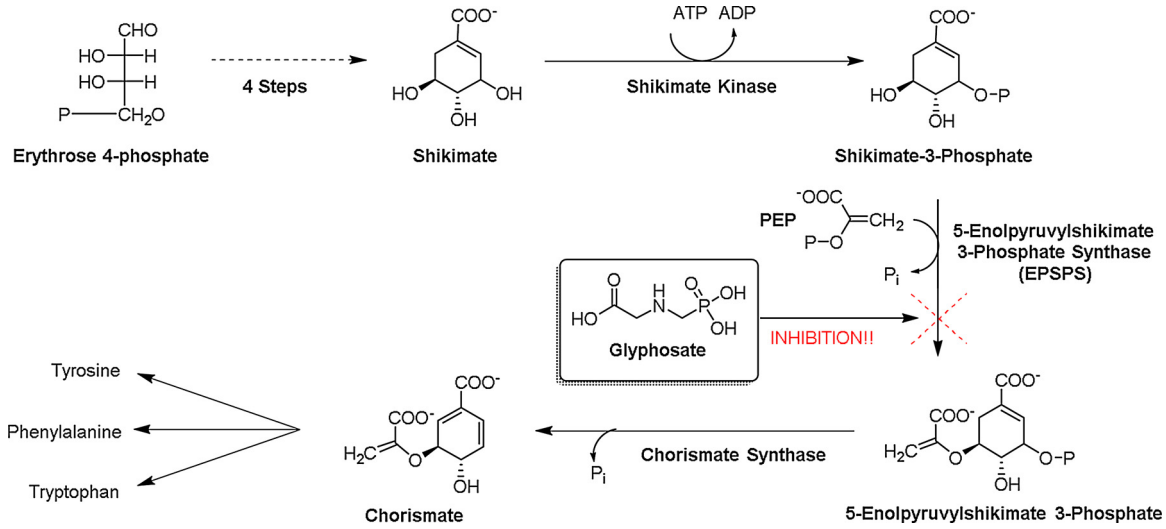

Fig. 1. Glyphosate mechanism of action inhibiting shikimic acid pathway.

occurring (De Roos et al., 2005; Eriksson et al., 2008). In addition, its exposure, from prenatal period to adulthood, induces endocrine effects (Manservisi et al., 2019), as diverse studies have confirmed effects on aromatase activity and reproductive alterations of Gly and GBH on human cell lines and rat testis (de Liz Oliveira Cavalli et al., 2013; Gasnier et al., 2009; Richard et al., 2005). Likewise, a proliferative effect on the hormone-dependent T47D cell line in the presence of Gly was found through activation of the estrogenic pathway (Thongprakaisang et al., 2013). Nevertheless, the European Food Safety Authority (EFSA) rejected the claim of Gly having hormonal disruption properties (European Food Safety Authority, 2017). Moreover, the European Union authorised its use for the next 5 years (European Commision, 2017). Further to this controversy, it has also been suggested that Gly can have a harmful effect on the bacterial enzymes of the intestine, which involves the shikimate pathway (Aitbali et al., 2018). Gly competitively inhibits EPSP synthase, blocking the formation of aromatic amino acids such as phenylalanine, tyrosine and tryptophan, essential for cellular survival (Schonbrunn et al., 2001).

Gly was specifically designed to eliminate grass and weeds which express EPSP. Similarly, yeast, algae, bacterial strains and fungi also express EPSP, therefore glyphosate also affects them (Bode et al., 1984; Cao et al., 2012; Du et al., 2000; Helander et al., 2018; Smedbol et al., 2017). Conversely, this enzyme is absent in mammals, since they obtain the three amino acids from diet, meaning a toxic effect of Gly through the shikimate pathway is improbable. Chemical compounds that interfere with the activity of EPSP synthase in this route are considered "safe" for humans when handled in reasonable concentrations. However, there is emerging evidence of the harmful effect on gut microbiota in animal models, suggesting Gly having a noxious role in human homeostasis.

It has been largely proposed that Gly modifies the microorganism's composition in the gut by beneficial bacteria generating a gut microbial imbalance or dysbiosis (Table 1). A decrease in the overall number of intestinal bacteria has been reported after repeated oral ingestion of both chronic and subchronic GBH doses in mice. Their gut microbiota showed high levels of *Firmicutes* and *Corynebacterium* phylum in comparison with control animals (Aitbali et al., 2018). A similar study proposes a relationship between sex and dysbiosis induced by Gly, characterised by an increase in *Bacteroidetes* over *Lactobacillus*, principally affecting female rats (Lozano et al., 2018). Moreover, changes in *Firmicutes* phylum has also been seen in rat pups after maternal exposure to GBH through drinking water (Mao et al., 2018). Honeybees' gut microbiota is also vulnerable to Gly, producing a weakening in beneficial bacterial composition. The bacterial community is reduced in the presence of Gly, making bees more susceptible to infection by *Serratia*, an opportunistic pathogen that promotes mortality (Motta et al., 2018). Likewise, high levels of botulinum neurotoxin were found in the rumen fluid of cow microbiota following the consumption of Gly-treated water, as well as a reduction in *Entodinium* spp, *Epidinium* spp,

Table 1
Altered intestinal microbiota following Gly and GBH exposure.

Model	Exposition	Changes in microbiota composition	References
Mouse	Oral ingestion of Gly and GBH	↑ *Firmicutes* spp. ↑ *Corynebacterium* spp. ↓ *Bacteroidetes* spp. ↓ *Lactobacillus* spp.	(Aitbali et al., 2018)
Rat	Oral ingestion of GBH	↑ *Bacteroidetes* spp. ↓ *Lactobacillus* spp. (mainly on females)	(Lozano et al., 2018)
Rat pup	Maternal oral ingestion of Gly and GBH	↑ *Prevotella* genus ↑ *Muscispirillum* genus ↓ Lactobacillus genus ↑ *Aggregatibacter* genus	(Mao et al., 2018)
Honey bee	Oral ingestion of Gly	↑ Guilliamella apicola ↓ Snodgrasella alvi ↓ Bifidobacterium ↓ Lactobacillus	(Motta et al., 2018)
Cow	Oral ingestion of Gly	↓ *Entodinium* ↓ *Epidinium* ↓ *Ophryoscolex* ↓ *Dasytricha*	(Ackermann et al., 2015)
Enterococcus strains	Incubation with Gly and GBH	↓ *Enterococcus* spp.	(Krüger et al., 2013)
Poultry microbiota in vitro	Incubation with GBH	↑ *Echerichia coli* ↑ *Salmonella enteriditis* ↑ *Salmonella typhiminium* ↑ *Salmonella gallinarum* ↑ *Clostridia* spp. ↓ *Enterococcus faecalis* ↓ *Enterococcus faecium* ↓ *Bacillus badius* ↓ *Bacillus cereus*	(Shehata et al., 2013)

Ophryoscolex spp and *Dasytricha* spp (Ackermann et al., 2015). However, changes in the microbial composition in the cow rumen have not been discovered in another study (Riede et al., 2016). The presence of Gly on strains of enterococcal isolated from horse and cattle induced low levels of *Enterococcus* spp., related to an alteration in its inhibitory role against *Clostridium botulinum*, causing diseases such as diarrhoea or pseudomembranous colitis (Krüger et al., 2013).

Beneficial intestinal bacteria such as *Enterococcus faecalis*, *E. faecium*, *Bacillus badius* and *B. cereus* were found to be sensitive to Gly, whereas pathogenic bacteria such as *Escherichia coli*, *Salmonella enteritidis*, *S. typhimurium*, *S. galliarum* and the Clostridia species have shown marked resistance to Gly in experiments on poultry microbiota (Shehata et al., 2013). The mechanisms underlying their resistance are yet to be ascertained. Herein, the different types of EPSP synthases (class I and II) are noteworthy. The class I enzyme is found in many bacteria and plants, while the class II enzyme has only been found in some Gly-resistant bacteria. Both have a similar structure but a different amino acid sequence. It has been suggested that the difference in various amino acid residues could lead to sensitivity to Gly in the class I EPSPS, while the class II enzyme would be involved in resistance to this herbicide (Li et al., 2009). Furthermore, it has been reported that mutations in the class II EPSP synthase and the production of mycotiol, a molecule involved in the detoxification of antibiotics, heavy metals or aromatic compounds of *Actinobacteria*, may also be involved in Gly resistance (Van Bruggen et al., 2018).

A striking case of voluntary ingestion of Gly was reported and indicated a predisposition to the development of *Clostridium* tertium bacteraemia in humans together with an effect on intestinal mucosa (You et al., 2015). Few studies have been performed on Gly or GBH on microbiota in mammals, but so far, all findings indicate an overgrowth of Gly-resistant microorganisms such as *Clostridium spp* and possibly several *Salmonella* strains (Krüger et al., 2013; Shehata et al., 2013).

4. Gly and microbiota-related disorders

It is worth emphasising that Gly directly induces neurotoxicity in CNS. For instance, Gly and GBH generate biochemical, neurophysiological and developmental cerebral deviations, such as changes in the levels of monoaminergic neurotransmitters (Martínez et al., 2018), generation of an oxidative environment (El-Shenawy, 2009), glutamatergic excitotoxicity (Cattani et al., 2017) and deformities (Paganelli et al., 2010). These changes may be related to a disruption of the gut-brain axis, as shown in both the preclinical and clinical studies reviewed below.

4.1. Preclinical findings

It is increasingly clear that a disturbance in the diversity and the richness of gut microbes is strongly related to depression/anxiety by the gut-brain axis. In fact, the transplantation of faecal microbiota from depressed patients to rodents can provoke depression-like symptoms (Kelly et al., 2016; Zheng et al., 2016). Germ-free mice exhibited anxiolytic behaviours compared with specific pathogen-free mice, accompanied by neurochemical shifts in the amygdala and dentate granule layer of the hippocampus (Neufeld et al., 2011). In addition, after a 7-day treatment with enrofloxacin in rodents, alteration of the microbial composition was induced, causing aggressive behaviour (Sylvia et al., 2017). Infection with pathogens such as *Campylobacter jejuni*, *Citrobacter rodentium* or *Trichuris muris* increased anxiety-like behaviours in animals (Bercik et al., 2010; Goehler et al., 2008; Lyte et al., 2006). Moreover, oral exposure to GBH reduced the abundance of *Firmicutes*, *Corynebacterium*, *Bacteroidetes* and *Lactobacillus* and it was associated with the appearance of anxiogenic and depressive behaviours in mice (Aitbali et al., 2018). Depressive and anxiety-like disorders have been associated with changes in the diversity and richness of gut microbes in rats together with an increase in proinflammatory cytokines (O'Mahony et al., 2009). Furthermore, neurochemical alterations were discovered following subchronic exposure to GBH in rats' offspring, such as glutamatergic overactivity and oxidative damage in the hippocampus, which were associated with depressive behaviours in adult offspring (Cattani et al., 2017).

Accumulating evidence indicates that bacterial-wall compounds participate in the neuropathological changes of ASD. For instance, lipopolysaccharides (LPS) are glycolipid endotoxins anchored in the outer cell wall of Gram-negative enterobacteria which are released into intestinal lumen during multiplication or lysis processes (Alexander and Rietschel, 2001). It is well-established that LPS binds to LPS binding protein (LPB) or CD4 to activate TLR4, triggering an increase in NF-KB activity, which induces the transcription of inflammatory signalling and immune-related genes in different tissues. Besides the effect of cytokines, it has been suggested that LPS is also able to reach the brain minimally by crossing the blood-brain barrier (Banks and Robinson, 2010; Zhou et al., 2014) or by modulating afferent vagal fibres (Hansen et al., 2000). Concerning the present review, *Clostridium* is resistant to Gly, so it is not implicated in LPS-induced cerebral toxicity, because it belongs to the Gram-positive bacteria group. *E. coli*, *S. enteritidis*, *S. typhimurium* and *S. galliarum*, which are significantly elevated in the presence of Gly in the poultry gut (Shehata et al., 2013), may be involved in the predisposition to brain dysfunction by LPS in their cellular walls. In rats subjected to oral ingestion of LPS, an increase in anxiety-like behaviours was discovered (Fields et al., 2018). Consequently, the reconstitution of intestinal microbiota through treatment with *Lactobacillus reuteri* and *Bifidobacterium adolescentis* decreased depressive and anxiety-like symptoms in stress-induced mice displaying anxiety/depression (Jang et al., 2019; Kantak et al., 2014).

Disruption of faecal mucosa induced by rotenone was found in the mouse model for Parkinson´s disease (PD) with a lower ratio of *Firmicutes/Bacteroidetes* than in the control group (Perez-Pardo et al., 2018). Another study showed how phylum *Firmicutes* decrease and phylum *Proteobacteria* increase in PD mice along with a marked augmentation of bacterial metabolites. In these PD animals, faecal microbiota transplantation diminished the motor impairment and microglial activity of substantia nigra (Sun et al., 2018).

The role of intestinal microbiota was also related to the pathogenesis of Alzheimer´s disease (AD), mainly by neuroinflammatory mechanisms. Bacterial faecal composition changed in Aβ precursor protein (APP) transgenic mice with an increase in a genus of *Rikenellaceae* and a reduction in the proportion of *Akkermansia* and *Allobaculum* (Harach et al., 2017). The administration of probiotics such as *Lactobacillus* and *Bifidobacterium* improved memory, behavioural deficits and mitigated the Aβ plaque formation in AD rodent models (Athari Nik Azm et al., 2018; Kobayashi et al., 2017). All in all, these findings suggest that compositional problems associated with the intestinal microbiota may be triggers for behavioural disorders.

4.2. Clinical findings

Regarding depression/anxiety, the clinical data so far correspond with the results observed in animals concerning the relationship between gut microbiota and these disorders. Depressive patients both resistant and non-resistant to the treatment, presented alterations of the intestinal composition with a marked reduction in *Firmicutes*, *Bacteroidetes* and *Proteobacteria* compared with healthy individuals (Jiang et al., 2015; Kelly et al., 2016). Combined administration of probiotics containing *Lactobacillus helveticus* and *Bifidobacterium longum* had anti-depressive and anxiolytic effects in comparison with the non-treated group (Messaoudi et al., 2011). As shown in section 2, Gly can provoke several emotional diseases (Van Bruggen et al., 2018) as well as intestinal dysbiosis. One possibility is that these changes in gut bacterial composition may generate alterations in the production of short-chain fatty acids (SCFA) in the intestinal lumen. SCFAs such as butyric (BA), propionic (PPA) or acetic acid (AA) are metabolites

derived from the bacterial fermentation of dietary fibre and play a variety of roles in health maintenance, not only on the intestine itself as an energy source improving transit, but also in supporting the immune system (Wichmann et al., 2013). Moreover, unsuitable levels of SCFA have been suggested as predisposing factors to suffering from neuropsychiatric diseases (MacFabe et al., 2007; Zhang et al., 2017).

It was also found that Gly severely depletes manganese (Mn) levels in plants. Glyphosate's disruption of Mn homeostasis selectively affects *Lactobacillus* and can lead to several disorders such as PD (Samsel and Seneff, 2015). In fact, there is an increase in the incidence of PD in urban areas with a higher industrial release of manganese (Willis et al., 2010), and there is some accidental exposure to Gly that causes symptomatology related to PD (Barbosa et al., 2001). Although the mechanism remains unclear, it could be related to dysbiosis. Intestinal dysbiosis and increased permeability in PD patients cause an immunological stimulation, whereas the activation of enteric neurons by microbiota-derived metabolites may contribute to the initiation of incorrect α-synuclein folding (Caputi and Giron, 2018; Poirier et al., 2016). Moreover, a correlation was found between metabolites from intestinal microorganisms and the development of symptoms in autistic patients during a situation of dysbiosis (Roman et al., 2018). In this regard, an interrelation between Clostridium bacteria's colonization of the intestinal tract and autism has been demonstrated (Argou-Cardozo and Zeidán-Chuliá, 2018).

Human gut dysbiosis is also linked to the aetiology of AD in animals exposed to Gly, where there is an imbalance in the intestinal bacterial composition. AD patients showed a reduced level of both *Firmicutes* and *Bifidobacterium* and an increase in Bacteroidetes (Vogt et al., 2017). Moreover, a correlation was noted between an abundance of *Escherichia* and *Shigella* in the guts of AD individuals and brain amyloidosis and behavioural impairment (Cattaneo et al., 2017). Additionally, elevated levels of LPS have been found in the brain and in plasma of patients with AD (Zhan et al., 2016; Zhang et al., 2009).

Additionally, Gly exposure has recently been associated with an increase in coeliac disease, probably owing to the reduction in levels of *Lactobacillus* and *Bifidobacteria* and to the *p*-cresol toxicity (Samsel and Seneff, 2013). It was also revealed that *p*-cresol and 3-[3-hydroxyphenyl]-3-hydroxypropionic acid (HPHPA), another Clostridia phenolic metabolite, used as a urinary marker in ASD-children (Shaw, 2010; Xiong et al., 2016), inhibit dopamine-beta-hydroxylase, which converts dopamine to noradrenaline (DeWolf et al., 1988; Goodhart et al., 1987). Consequently, a positive correlation between the use of Gly and ASD was proposed. The suggested mechanism was similar to Parkinson´s disease, in that the overproduction of metabolites from *Clostridium* spp. provokes an excess of dopamine and its metabolites, generating ROS through formation of dopamine quinone species, leading mitochondrial dysfunction and oxidative stress (Shaw, 2017).

The developing healthy population is considered to be one of the most vulnerable to environmental pollutants because they do not have fully developed immune, digestive and neurological systems. Exposure to any contaminant from air, water or soil might cause damage to their physical or mental development, which is attributable to Gly. Children may consume Gly in breakfast cereals, causing gut microbial disbalance. The idea that Gly is able to induce dysbiosis in young individuals is compounded in experiments on mammals. Although there are few studies, a disturbance in bowel microorganism levels was noted after exposure to Gly that induced an increase in anxiety and depression-related behaviours (Aitbali et al., 2018). Gastrointestinal symptoms present in autistic children have been related to an intestinal microbiome alteration showing high levels of pathogenic bacteria and gastrointestinal problems (Roman et al., 2018). Evidence of correlations between neurodevelopmental disorders and Gly perinatal exposure were reported in pregnant rodents treated with Gly (Yu et al., 2018), where processes of enzymatic disruption (Daruich et al., 2001; Gallegos et al., 2018) and induction of glutamate release emerged in offspring (Cattani et al., 2014).

It is well known that autistic children's gut microbial density differs to that of healthy children, with a decrease in the beneficial bacterial group, *Bifidobacterium* (De Angelis et al., 2013). Recent findings indicate a close connection between metabolites from gut microbiota and autistic disorders. Thus, perinatal exposure to LPS was revealed as risk factor in the presence of ASD in progeny (Knuesel et al., 2014) and the presence of *p*-cresol in faeces or urine is used as marker of ASD (Altieri et al., 2011; Gabriele et al., 2016). However, it is still unclear whether a bacterial imbalance is produced after the appearance of ASD or it is a trigger in the symptomatology of this pathology.

Finally, relating to most studies, the main limitation is the use of high doses of Gly or GBH, which is difficult to obtain in mammalian tissues, calling into question clinical relevance. Additionally, it has been suggested that the harmful effects of Gly may be due to the adjuvants in formulations of GBH like Roundup®, among others. For instance, in order for Gly to penetrate the plant cell, the presence of surfactants, such as polyoxyethyleneamine, which exhibits a potent cytotoxic effect, is essential (Defarge et al., 2018). In line with this, many investigations have been conducted with Gly instead of commercial formulations based on Gly.

To our knowledge, the lack of information, contradictory data and independency of studies have generated controversy concerning the safety of Gly for humans. Despite the usage of Gly being permitted, its indiscriminate utilisation may have a harmful effect on human health. We have assessed the mechanisms by which a Gly-induced intestinal microbiome disturbance could be involved in emotional disorders and neurological diseases such as ASD. However, more research is certainly required to expound the role of Gly on the gut's bacterial community and its outcomes in neurobehavioral diseases. Moreover, owing to a lack of existing literature, future research should evaluate the role of innovative approaches such as the utilisation of NAC, vitamin C, vitamin E, cyclophosphamide or probiotics to treat herbicide poisoning (Cherukuri et al., 2014).

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Declaration of Competing Interest

The authors declare that there have no conflicts of interest.

References

Ackermann, W., Coenen, M., Schrödl, W., Shehata, A.A., Krüger, M., 2015. The influence of glyphosate on the microbiota and production of *botulinum* neurotoxin during ruminal fermentation. Curr. Microbiol. 70, 374–382. https://doi.org/10.1007/s00284-014-0732-3.

Aitbali, Y., Ba-M'hamed, S., Elhidar, N., Nafis, A., Soraa, N., Bennis, M., 2018. Glyphosate based- herbicide exposure affects gut microbiota, anxiety and depression-like behaviors in mice. Neurotoxicol. Teratol. 67, 44–49. https://doi.org/10.1016/J.NTT.2018.04.002.

Alexander, C., Rietschel, E.T., 2001. Bacterial lipopolysaccharides and innate immunity. J. Endotoxin Res. 7, 167–202.

Altieri, L., Neri, C., Sacco, R., Curatolo, P., Benvenuto, A., Muratori, F., Santocchi, E., Bravaccio, C., Lenti, C., Saccani, M., Rigardetto, R., Gandione, M., Urbani, A., Persico, A.M., 2011. Urinary p-cresol is elevated in small children with severe autism spectrum disorder. Biomarkers 16, 252–260. https://doi.org/10.3109/1354750X.2010.548010.

Argou-Cardozo, I., Zeidán-Chuliá, F., 2018. *Clostridium* Bacteria and autism Spectrum conditions: a systematic review and hypothetical contribution of environmental glyphosate levels. Med. Sci. 6, 29. https://doi.org/10.3390/medsci6020029.

Athari Nik Azm, S., Djazayeri, A., Safa, M., Azami, K., Ahmadvand, B., Sabbaghziarani, F., Sharifzadeh, M., Vafa, M., 2018. *Lactobacilli* and *bifidobacteria* ameliorate memory and learning deficits and oxidative stress in β-amyloid (1-42) injected rats. Appl. Physiol. Nutr. Metab. 43, 718–726. https://doi.org/10.1139/apnm-2017-0648.

Banks, W.A., Robinson, S.M., 2010. Minimal penetration of lipopolysaccharide across the murine blood-brain barrier. Brain Behav. Immun. 24, 102–109. https://doi.org/10.1016/j.bbi.2009.09.001.

Barbosa, E.R., Leiros da Costa, M.D., Bacheschi, L.A., Scaff, M., Leite, C.C., 2001. Parkinsonism after glycine-derivate exposure. Mov. Disord. 16, 565–568.

Bercik, P., Verdu, E.F., Foster, J.A., Macri, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K.A., Lu, J., Khan, W.I., Corthesy-Theulaz, I., Cherbut, C., Bergonzelli, G.E., Collins, S.M., 2010. Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139, 2102–2112. https://doi.org/10.1053/j.gastro.2010.06.063. e1.

Bode, R., Melo, C., Birnbaum, D., 1984. Mode of action of glyphosate in Candida maltosa. Arch. Microbiol. 140, 83–85.

Bonaz, B., 2013. Inflammatory bowel diseases: a dysfunction of brain-gut interactions? Minerva Gastroenterol. Dietol. 59, 241–259.

Brewster, D.W., Warren, J., Hopkins, W.E., 1991. Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. Fundam. Appl. Toxicol. 17, 43–51.

Bridi, D., Altenhofen, S., Gonzalez, J.B., Reolon, G.K., Bonan, C.D., 2017. Glyphosate and Roundup® alter morphology and behavior in zebrafish. Toxicology 392, 32–39. https://doi.org/10.1016/j.tox.2017.10.007.

Cao, G., Liu, Y., Zhang, S., Yang, X., Chen, R., Zhang, Y., Lu, W., Liu, Y., Wang, J., Lin, M., Wang, G., 2012. A novel 5-Enolpyruvylshikimate-3-Phosphate synthase shows high glyphosate tolerance in *Escherichia coli* and tobacco plants. PLoS One 7, e38718. https://doi.org/10.1371/journal.pone.0038718.

Caputi, V., Giron, M., 2018. Microbiome-gut-Brain Axis and toll-like receptors in Parkinson's disease. Int. J. Mol. Sci. 19, 1689. https://doi.org/10.3390/ijms19061689.

Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C., Guerra, U.P., Paghera, B., Muscio, C., Bianchetti, A., Volta, G.D., Turla, M., Cotelli, M.S., Gennuso, M., Prelle, A., Zanetti, O., Lussignoli, G., Mirabile, D., Bellandi, D., Gentile, S., Belotti, G., Villani, D., Harach, T., Bolmont, T., Padovani, A., Boccardi, M., Frisoni, G.B., INDIA-FBP Group, 2017. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol. Aging 49, 60–68. https://doi.org/10.1016/j.neurobiolaging.2016.08.019.

Cattani, D., Cesconetto, P.A., Tavares, M.K., Parisotto, E.B., De Oliveira, P.A., Rieg, C.E.H., Leite, M.C., Prediger, R.D.S., Wendt, N.C., Razzera, G., Filho, D.W., Zamoner, A., 2017. Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: implication of glutamate excitotoxicity and oxidative stress. Toxicology 387, 67–80. https://doi.org/10.1016/j.tox.2017.06.001.

Cattani, D., de Liz Oliveira Cavalli, V.L., Heinz Rieg, C.E., Domingues, J.T., Dal-Cim, T., Tasca, C.I., Mena Barreto Silva, F.R., Zamoner, A., 2014. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. Toxicology 320, 34–45. https://doi.org/10.1016/j.tox.2014.03.001.

Cherukuri, H., Pramoda, K., Rohini, D., Thunga, G., Vijaynarayana, K., Sreedharan, N., Varma, M., Pandit, V., 2014. Demographics, clinical characteristics and management of herbicide poisoning in tertiary care hospital. Toxicol. Int. 21, 209–213. https://doi.org/10.4103/0971-6580.139813.

Cook, K., 2019. Glyphosate in Beer and Wine.

Daruich, J., Zirulnik, F., Gimenez, M.S., 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses. Environ. Res. 85, 226–231. https://doi.org/10.1006/enrs.2000.4229.

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazzanetti, D.I., Cristofori, F., Guerzoni, M.E., Gobbetti, M., Francavilla, R., 2013. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS One 8, e76993. https://doi.org/10.1371/journal.pone.0076993.

de Liz Oliveira Cavalli, V.L., Cattani, D., Heinz Rieg, C.E., Pierozan, P., Zanatta, L., Benedetti Parisotto, E., Wilhelm Filho, D., Mena Barreto Silva, F.R., Pessoa-Pureur, R., Zamoner, A., 2013. Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. Free Radic. Biol. Med. 65, 335–346. https://doi.org/10.1016/j.freeradbiomed.2013.06.043.

De Roos, A.J., Blair, A., Rusiecki, J.A., Hoppin, J.A., Svec, M., Dosemeci, M., Sandler, D.P., Alavanja, M.C., 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environ. Health Perspect. 113, 49–54. https://doi.org/10.1289/ehp.7340.

Defarge, N., Spiroux de Vendômois, J., Séralini, G.E., 2018. Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. Toxicol. reports 5, 156–163. https://doi.org/10.1016/j.toxrep.2017.12.025.

DeWolf, W.E., Carr, S.A., Varrichio, A., Goodhart, P.J., Mentzer, M.A., Roberts, G.D., Southan, C., Dolle, R.E., Kruse, L.I., 1988. Inactivation of dopamine beta-hydroxylase by p-cresol: isolation and characterization of covalently modified active site peptides. Biochemistry 27, 9093–9101.

Du, W., Wallis, N.G., Mazzulla, M.J., Chalker, A.F., Zhang, L., Liu, W.S., Kallender, H., Payne, D.J., 2000. Characterization of *Streptococcus pneumoniae* 5-enolpyruvylshikimate 3-phosphate synthase and its activation by univalent cations. Eur. J. Biochem. 267, 222–227.

El-Shenawy, N.S., 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environ. Toxicol. Pharmacol. 28, 379–385. https://doi.org/10.1016/j.etap.2009.06.001.

Eriksson, M., Hardell, L., Carlberg, M., Akerman, M., 2008. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. Int. J. Cancer 123, 1657–1663. https://doi.org/10.1002/ijc.23589.

European Commision, 2017. Health and Food Safety Directorate-general. Summary Report of the Appeal Committe - Phytopharmaceuticals - Plan Protection Products - Legislation.

European Food Safety Authority, 2017. Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA J. 15. https://doi.org/10.2903/j.efsa.2017.4979.

FAO/WHO Joint Meeting on Pesticide Residues, 2016. Summary Report for Diazinon, Glyphosate, Malathion. food and agriculture organization of the United Nations/Geneva, World Health Organization (WHO), Geneva, Switzerland.

Fields, C.T., Chassaing, B., Castillo-Ruiz, A., Osan, R., Gewirtz, A.T., de Vries, G.J., 2018. Effects of gut-derived endotoxin on anxiety-like and repetitive behaviors in male and female mice. Biol. Sex Differ. 9, 7. https://doi.org/10.1186/s13293-018-0166-x.

Forsythe, P., Bienenstock, J., Kunze, W.A., 2014. Vagal pathways for microbiome-brain-gut axis communication. Advances in Experimental Medicine and Biology. pp. 115–133. https://doi.org/10.1007/978-1-4939-0897-4_5.

Frontera, J.L., Vatnick, I., Chaulet, A., Rodríguez, E.M., 2011. Effects of glyphosate and polyoxyethylenamine on growth and energetic reserves in the freshwater crayfish Cherax quadricarinatus (Decapoda, Parastacidae). Arch. Environ. Contam. Toxicol. 61, 590–598. https://doi.org/10.1007/s00244-011-9661-3.

Furness, J.B., Callaghan, B.P., Rivera, L.R., Cho, H.-J., 2014. The enteric nervous system and gastrointestinal innervation: integrated local and central control. Advances in Experimental Medicine and Biology. pp. 39–71. https://doi.org/10.1007/978-1-4939-0897-4_3.

Gabriele, S., Sacco, R., Altieri, L., Neri, C., Urbani, A., Bravaccio, C., Riccio, M.P., Iovene, M.R., Bombace, F., De Magistris, L., Persico, A.M., 2016. Slow intestinal transit contributes to elevate urinary *p* -cresol level in Italian autistic children. Autism Res. 9, 752–759. https://doi.org/10.1002/aur.1571.

Gallegos, C.E., Baier, C.J., Bartos, M., Bras, C., Domínguez, S., Mónaco, N., Gumilar, F., Giménez, M.S., Minetti, A., 2018. Perinatal glyphosate-based herbicide exposure in rats alters brain antioxidant status, glutamate and acetylcholine metabolism and affects recognition memory. Neurotox. Res. 34, 363–374. https://doi.org/10.1007/s12640-018-9894-2.

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.-C., Séralini, G.-E., 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262, 184–191. https://doi.org/10.1016/j.tox.2009.06.006.

Goehler, L.E., Park, S.M., Opitz, N., Lyte, M., Gaykema, R.P.A., 2008. *Campylobacter jejuni* infection increases anxiety-like behavior in the holeboard: possible anatomical substrates for viscerosensory modulation of exploratory behavior. Brain Behav. Immun. 22, 354–366. https://doi.org/10.1016/j.bbi.2007.08.009.

Goodhart, P.J., DeWolf, W.E., Kruse, L.I., 1987. Mechanism-based inactivation of dopamine beta-hydroxylase by p-cresol and related alkylphenols. Biochemistry 26, 2576–2583.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K., International Agency for Research on Cancer Monograph Working Group, IARC, Lyon, France, 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490–491. https://doi.org/10.1016/S1470-2045(15)70134-8.

Hansen, M.K., Nguyen, K.T., Goehler, L.E., Gaykema, R.P., Fleshner, M., Maier, S.F., Watkins, L.R., 2000. Effects of vagotomy on lipopolysaccharide-induced brain interleukin-1beta protein in rats. Auton. Neurosci. 85, 119–126.

Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., Mc Coy, K.D., Frisoni, G., Neher, J.J., Fåk, F., Jucker, M., Lasser, T., Bolmont, T., 2017. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci. Rep. 7, 41802. https://doi.org/10.1038/srep41802.

Helander, M., Saloniemi, I., Omacini, M., Druille, M., Salminen, J.-P., Saikkonen, K., 2018. Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback. Sci. Total Environ. 642, 285–291. https://doi.org/10.1016/j.scitotenv.2018.05.377.

Herrmann, K.M., 1995. The shikimate pathway: early steps in the biosynthesis of aromatic compounds. Plant Cell 7, 907. https://doi.org/10.2307/3870046.

Herrmann, K.M., Weaver, L.M., 1999. The shikimate pathway. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 473–503. https://doi.org/10.1146/annurev.arplant.50.1.473.

Huang, Y., Shi, X., Li, Z., Shen, Y., Shi, X., Wang, L., Li, G., Yuan, Y., Wang, J., Zhang, Y., Zhao, L., Zhang, M., Kang, Y., Liang, Y., 2018. Possible association of *Firmicutes* in the gut microbiota of patients with major depressive disorder. Neuropsychiatr. Dis. Treat. 14, 3329–3337. https://doi.org/10.2147/NDT.S188340.

Icaza-Chávez, M.E., 2013. [Gut microbiota in health and disease]. Rev. Gastroenterol. Mex. 78, 240–248. https://doi.org/10.1016/j.rgmx.2013.04.004.

International Agency for Research on Cancer, 2015. IARC Monographs Volume 112: Evaluation of Five Organophosphate Insecticides and Herbicides. World Heal. Organ., Lyon.

Jang, H.-M., Lee, K.-E., Kim, D.-H., 2019. The preventive and curative effects of *Lactobacillus reuteri* NK33 and *Bifidobacterium adolescentis* NK98 on immobilization stress-induced anxiety/depression and colitis in mice. Nutrients 11, 819. https://doi.org/10.3390/nu11040819.

Jia, L., Jia, Q., Yang, J., Jia, R., Zhang, H., 2018. Efficacy of probiotics supplementation on chronic kidney disease: a systematic review and meta-analysis. Kidney Blood Press. Res. 43, 1623–1635. https://doi.org/10.1159/000494677.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., Ruan, B., 2015. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav. Immun. 48, 186–194. https://doi.org/10.1016/j.bbi.2015.03.016.

Kantak, P.A., Bobrow, D.N., Nyby, J.G., 2014. Obsessive-compulsive-like behaviors in house mice are attenuated by a probiotic (*Lactobacillus rhamnosus* GG). Behav. Pharmacol. 25, 71–79. https://doi.org/10.1097/FBP.0000000000000013.

Kassinen, A., Krogius-Kurikka, L., Mäkivuokko, H., Rinttilä, T., Paulin, L., Corander, J., Malinen, E., Apajalahti, J., Palva, A., 2007. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology 133, 24–33. https://doi.org/10.1053/j.gastro.2007.04.005.

Kašuba, V., Milić, M., Rozgaj, R., Kopjar, N., Mladinić, M., Žunec, S., Vrdoljak, A.L., Pavičić, I., Čermak, A.M.M., Pizent, A., Lovaković, B.T., Želježić, D., 2017. Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line. Environ. Sci. Pollut. Res. 24, 19267–19281. https://doi.org/10.

1007/s11356-017-9438-y.
Kelly, J.R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., Hoban, A.E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J.F., Dinan, T.G., 2016. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. J. Psychiatr. Res. 82, 109–118. https://doi.org/10.1016/j.jpsychires.2016.07.019.
Knuesel, I., Chicha, L., Britschgi, M., Schobel, S.A., Bodmer, M., Hellings, J.A., Toovey, S., Prinssen, E.P., 2014. Maternal immune activation and abnormal brain development across CNS disorders. Nat. Rev. Neurol. 10, 643–660. https://doi.org/10.1038/nrneurol.2014.187.
Kobayashi, Y., Sugahara, H., Shimada, K., Mitsuyama, E., Kuhara, T., Yasuoka, A., Kondo, T., Abe, K., Xiao, J., 2017. Therapeutic potential of *Bifidobacterium breve* strain A1 for preventing cognitive impairment in Alzheimer's disease. Sci. Rep. 7, 13510. https://doi.org/10.1038/s41598-017-13368-2.
Kraehmer, H., Laber, B., Rosinger, C., Schulz, A., 2014. Herbicides as weed control agents: state of the art: I. Weed control research and safener technology: the path to modern agriculture. Plant Physiol. 166, 1119–1131. https://doi.org/10.1104/pp.114.241901.
Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H.W., Lutz, W., Shehata, A., 2014a. Detection of glyphosate residues in animals and humans. Environ. Anal. Toxicol. 4, 1. https://doi.org/10.4172/2161-0525.1000210.
Krüger, M., Schrödl, W., Pedersen, I., 2014b. Detection of glyphosate in malformed piglets. J. Environ. Anal. Toxicol. 4https://doi.org/10.4172/2161-0525.1000230. 2161–0525.
Krüger, M., Shehata, A.A., Schrödl, W., Rodloff, A., 2013. Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*. Anaerobe 20, 74–78. https://doi.org/10.1016/j.anaerobe.2013.01.005.
Li, L., Lu, W., Han, Y., Ping, S., Zhang, W., Chen, M., Zhao, Z., Yan, Y., Jiang, Y., Lin, M., 2009. A novel RPMXR motif among class II 5-enolpyruvylshikimate-3-phosphate synthases is required for enzymatic activity and glyphosate resistance. J. Biotechnol. 144, 330–336. https://doi.org/10.1016/J.JBIOTEC.2009.09.014.
Liu, Y., Li, Y., Hua, X., Müller, K., Wang, H., Yang, T., Wang, Q., Peng, X., Wang, M., Pang, Y., Qi, J., Yang, Y., 2018. Glyphosate application increased catabolic activity of gram-negative bacteria but impaired soil fungal community. Environ. Sci. Pollut. Res. Int. 25, 14762–14772. https://doi.org/10.1007/s11356-018-1676-0.
Lozano, V.L., Defarge, N., Rocque, L.-M., Mesnage, R., Hennequin, D., Cassier, R., de Vendômois, J.S., Panoff, J.-M., Séralini, G.-E., Amiel, C., 2018. Sex-dependent impact of Roundup on the rat gut microbiome. Toxicol. Reports 5, 96–107. https://doi.org/10.1016/j.toxrep.2017.12.005.
Lyte, M., Li, W., Opitz, N., Gaykema, R., Goehler, L., 2006. Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium. Physiol. Behav. 89, 350–357. https://doi.org/10.1016/j.physbeh.2006.06.019.
MacFabe, D., Cain, D., Rodriguez-Capote, K., Franklin, A., Hoffman, J., Boon, F., Taylor, A., Kavaliers, M., Ossenkopp, K., 2007. Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behav. Brain Res. 176, 149–169. https://doi.org/10.1016/j.bbr.2006.07.025.
Maeda, H., Dudareva, N., 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. Annu. Rev. Plant Biol. 63, 73–105. https://doi.org/10.1146/annurev-arplant-042811-105439.
Mañas, F., González, M.B., García, H., Weyers, I., Ugnia, L., Larripa, I.B., Gorla, N., 2006. Evaluation of genotoxicity of the herbicide glyphosate quantitatively measures by the comet assay and micronucleius formation in treated mice. Theoria 15, 53–60.
Manco, M., Putignani, L., Bottazzo, G.F., 2010. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr. Rev. 31, 817–844. https://doi.org/10.1210/er.2009-0030.
Mangiola, F., Ianiro, G., Franceschi, F., Fagiuoli, S., Gasbarrini, G., Gasbarrini, A., 2016. Gut microbiota in autism and mood disorders. World J. Gastroenterol. 22, 361. https://doi.org/10.3748/wjg.v22.i1.361.
Manservisi, F., Lesseur, C., Panzacchi, S., Mandrioli, D., Falcioni, L., Bua, L., Manservigi, M., Spinaci, M., Galeati, G., Mantovani, A., Lorenzetti, S., Miglio, R., Andrade, A.M., Kristensen, D.M., Perry, M.J., Swan, S.H., Chen, J., Belpoggi, F., 2019. The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on development and endocrine system. Environ. Heal. 18, 15. https://doi.org/10.1186/s12940-019-0453-y.
Mao, Q., Manservisi, F., Panzacchi, S., Mandrioli, D., Menghetti, I., Vornoli, A., Bua, L., Falcioni, L., Lesseur, C., Chen, J., Belpoggi, F., Hu, J., 2018. The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. Environ. Health 17, 50. https://doi.org/10.1186/s12940-018-0394-x.
Martínez, M.-A., Ares, I., Rodríguez, J.-L., Martínez, M., Martínez-Larrañaga, M.-R., Anadón, A., 2018. Neurotransmitter changes in rat brain regions following glyphosate exposure. Environ. Res. 161, 212–219. https://doi.org/10.1016/J.ENVRES.2017.10.051.
Messaoudi, M., Violle, N., Bisson, J.-F., Desor, D., Javelot, H., Rougeot, C., 2011. Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. Gut Microbes 2, 256–261. https://doi.org/10.4161/gmic.2.4.16108.
Motta, E.V.S., Raymann, K., Moran, N.A., 2018. Glyphosate perturbs the gut microbiota of honey bees. Proc. Natl. Acad. Sci. U. S. A. 115, 10305–10310. https://doi.org/10.1073/pnas.1803880115.
Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., Rudi, K., 2014. Correlation between the human fecal microbiota and depression. Neurogastroenterol. Motil. 26, 1155–1162. https://doi.org/10.1111/nmo.12378.
Neufeld, K.M., Kang, N., Bienenstock, J., Foster, J.A., 2011. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol. Motil. 23https://doi.org/10.1111/j.1365-2982.2010.01620.x. 255-e119.
O'Mahony, S.M., Marchesi, J.R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E.M.M., Cryan, J.F., Dinan, T.G., 2009. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol. Psychiatry 65, 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026.
Paganelli, A., Gnazzo, V., Acosta, H., López, S.L., Carrasco, A.E., 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem. Res. Toxicol. 23, 1586–1595. https://doi.org/10.1021/tx1001749.
Perez-Pardo, P., Dodiya, H.B., Engen, P.A., Naqib, A., Forsyth, C.B., Green, S.J., Garssen, J., Keshavarzian, A., Kraneveld, A.D., 2018. Gut bacterial composition in a mouse model of Parkinson's disease. Benef. Microbes 9, 799–814. https://doi.org/10.3920/BM2017.0202.
Peruzzo, P.J., Porta, A.A., Ronco, A.E., 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ. Pollut. 156, 61–66. https://doi.org/10.1016/j.envpol.2008.01.015.
Poirier, A.-A., Aubé, B., Côté, M., Morin, N., Di Paolo, T., Soulet, D., 2016. Gastrointestinal dysfunctions in Parkinson's disease: symptoms and treatments. Parkinsons Dis. 2016, 6762528. https://doi.org/10.1155/2016/6762528.
Rajilić-Stojanović, M., Biagi, E., Heilig, H.G.H.J., Kajander, K., Kekkonen, R.A., Tims, S., de Vos, W.M., 2011. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology 141, 1792–1801. https://doi.org/10.1053/j.gastro.2011.07.043.
Ribeiro Marques, M., Henrique Pereira, J., Oliveira, J., Augusto Basso, L., Filgueira de Azevedo, W., Santiago Santos, D., Sergio Palma, M., 2007. The inhibition of 5-enolpyruvylshikimate-3-phosphate synthase as a model for development of novel antimicrobials. Curr. Drug Targets 8, 445–457. https://doi.org/10.2174/138945007780058951.
Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G.-E., 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ. Health Perspect. 113, 716–720. https://doi.org/10.1289/ehp.7728.
Riede, S., Toboldt, A., Breves, G., Metzner, M., Köhler, B., Bräunig, J., Schafft, H., Lahrssen-Wiederholt, M., Niemann, L., 2016. Investigations on the possible impact of a glyphosate-containing herbicide on ruminal metabolism and bacteria *in vitro* by means of the 'Rumen Simulation Technique'. J. Appl. Microbiol. 121, 644–656. https://doi.org/10.1111/jam.13190.
Roman, P., Rueda-Ruzafa, L., Cardona, D., Cortes-Rodríguez, A., 2018. Gut–brain axis in the executive function of austism spectrum disorder. Behav. Pharmacol. 29, 654–663. https://doi.org/10.1097/FBP.0000000000000428.
Rubio, F., Guo, E., Kamp, L., 2014. Survey of glyphosate residues in honey, corn and soy products. J. Environ. Anal. Toxicol. 5https://doi.org/10.4172/2161-0525.1000249. 2161–0525.
Samsel, A., Seneff, S., 2015. Glyphosate, pathways to modern diseases III: manganese, neurological diseases, and associated pathologies. Surg. Neurol. Int. 6, 45. https://doi.org/10.4103/2152-7806.153876.
Samsel, A., Seneff, S., 2013. Glyphosate, pathways to modern diseases II: celiac sprue and gluten intolerance. Interdiscip. Toxicol. 6, 159–184. https://doi.org/10.2478/intox-2013-0026.
Santovito, A., Ruberto, S., Gendusa, C., Cervella, P., 2018. In vitro evaluation of genomic damage induced by glyphosate on human lymphocytes. Environ. Sci. Pollut. Res. 1–8. https://doi.org/10.1007/s11356-018-3417-9.
Schonbrunn, E., Eschenburg, S., Shuttleworth, W.A., Schloss, J.V., Amrhein, N., Evans, J.N.S., Kabsch, W., 2001. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc. Natl. Acad. Sci. 98, 1376–1380. https://doi.org/10.1073/pnas.98.4.1376.
Shaw, W., 2017. Elevated urinary glyphosate and *Clostridia* metabolites with altered dopamine metabolism in triplets with autistic Spectrum disorder or suspected seizure disorder: a case study. Integr. Med. (Encinitas) 16, 50–57.
Shaw, W., 2010. Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of *Clostridia* spp. In the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. Nutr. Neurosci. 13, 135–143. https://doi.org/10.1179/147683010X12611460763968.
Shehata, A.A., Schrödl, W., Aldin, A.A., Hafez, H.M., Krüger, M., 2013. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr. Microbiol. 66, 350–358. https://doi.org/10.1007/s00284-012-0277-2.
Shreiner, A.B., Kao, J.Y., Young, V.B., 2015. The gut microbiome in health and in disease. Curr. Opin. Gastroenterol. 31, 69–75. https://doi.org/10.1097/MOG.0000000000000139.
Shuttleworth, W.A., Hough, C.D., Bertrand, K.P., Evans, J.N., 1992. Over-production of 5-enolpyruvylshikimate-3-phosphate synthase in *Escherichia coli*: use of the T7 promoter. Protein Eng. 5, 461–466.
Smedbol, É., Lucotte, M., Labrecque, M., Lepage, L., Juneau, P., 2017. Phytoplankton growth and PSII efficiency sensitivity to a glyphosate-based herbicide (Factor 540®). Aquat. Toxicol. 192, 265–273. https://doi.org/10.1016/j.aquatox.2017.09.021.
Srinivasan, P.R., Shigueura, H.T., Sprecher, M., Sprinson, D.B., Davis, B.D., 1956. The biosynthesis of shikimic acid from D-glucose. J. Biol. Chem. 220, 477–497.
Sun, M.-F., Zhu, Y.-L., Zhou, Z.-L., Jia, X.-B., Xu, Y.-D., Yang, Q., Cui, C., Shen, Y.-Q., 2018. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF-α signaling pathway. Brain Behav. Immun. 70, 48–60. https://doi.org/10.1016/j.bbi.2018.02.005.
Sylvia, K.E., Jewell, C.P., Rendon, N.M., St. John, E.A., Demas, G.E., 2017. Sex-specific modulation of the gut microbiome and behavior in Siberian hamsters. Brain Behav. Immun. 60, 51–62. https://doi.org/10.1016/j.bbi.2016.10.023.

Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., Satayavivad, J., 2013. Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem. Toxicol. 59, 129–136. https://doi.org/10.1016/j.fct.2013.05.057.

Thursby, E., Juge, N., 2017. Introduction to the human gut microbiota. Biochem. J. 474, 1823–1836. https://doi.org/10.1042/BCJ20160510.

Van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris, J.G., 2018. Environmental and health effects of the herbicide glyphosate. Sci. Total Environ. 616–617, 255–268. https://doi.org/10.1016/J.SCITOTENV.2017.10.309.

Van Stempvoort, D.R., Spoelstra, J., Senger, N.D., Brown, S.J., Post, R., Struger, J., 2016. Glyphosate residues in rural groundwater, Nottawasaga River Watershed, Ontario, Canada. Pest Manag. Sci. 72, 1862–1872. https://doi.org/10.1002/ps.4218.

Vigvári, S., Vincze, Á., Solt, J., Sipos, D., Feiszt, Z., Kovács, B., Kappéter, Á., Péterfi, Z., 2018. Experiences with fecal microbiota transplantation in *Clostridium difficile* infections via upper gastrointestinal tract. Acta Microbiol. Immunol. Hung. 1–10. https://doi.org/10.1556/030.65.2018.051.

Vogt, N.M., Kerby, R.L., Dill-McFarland, K.A., Harding, S.J., Merluzzi, A.P., Johnson, S.C., Carlsson, C.M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B.B., Rey, F.E., 2017. Gut microbiome alterations in Alzheimer's disease. Sci. Rep. 7, 13537. https://doi.org/10.1038/s41598-017-13601-y.

Wichmann, A., Allahyar, A., Greiner, T.U., Plovier, H., Lundén, G., Larsson, T., Drucker, D.J., Delzenne, N.M., Cani, P.D., Bäckhed, F., 2013. Microbial modulation of energy availability in the Colon Regulates intestinal transit. Cell Host Microbe 14, 582–590. https://doi.org/10.1016/j.chom.2013.09.012.

Williams, G.M., Kroes, R., Munro, I.C., 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul. Toxicol. Pharmacol. 31, 117–165. https://doi.org/10.1006/rtph.1999.1371.

Willis, A.W., Evanoff, B.A., Lian, M., Galarza, A., Wegrzyn, A., Schootman, M., Racette, B.A., 2010. Metal emissions and urban incident Parkinson disease: a community health study of Medicare beneficiaries by using geographic information systems. Am. J. Epidemiol. 172, 1357–1363. https://doi.org/10.1093/aje/kwq303.

Xiong, X., Liu, D., Wang, Y., Zeng, T., Peng, Y., 2016. Urinary 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, 3-Hydroxyphenylacetic acid, and 3-Hydroxyhippuric acid are elevated in children with autism Spectrum disorders. Biomed Res. Int. 2016, 1–8. https://doi.org/10.1155/2016/9485412.

You, M.J., Shin, G.W., Lee, C.S., 2015. *Clostridium tertium* bacteremia in a patient with glyphosate ingestion. Am. J. Case Rep. 16, 4–7. https://doi.org/10.12659/AJCR.891287.

Yu, N., Tong, Y., Zhang, D., Zhao, S., Fan, X., Wu, L., Ji, H., 2018. Circular RNA expression profiles in hippocampus from mice with perinatal glyphosate exposure. Biochem. Biophys. Res. Commun. 501, 838–845. https://doi.org/10.1016/j.bbrc.2018.04.200.

Zhan, H., Feng, Y., Fan, X., Chen, S., 2018. Recent advances in glyphosate biodegradation. Appl. Microbiol. Biotechnol. 102, 5033–5043. https://doi.org/10.1007/s00253-018-9035-0.

Zhan, X., Stamova, B., Jin, L.-W., DeCarli, C., Phinney, B., Sharp, F.R., 2016. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology 87, 2324–2332. https://doi.org/10.1212/WNL.0000000000003391.

Zhang, L., Wang, Y., Xiayu, X., Shi, C., Chen, W., Song, N., Fu, X., Zhou, R., Xu, Y.-F., Huang, L., Zhu, H., Han, Y., Qin, C., 2017. Altered gut microbiota in a mouse model of Alzheimer's disease. J. Alzheimers Dis. 60, 1241–1257. https://doi.org/10.3233/JAD-170020.

Zhang, R., Miller, R.G., Gascon, R., Champion, S., Katz, J., Lancero, M., Narvaez, A., Honrada, R., Ruvalcaba, D., McGrath, M.S., 2009. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). J. Neuroimmunol. 206, 121–124. https://doi.org/10.1016/j.jneuroim.2008.09.017.

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N.D., Licinio, J., Wei, H., Xie, P., 2016. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol. Psychiatry 21, 786–796. https://doi.org/10.1038/mp.2016.44.

Zhou, T., Zhao, L., Zhan, R., He, Q., Tong, Y., Tian, X., Wang, H., Zhang, T., Fu, Y., Sun, Y., Xu, F., Guo, X., Fan, D., Han, H., Chui, D., 2014. Blood–brain barrier dysfunction in mice induced by lipopolysaccharide is attenuated by dapsone. Biochem. Biophys. Res. Commun. 453, 419–424. https://doi.org/10.1016/j.bbrc.2014.09.093.