Nanoplastic and the Gut-Brain Axis

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The widespread usage of plastic places a significant burden on the environment and impacts numerous aquatic and terrestrial species. Humans in particular can be affected by plastic pollution, predominantly via inhalation and ingestion, as well as trophic transfer along the food chain. Under natural conditions synthetic materials undergo degradation into micro- and nanoparticles, especially prone to interact with biological systems. Organisms exposed to nanoplastic accumulate it in multiple tissues, including the gut and the brain. The scarce but consistent evidence shows that exposure to plastic nanoparticles can indeed affect both the digestive and the nervous system, therefore, potentially pose a threat to the complex network of mutual interactions between them, known as the gut-brain axis.

nanoplastic gut-brain axis inflammation microbiome intestinal barrier permeability oxidative stress neurotoxicity

1. Plastics in Human Environment

The current era of the Earth's history is frequently referred to as the Plasticene, the "Plastic Age". Although coined informally, the term seems to appropriately reflect the state of the global environment, ubiquitously polluted with synthetic litter [1]. During the last decades plastic production worldwide has been steadily increasing, reaching 368 million tons in 2019 [2][3]. The total amount of plastic ever produced by humans has been estimated to be more than 8 billion tons. Approximately 60% of that amount was discarded as waste and accumulated in the environment [4]. This rejected, unrecycled material not only contaminates the land, but also ends up in the aquatic biome, forming plastic debris both on and beneath the water surface [2]. Due to physicochemical and biological processes, such as UV-induced decomposition and digestion by marine species, under environmental conditions plastic can undergo degradation into micro- (defined as smaller than 5 mm in diameter) and nanoparticles (defined as smaller than 1000 or 100 nm in diameter). Compared with larger fragments, these micro- and nanoplastics (MNPs) pose a less tangible, but not less dangerous threat to organisms. As it turns out, they can be harmful especially in regards to digestive and nervous systems of aquatic organisms and other elements of the food chain, probably also including humans [2][5][6][7].

2. The Gut-Brain Axis

The gastrointestinal tract (GI tract) and the central nervous system (CNS) are connected by a complex network of mutual interactions, known as the gut-brain axis (GB axis) [8]. The core of the GB axis consists of the vagus nerve,

the X cranial nerve and simultaneously a branch of the autonomic nervous system. It sends information about the state of the inner organs, via afferent fibers, to the brain and connects the CNS to the enteric nervous system (ENS) [8][9][10]. Simultaneously, the CNS interacts with the GI tract via the hypothalamic-pituitary-adrenal axis (HPA axis), also integrated into the gut-brain communication pathways [9]. Direct effects of diverse neuronal and hormonal stimulation on the GI tract are possible due to the ENS, an intricate complex of nerves situated beneath the intestinal mucosa [9][10]. The ENS is located in close vicinity to the epithelium, which creates a tight barrier between the gut lumen and underlying tissues, preventing the unwanted passage of food and microbial antigens deep into the mucosa. A disruption of the barrier has been observed in several psychiatric disorders, including anxiety and depression, which suggests a link between gut permeability and CNS function [11][10].

Besides the physical defense provided by the intestinal barrier, the digestive tract is protected against multiple environmental factors by immune cells in the gut-associated lymphoid tissue (GALT) [9][11][12]. GALT contains the highest concentration of immune cells in the entire human organism and provides the primary space of exposure to microbial agents and their metabolites [11][12]. The totality of these microorganisms is known as the gut microbiota and, as a growing body of evidence demonstrates, heavily affects CNS functioning, blood-brain barrier (BBB) permeability, brain cells development and neuron maturation [11][12][13][14]. Additionally, microbial metabolites act as neuromodulators, while short-chain fatty acids (SCFA) produced by gut bacteria might stimulate the vagus nerve, affect neurotransmitter metabolism and have an impact on behavior [11]. Changes in microbiota have been linked to the development of different CNS-related disorders, including pathogeneses of Alzheimer's disease, Parkinson's disease, autism and depression [10]. Taken together, there are multifarious ways of communication between the gut and the brain. Given the crucial physiological function of the GB axis and its involvement in numerous neurological disorders, any potential disrupting agents are a cause of particular concern.

3. Impact of Nanoplastic Exposure on the Gut-Brain Axis

Data regarding nanoplastics (NPs) and their impact on living organisms indicate that the main risk associated with plastic exposure is a non-acute toxicity, particularly with respect to the digestive and the nervous system [6][7]. Moreover, there is a parallelism between the effects provoked by NPs and other nanoparticles, since many nanomaterials, especially metallic nanoparticles, have well-documented neurotoxic properties [15][16]. Therefore, NPs of similar size can be expected to produce analogous outcomes. The following is the description of experimental studies investigating NPs-related toxicity in regards to several elements of the GB axis.

3.1. In Vitro Studies on Cellular Cultures

Currently, in vitro research exploring the toxicity of NPs with regards to the GI tract and CNS cells is still scarce. In addition, in the limited number of existing studies different types, sizes and concentrations of nanoplastics have been used, which complicates direct comparisons. Nevertheless, those preliminary findings share certain commonalities, allowing for some generalization. NPs seem to be able to penetrate gut cells, creating an opportunity for further distribution. In brain cells models nanoplastic is internalized, elicits oxidative stress and

reduces cell viability, particularly in the more realistic, “aged” form. The summary of these research is presented in Table 1.

Table 1. In vitro NPs toxicity related to the GB axis.

Cell Models	NPs Type and Size	Exposure	Effects Related to the GB axis	Reference
Human intestinal Caco-2, HT29-MTX-E12 and THP-1 monocultures; triple culture human intestinal Caco-2/HT29-MTX-E12/THP-1 model (healthy or inflamed)	Pristine/amino-modified polystyrene NPs (59 nm); polyvinyl chloride NPs (279 nm)	24 h (1–50 µg/mL)	Monocultures/amino-modified polystyrene: metabolic disruption, inflammation, DNA damage; healthy triple culture model/amino-modified polystyrene: increased cytotoxicity, decrease of tight junction protein 1; inflamed triple culture model/polyvinyl chloride: loss of nuclei	[17]
Human intestinal Caco-2/HT29 and Caco-2/HT29 + Raji-B cells	Polystyrene NPs (5–100 nm)	24 h (1–100 µg/mL)	No significant toxic effects	[18]
Human intestinal Caco-2/HT29-MTX-E12 co-culture model	Carboxylated polystyrene NPs (50 and 500 nm)	24 h (0.1–100 µg/mL)	Uptake of NPs	[19]

Human intestinal Caco-2/HT29-MTX co-culture model	Pristine/positively/ negatively charged polystyrene NPs (50 nm), non-digested or digested in vitro	24 h (250 µg/mL)	Digested NPs: enhanced translocation across cells; positively charged NPs: increased intestinal barrier permeability	[20]
Murine mixed neuronal cells; primary astrocytes	Polystyrene NPs (100 nm)	48 h (50–200 µg/mL)	Uptake of NPs; mixed neuronal cells: reduced cell viability, altered expression of *Tubb3* and *Gfap* genes; primary astrocytes: increased expression of *Tnfa* and *Il1b* genes	[21]
Human neuronal T98G cells	Polyethylene NPs (100–600 nm); polystyrene NPs (40–250 nm)	24 h (0.05–10 µg/mL)	Increased reactive oxygen species generation	[22]
Murine NE-4C Neuroectodermal stem cells; neuron-enriched primary brain cell cultures; primary astrocytes; microglial cultures; brain vascular	Carboxylated/PEGylated polystyrene NPs (45–70 nm), “fresh” or “aged” (6 months< of storage)	1 h (50 µg/mL) 24 h (7.8–250 µg/mL)	“Fresh” carboxylated NPs: uptake by microglia; “aged” NPs: uptake and cytotoxicity in NE-4C neuronal stem cells and microglia; enhanced cellular uptake of NPs caused by lipopolysaccharide adsorption	[23]

endothelial cell cultures				
Embryonic stem cell (hESC)-derived 3-dimensional model of human neural development	Polyethylene NPs (33 nm)	48 h (5.6–1440 µg/mL); 18 days (5.6–360 µg/mL)	Uptake of NPs; reduced cell viability; oxidative stress; down-regulation of *HES5*, *NOTCH1*, *FOXG1*, *NEUROD1* and *ASCL1* genes	[24]

3.2. In Vivo Studies on Fish

Data regarding NPs impact on GB axis derived from in vivo studies on aquatic vertebrates are still insufficient to draw definitive conclusions. Furthermore, the size and concentration of particles applied are often too dissimilar to confidently compare results derived from different experiments. However, studies conducted up to date are consistent in at least several aspects. They clearly show size-dependent differences in toxicity, NPs being more harmful than microplastics (MPs), possibly due to their smaller diameter and, consequently, higher bioactivity. There is also convincing preliminary evidence for translocation of NPs from the gut to the brain and their ability to cross the BBB. In conjunction with behavioral alterations, possible neurodevelopmental disturbances, impact on enzymatic activity, induction of oxidative stress and immune system activation, the influence of nanoplastic on the GB axis becomes a plausible phenomenon. The summary of these research is presented in Table 2.

Table 2. Summarized data derived from in vivo experiments on fish regarding toxic effects of NPs related to the GB axis.

Fish	NPs Type and Size	Exposure	Effects Related to the GB Axis	Reference
Zebrafish (*D. rerio*)	Polystyrene NPs (700 nm)	Single-dose injection (5 mg/mL)	Altered expression of 26 genes 1 day and 51 genes 3 days post-injection; activation of the complement system; activation of oxidative stress-related pathways	[25]

Marine medaka (*O. melastigma*)	Polystyrene NPs (50 nm)	In water for 24 h (10 μg/mL) or 14 days (2.5 μg/mL)	NPs accumulation in the digestive system; induction of apoptosis in the gut; increased activity of superoxide dismutase and catalase in the gut	[26]
Japanese medaka (*O. latipes*)	Polystyrene NPs (39.4 nm)	In water for 7 days (10 μg/mL)	NPs accumulation in the gut and brain	[27]
Zebrafish (*D. rerio*)	Polystyrene NPs (51 nm)	In water for 114 h (0.1–10 μg/mL)	NPs accumulation in the gut and head; behavioral alterations	[28]
Chinese medaka (*O. sinensis*); Dark chub (*Z. temminckii*)	Polystyrene NPs (51 nm)	In water for 7 days (5 μg/mL, individual toxicity); for 48 h (*O. sinensis*) or 24 h (*Z. temminckii*) via trophic transfer (*C. reinhardtii* → *D. magna* → *O. sinensis* → *Z. temminckii*)	Individual toxicity: behavioral alterations; *O. sinensis*/trophic transfer: NPs accumulation in the gut; *Z. temminckii*/trophic transfer: NPs accumulation in the gut and stomach	[29]
Crucian carp (*C. carassius*)	Sulfonated polystyrene NPs (24 and 27 nm)	For 61 days via trophic transfer (*Scenedesmus sp.* → *D. magna* → *C. carassius*)	Histological changes in the brain; behavioral alterations	[30]

Crucian carp (*C. carassius*)	Amino-modified polystyrene NPs (53 and 180 nm)	For 67 days via trophic transfer (*Scenedesmus sp.→D. magna→C. carassius*)	NPs accumulation in the brain; behavioral alterations	[31]
Zebrafish (*D. rerio*)	Polystyrene NPs (50 nm)	In water for 117 h (1 µg/mL)	Up-regulation of *Gfap* and *α1-tubulin* genes; decreased acetylcholinesterase activity; decreased levels of reduced glutathione; decreased body length; behavioral alterations	[32]
Zebrafish (*D. rerio*)	Polystyrene NPs (70 nm)	7 days (0.5 and 1.5 µg/mL); 30 days (1.5 µg/mL); 7 weeks (5 µg/mL)	NPs accumulation in the gut and brain; lowered levels of acetylcholinesterase, dopamine, melatonin, vasopressin, 5-hydroxytryptophan, kisspeptin, γ-aminobutyric acid and oxytocin; behavioral alterations	[33]

3.3. In Vivo Studies on Rodents

Research into plastic toxicity in mammalian species is currently very scarce and in most part focused on the effects caused by MPs. Consequently, studies investigating nanoplastic effects in regards to the GB axis are even more lacking. In fact, a recent review by Yong et al. mentioned only 10 articles describing MNPs effects in mice, whereas another review by Prüst et al. identified only one such publication directly related to NPs neurotoxicity [6][7]. Nevertheless, the existing evidence allows to formulate some initial remarks and is definitely worth exploring. Summarized data of in vivo studies on rodents are presented in Table 3.

Table 3. Summarized data derived from in vivo experiments on rodents regarding toxic effects of NPs related to the GB axis.

Rodent	NPs Type and Size	Exposure	Effects Related to the GB Axis	Reference
Fischer rat	Pristine/positively/ negatively charged polystyrene NPs (50 nm)	Single-dose orally (125 mg/kg bw)	NPs accumulation in the gut	[34]
Sprague-Dawley rat	Polystyrene NPs (500 nm)	Orally for 5 h (100–125 mg/kg bw)	Accumulation in the GI tract and brain	[35]
Sprague-Dawley rat (pregnant)	Polystyrene NPs (20 nm)	Single-dose inhalation (2.64×10^{14} particles)	NPs accumulation in fetal brain	[36]
ICR mouse	Polystyrene NPs (500 nm)	Orally in drinking water for 5 weeks (0.1 or 1 µg/mL)	Higher load: decreased body weight; decrease in gut mucin secretion; lowered expression of *Muc1* and *Klf4* genes; dysbiosis	[37]
Wistar rat	Polystyrene NPs (38.9 nm)	Orally for 35 days (1–10 mg/kg bw)	No changes in behavior	[38]
C57BL/6J mice	Polystyrene NPs (around 50 nm)	Orally for 30 days (0.2–10 mg/kg bw)	No changes in behavior; no inflammation/oxidative stress in the gut and brain; highest dose: damage to the intestinal	[39]

			wall; changes in microbiota composition	
BALB/c mice	Pristine/carboxyl-/amino-modified polystyrene NPs (100 nm)	Orally for 28 days (1 mg/day)	NPs accumulation in the gut and brain; histological damage to the gut and brain; inflammation in the brain; intestinal cells penetration confirmed in vitro	[40]

Research regarding the influence of NPs on different components of the GB axis is scarce and only begins to scratch the surface of possible toxicity. Available data come exclusively from experiments performed on cellular cultures and animal models, therefore, any indications of potential risks for human health have to be extrapolated from these results. In vitro studies demonstrate that nanoscale plastic particles undergo internalization, both in intestinal and cerebral cells, provoking reduced viability and oxidative damage. Moreover, under environmentally realistic conditions, they are able to adsorb other toxins, which contribute to their harmfulness. In vivo experiments on aquatic vertebrates confirm these observations, proving NPs capable of effectively distribute over the body, affecting the digestive tract and the brain. They trigger the immune response, disturb the intestinal microbiota homeostasis, induce oxidative stress and cause behavioral alterations. Finally, the few studies conducted on rodents are in line with the aforementioned research and show several alarming effects taking place upon exposure to NPs. In mammals nanoplastic accumulates in the GI tract, induces dysbiosis and undermines the intestinal barrier integrity. Furthermore, it translocates to multiple organs and passes across biological barriers, including the placental-blood barrier and the BBB, to ultimately enter the brain. Summarized effects of NPs exposure on the GB axis are depicted in Figure 1.

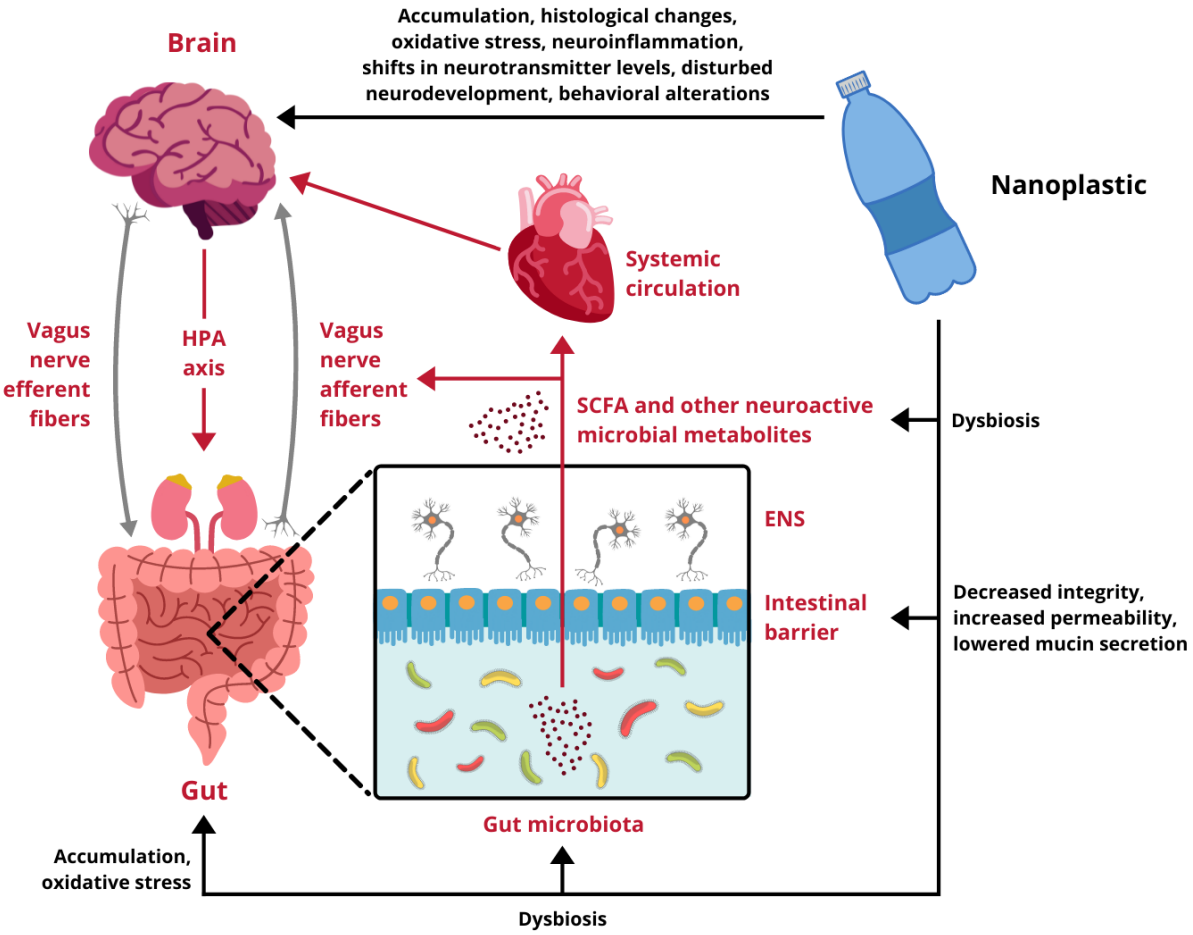

Figure 1. Impact of nanoplastic exposure on the gut-brain axis. HPA axis, hypothalamic-pituitary-adrenal axis; ENS, enteric nervous system; SCFA, short-chain fatty acids.

4. Future Perspectives

Although the experimental data regarding nanoplastic impact on mammalian systems are just beginning to build up, the evidence gathered up to date sheds some light on the consequences NPs exposure could have for both the GI tract and the CNS. The accumulation in the digestive system seems to be a factual phenomenon that might lead to dysbiosis and jeopardize the integrity of the intestinal barrier. Further biodistribution of NPs also takes place, as their presence in multiple tissues is shown consistently. One of the target organs is the brain, which suggests that nanoparticulate plastic possesses the ability to cross the BBB. Even though specific behavioral or biochemical alterations in the CNS are yet to be proven, the fact that NPs can reach cerebral compartments and affect the gut environment opens up the alarming possibility of compromised functioning of the GB axis. Toxicity determinants, such as plastic type, particle size and load, surface modification or adsorption of chemicals, as well as impact on gene expression and specific biochemical pathways involved in the gut-brain communication are examples of

topics that future investigation should aim to address. Regardless of the outcomes, the widespread plastic contamination in the human environment makes preventive measures and caution highly advisable.

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