Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(9): 204
https://doi.org/10.31083/j.fbl2809204

Review

From Mechanisms to Implications: Understanding the Molecular Neurotoxicity of Titanium Dioxide Nanoparticles

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Academic Editor: Simone Battaglia
Submitted: 19 June 2023 Revised: 3 August 2023 Accepted: 24 August 2023 Published: 15 September 2023

Abstract

Titanium dioxide nanoparticles (TiO_2NPs) are widely produced and used nanoparticles. Yet, TiO_2NP exposure may possess toxic effects to different cells and tissues, including the brain. Recent studies significantly expanded the understanding of the molecular mechanisms underlying TiO_2NP neurotoxicity implicating a number of both direct and indirect mechanisms. In view of the significant recent progress in research on TiO_2NP neurotoxicity, the objective of the present study is to provide a narrative review on the molecular mechanisms involved in its neurotoxicity, with a special focus on the studies published in the last decade. The existing data demosntrate that although TiO_2NP may cross blood-brain barrier and accumulate in brain, its neurotoxic effects may be mediated by systemic toxicity. In addition to neuronal damage and impaired neurogenesis, TiO_2NP exposure also results in reduced neurite outgrowth and impaired neurotransmitter metabolism, especially dopamine and glutamate. TiO_2NP exposure was also shown to promote α-synuclein and β-amyloid aggregation, thus increasing its toxicity. Recent findings also suggest that epigenetic effects and alterations in gut microbiota biodiversity contribute to TiO_2NP neurotoxicity. Correspondingly, *in vivo* studies demosntrated that TiO_2NPs induce a wide spectrum of adverse neurobehavioral effects, while epidemiological data are lacking. In addition, TiO_2NPs were shown to promote neurotoxic effects of other toxic compounds. Here we show the contribution of a wide spectrum of molecular mechanisms to TiO_2NP-induced neurotoxicity; yet, the role of TiO_2NP exposure in adverse neurological outcomes in humans has yet to be fully appreciated.

Keywords: titanium; nanoparticle; neurotoxicity; neuroinflammation; neurodegeneration; brain

1. Introduction

Nanoparticles are considered as small-sized particles with a diameter lesser than 100 nm [1]. Due to their unique physico-chemical properties including high surface size and optical properties, nanoparticles are widely used in industry, medicine, environmental applications [2]. In medicine, nanoparticles are used for drug delivery, cancer therapy, bioimaging, and photoablation therapy, to name a few [3]. Due to the increasing production and use of nanoparticles, their safety for environment and human health has been of particular interest [4].

Titanium dioxide (TiO_2) is considered one of the most produced nanoparticles [5] with a global production of approximately 4 million tons per year [6]. TiO_2 nanoparticles (TiO_2NPs) are found in three crystalline forms, including anatase, rutile, and brookite [7]. Anatase and rutile are the main forms of TiO_2, and are widely used in industry for the production of personal care products (toothpaste, sunscreens), cosmetics, paints, optics, and photocatalysts, to name a few [8]. Due to their relatively low toxicity, TiO_2NPs are used in the food industry as an additive during food processing, production, and packaging [9], referred to as an additive–E171. Correspondingly, certain dietary items including chewing gum, candy, jelly, cookies, chocolates, may significantly contribute to increased dietary TiO_2NP intake [10]. Medical applications

of TiO_2NPs include their use as a photosensitizing agents in cancer treatment, antibacterial agents, and a components of implants and drug delivery systems [6].

Despite being relatively non-toxic upon dermal exposure due to their inability to penetrate skin and enter systemic circulation [11], recent studies have demonstrated that oral exposure to these NPs was associated with TiO_2NPs accumulation in the organism and subsequent systemic toxicity [12].

Increased evidence demonstrates that overexposure to TiO_2NPs can result in significant adverse health effects due to their accumulation [13] and subsequent toxic effects in a number of cells and tissues [14]. Specifically, TiO_2NPs exposure was shown to induce cytotoxicity due to oxidative stress, mitochondrial dysfunction and genotoxicity in epidermal cells [15], gastric epithelial cells [16], hepatocytes [17], alveolar macrophages [18], and endothelial cells [19], to name a few. Correspondingly, *in vivo* studies demonstrated that TiO_2NPs exposure may result in genotoxicity [20], altered lipid metabolism [21], cardiovascular damage [22], liver hepatotoxicity [23], intestinal inflammation [24]. TiO_2NPs were also shown to enter the fetus and neonate through transplacental transport or during breastfeeding, respectively [25], thus posing a significant risk for developing offspring, especially in view of higher susceptibility of the younger organism to toxic effects [26]. At the same time, epidemiological evidence on the health hazards of TiO_2NP exposure is lacking [27].

In view of its potential genotoxicity, the use of TiO_2NPs as a food additive has been limited in certain countries since 2020 [25] until European Food Safety Authority concluded that TiO_2NPs cannot be considered as a safe food additive in 2021 [28].

An increasing body of evidence has demonstrated that the brain should be considered as a primary target for TiO_2NP toxicity [29,30]. Several studies have shown that TiO_2NPs can accumulate in the brain upon exposure [31], while other studies studied failed to ascertain detectable TiO_2NP levels in the brain [32]. A number of excellent reviews published in 2015–2016 demonstrated that TiO_2NPs exposure can also induce neurotoxicity through a number of mechanisms, including oxidative stress, apoptosis, neuroinflammation, neurotransmitter metabolism dysregulation, and impaired synaptic plasticity, to name a few [29,30,33]. Nonetheless, most recent findings demonstrate that other mechanisms including epigenetic effects [34] as well as modulation of gut microbiota [35] may be involved in TiO_2NPs neurotoxicity. Therefore, in view of the significant recent progress in research on TiO_2NP neurotoxicity, the objective of the present study is to provide a narrative review on the molecular mechanisms involved in TiO_2NP neurotoxicity with special emphasis on studies published in the last decade.

We have performed a search in the PubMed-Medline database using the MeSH terms "titanium", "TiO_2", "titanium dioxide" and "neurotoxicity", "neuroinflammation", "brain", "neurodegeneration", "brain", "synapse", "neurite", "axon", "neurodevelopment", "amyloid", "synuclein", "neuron", "glia", "astrocyte", "neurogenesis", "neurotransmitter", "dopamine", "glutamate", "glutamine", "serotonin".

2. Brain Accumulation

TiO_2 has been shown to readily accumulate in the brain following oral exposure due to its ability to cross BBB and accumulate in the brain [36]. Subsequently, it can lead to induction of oxidative stress, apoptosis, neuroinflammation, and neuronal degeneration [36]. Oral TiO_2NPs have been shown to induce hippocampal, cortical, and cerebellar neuron apoptosis, oxidative stress, neuroinflammation, as well as impaired neurotransmitter metabolism [37].

The existing data on TiO_2NPs accumulation in brain are inconsistent (Table 1, Ref. [17,31–33,38]). Specifically, prenatal exposure to TiO_2NPs via maternal intravenous (i.v.) injection has been shown to result in a significant increase in brain and liver Ti accumulation in the offspring [31]. However, other studies have implied that intravenously injected TiO_2NPs accumulated predominantly in liver, followed by spleen, and to a lesser extent lungs, kidneys, and heart, whereas no translocation to the brain was detected [32], corroborating the findings by Fabian *et al*. (2008) [39]. Such a lack of detectable TiO_2 in brain may be associated with different rates of TiO_2 uptake in brain cells in comparison to other tissues.

It is also noteworthy that even without significant brain accumulation of TiO_2 following inhalation exposure, TiO_2 can induce systemic response associated with increased blood-brain barrier (BBB) permeability, neuroinflammation, and reduced synaptophysin expression, being more profound in aged rats [40]. Correspondingly, TiO_2NP accumulation in liver without detectable deposition of the metal in brain tissue was associated with a proinflammatory response in brain microvasculature endothelial cells characterized by up-regulation of IL-1β, CXCL1, and CXCL10 expression, as well as increased IL-1β levels in brain parenchyma, although BBB integrity was not affected in this study [41]. These observations implicate a role for systemic proinflammatory response in the early stages of TiO_2NPs neurotoxicity.

Cell culture studies demonstrated that neural cells, and especially neurons, are highly sensitive to TiO_2 toxicity. Brandão *et al*. (2020) [38] demonstrated that although SH-SY5Y cells accumulated fewer TiO_2NPs after 3 hours of exposure as compared to A549, HepG2, and A172 cells after 24 hours of exposure A172, and especially SH-SY5Y cells, accumulated TiO_2NPs more actively than did A549 and HepG2 cells. It has been also demonstrated that neuroblastoma cells, SH-SY5Y, were subjected to TiO_2NP-induced reduction in cell viability, being more sensitive to toxic effects than HepG2 cells [42]. At the same time, dis-

Table 1. A summary of studies demonstrating accumulation of Titanium dioxide nanoparticles (TiO_2NPs) in brain.

Model	Nanoparticle	Dose	Exposure	Tissue level	Ref.
BALB/c mice	Rutile-type TiO_2NPs, size—35 nm	0.8 mg per mouse	prenatal exposure through injection into maternal tail vein	Brain + (qualitative) Liver + (qualitative)	[31]
F344/DuCrlCrlj rats	Degussa P25 TiO_2NPs consisted of both anatase and rutile forms (70/30), size—21 nm	1 mg/kg b.w.	injected intravenously into the tail vein daily	After 24 h: Liver –230,000 mg/organ Spleen –4000 mg/organ Kidney –56 mg/organ Lungs –380 mg/organ Heart –14 mg/organ Brain –not detected On day 30: Liver –220,000 mg/organ Spleen –4300 mg/organ Kidney –24 mg/organ Lungs –100 mg/organ Heart –9.3 mg/organ Brain –not detected	[32]
Wistar rats	TiO_2NPs consisted of both anataseand rutile forms (70/30), size—20–30 nm	5 mg/kg b.w.	injected intravenously into the tail vein daily	On day 1: Liver –133.8 µg/g Spleen –78.7 µg/g Lung –8.8 µg/g Kidney –0.67 µg/g Brain –not detected On day 14: Liver –99.5 µg/g Spleen –48.8 µg/g Lung –2.8 µg/g Kidney –0.2 µg/g Brain –not detected On day 28: Liver –111.3 µg/g Spleen –33.3 µg/g Lung –2.3 µg/g Kidney –0.2 µg/g Brain –not detected	[33]

Table 1. Continued.

Model	Nanoparticle	Dose	Exposure	Tissue level	Ref.
Fisher F344 rats	Aeroxide TiO_2NPs P25 NPs consisted of both anatase and rutile forms (75/25)	1 mg/kg b.w.	single-dose intravenous administration	After 24 hours: Brain ~70 ng/g Liver ~9500 ng/g Lungs ~7500 ng/g Spleen ~5500 ng/g Kidneys ~250 ng/g After 365 days: Brain ~40 ng/g Liver ~3500 ng/g Lungs ~500 ng/g Spleen ~2000 ng/g Kidneys ~40 ng/g	[17]
Cell cultures	Degussa Aeroxide TiO_2NPs P25 consisted of both anatase and rutile forms (80/20), size—25 nm	10–200 µg/mL	24 h exposure *in vitro*	After 3 h of exposure to 200 µg/mL: A549 ~52% HepG2 ~13% A172 ~33% SH-SY5Y ~8% cells with NPs After 24 h of exposure to 200 µg/mL: A549 ~83% HepG2 ~50% A172 ~77% SH-SY5Y ~82% cells with NPs	[38]

tinct differences in susceptibility to the toxic effects of TiO_2NPs were also observed even between different brain cell types. Specifically, comparative analysis demonstrated that neuronal (SH-SY5Y) cells were more sensitive to TiO_2NP exposure than the human glial (D384) cell line, both upon acute and chronic low-dose exposure [43].

The effects of TiO_2 on the brain were also shown to be particle size-dependent. Specifically, TiO_2NPs sized 10–20 nm significantly induced brain damage, BBB disruption, and glial cell damage, along with up-regulation of IL-1β, TNFα, and IL-10 production in brain tissue, whereas large TiO_2 particles sized 200 nm did not possess such effect [44]. Small-sized TiO_2NPs were shown to penetrate BBB more effectively than the larger ones [45]. Another study demonstrated that large TiO_2NPs (90 nm) induced greater developmental toxicity and increased number of malformations in *ex vivo* mouse embryo models as compared to smaller particles, although the effects of TiO_2NPs were more profound than those of micro TiO_2 [46]. In contrast, exposure of human neural stem cells (hNSC) to both TiO_2NP (80 nm) and micro-TiO_2 (<44 µm) significantly altered cellular morphology, and increased nestin and neurofilament heavy polypeptide gene expression, whereas no effect on *HMGA1* gene, involved in regulation of neuronal differentiation, was observed [47].

While considering the role of exposure duration, it has been also demonstrated that acute and subacute exposure to TiO_2NPs did not cause neurotoxicity, and only chronic exposure induced modest neuronal dysfunction [48].

The neurotoxic effects of TiO_2NPs are dependent not only on the dose and duration of exposure but also on crystalline structure [49]. Following intranasal instillation, TiO_2NPs including rutile (80 nm) and anatase (155 nm) were shown to accumulate in hippocampus through the olfactory bulb, although anatase induced a more profound oxidative stress and neuroinflammation, indicative of the influence of not only size, but also the crystalline structure of TiO_2NPs on its neurotoxicity [50].

Although multiple studies demonstrate significant neurotoxic effects of TiO_2NPs, it is noteworthy that several studies have demonstrated that low-dose TiO_2NPs do not possess significant neurotoxicity in zebrafish [51] and murine models [52]. These findings, despite being contradictory to the majority of studies, demonstrate that adequate monitoring of the exposure doses and nanoparticle accumulation in the organism is sufficient to prevent the neurotoxic effects of TiO_2NPs.

3. Blood-Brain Barrier

Existing data demonstrate that TiO_2NPs not only cross the BBB but also impair its permeability. Specifically, TiO_2NPs were shown to accumulate in the brain secondary to increased BBB permeability through a number of mechanisms, including down-regulation of tight junction proteins such as zonula occludens 1 (ZO-1) and occludin, paracellular gap formation, and associated ROCKII activation [53]. Inhibition of claudin-5 expression can also mediate the adverse effects of TiO_2NPs on BBB permeability [54]. Shelly *et al*. (2021) [55] demonstrated that in a model of BBB, TiO_2NP exposure resulted in increased leakiness due to its accumulation in brain-like endothelial cells and induction of proinflammatory signaling mediated by IL-1R and IL-6. Correspondingly, TiO_2NP-induced impairment in BBB integrity was shown to involve increased production of proinflammatory cytokines and chemokines, including up-regulation of CXCL1 and CXCL2 expression that may bind to CXCR2 receptor subsequently stimulating expression of CCL2 and TGFβ. The latter is capable of down-regulating tight junction proteins expression (ZO-1, Occludin, claudin 5), thus increasing BBB permeability, whereas CCL2 is known to up-regulate expression of adhesion molecules and subsequent leukocyte adhesion [56]. Nonetheless, despite a significant decrease in intact BBB integrity upon exposure to TiO_2NPs, the latter did not cause any significant effect on integrity of lipopolysaccharide (LPS)-treated BBB [45].

These findings demonstrate that TiO_2NP exposure significantly increases BBB permeability through alteration of tight junction protein expression due to its proinflammatory activity. Such effects may be associated with increased transport of TiO_2NPs [57] as well as other potentially hazardous substances to the brain.

4. Brain Cells as Targets for TiO_2NPs Toxicity: The Role of Redox Mechanisms and Apoptosis in Cell Damage

4.1 Neurons

The role of TiO_2NPs in the induction of neuronal cell death through oxidative stress and apoptosis has been clearly demonstrated [58], while the understanding of the participating mechanisms is evolving. The inhibitory effect of TiO_2 on hippocampal antioxidant enzymes and mitochondrial complex I, II, III, and IV activity, as well as ROS overproduction through down-regulation of Keap1 expression and subsequent Nrf2 activation, was shown to be associated with neuronal apoptosis and neuroinflammation, leading to both anxiety and motor deficits [59]. TiO_2NP exposure also inhibited complex V activity and resulted in reduced mitochondrial ATP generation [60]. Correspondingly, TiO_2 exposure was shown to induce brain oxidative stress through mitochondrial dysfunction, depression of antioxidant enzymes including NQO1, and down-regulation of Nrf2 mRNA expression, as well as stimulating Fas- and caspase-3-dependent apoptosis [61]. It has been also demonstrated that Nrf2 may be up-regulated upon TiO_2NPs exposure as a compensatory mechanism to overcome TiO_2NP-induced oxidative stress in mouse brains [62]. Nalika *et al*. (2023) [63] demonstrated that both melatonin and quercetin ameliorated neurobehavioral deficiency in TiO_2NP by preventing respiratory complex activity dysregulation, mitochondrial respiration, and ox-

idative stress, thus confirming the role of mitochondrial dysfunction-associated oxidative stress in TiO_2 neurotoxicity. Another mechanism of Ti-induced neuronal apoptosis may involve up-regulation of JNK and P53 phosphorylation [64].

Endoplasmic reticulum stress was also shown to play a significant role in the neurotoxic effect of TiO_2NPs in human neuroblastoma (SH-SY5Y) cell line [65]. In hippocampal neuron HT22 cells TiO_2NPs resulted in a significant increase in ROS generation and intracellular Ca^{2+} levels ultimately leading to ERS characterized by up-regulation of GRP78, IRE-1α, ATF6, CHOP, and caspase-12 mRNA and protein expression and subsequent apoptosis [66]. It has been demonstrated that TiO_2NP-induced neuronal mitochondrial dysfunction and endoplasmic reticulum stress and apoptosis through an increase in intracellular Ca^{2+} and cytochrome c levels with subsequent up-regulation of Bax and caspase-3 expression, as well as down-regulation of antiapoptotic Bcl2 [67].

In addition to neuronal damage through stimulation of proapoptotic signaling, TiO_2NP exposure also affects neurogenesis [68]. Valentini *et al*. (2018) [69] demonstrated TiO_2NP-induced inhibition of neuroblast proliferation both *in vivo* and *in vitro*. Inhibition of neuroblast proliferation in response to TiO_2 exposure was also demonstrated in cell culture from embryonic cortical brain [70]. Correspondingly, reduced hippocampal cell proliferation as evidenced by Ki-67 protein immunolabelling was associated with altered learning and memory in prenatally TiO_2NP-exposed rats [71].

4.2 Glia

Glial cells were shown to uptake TiO_2NPs through Cav-1-dependent endocytosis and activation of cysteine string proteins (CSPs) [72] with subsequent mitochondrial dysfunction, ROS overproduction, and lipid peroxidation in rat C6 and human U373 glial cell lines [73], as well as murine microglial cells (BV-2) [74]. In addition, both in rat C6 and human U373 glial cells, TiO_2 exposure inhibited cell proliferation and promoted apoptosis [75].

It has been also demonstrated that even "non-cytotoxic" concentrations of TiO_2NPs (2.5–120 ppm) that do not affect cell viability over an 18-h period, induced ROS overproduction associated with dysregulation of mitochondrial electron-transport chain in brain microglia (BV2) cells [76]. The authors also demonstrated that despite the lack of significant toxicity of TiO_2NPs (2.5–120 ppm) to isolated N27 neurons, in primary cultures of embryonic rat striatum damage in response to these particles was characterized by neuronal apoptosis, indicative of the potential role of prooxidant response of glial cells as a mediator of TiO_2NP neurotoxicity [77]. Furthermore, TiO_2NPs did not affect N2a neuroblastoma cell viability, although in co-cultures with BV-2 microglia, but not astrocytes, neuronal damage was induced by Ti-induced microglial overproduction of ROS and proinflammatory cytokines [78].

In primary rat astrocytes TiO_2 exposure induced oxidative stress and mitochondrial dysfunction [79] characterized by altered mitochondrial morphology, reduced mitochondrial membrane potential, as well as up-regulation of Mfn1, Mfn2, and Drp1 expression being markers of mitochondrial fission and fusion [80]. In agreement with mitochondrial toxicity of TiO_2NPs in astrocytes, exposure of human D384 astrocytes to TiO_2NPs induced apoptotic signaling characterized by up-regulation of p-p53, p53, p21, Bax, and caspase 3 expression, whereas Bcl-2 expression was reduced [81]. TiO_2-induced toxic effects in astrocytes may also contribute to neuronal damage due to the role of astrocytes in neuronal support.

Therefore, the existing data demonstrate not only toxic effects of TiO_2 in glial cells due to induction of mitochondrial dysfunction, oxidative stress, and apoptosis, but also a significant role of glia in mediatiing neuronal toxicity following TiO_2NP exposure.

5. Targets and Mechanisms of TiO_2NP Neurotoxicity

5.1 Axonal Growth

In addition to induction of neuronal damage and death, as well as inhibition of neuroblast proliferation, in laboratory rodents TiO_2NP exposure significantly affected neurite outgrowth. Impaired neurite outgrowth in mice prenatally exposed to TiO_2 was shown to be mediated by activation of ERK1/2/MAPK pathway as evidenced by up-regulated expression of phosphorylated ERK1/2, p38, and JNK in hippocampus [82]. Correspondingly, hippocampal neuron dendritic length was found to be reduced in association with oxidative stress, apoptosis, and excessive LC3II-mediated autophagy in mice prenatally exposed to TiO_2NPs [83]. Thinning of cerebral and cerebellar cortex, as well as hippocampal pyramidal layer, and pyramidal cell neurite dysplasia, was associated with increased RhoA [84], ROCK1, and cyclin Cdk5 protein expression in parallel with inhibition of RhoGTPase, Ras-related C1 botulinum toxin substrate (Rac1), cell division cycle42 (Cdc42), phosphorylated cAMP response element binding protein (p-CREB), p21-activated kinase (PAK) 1 and 3, LIMK (LIM kinase) 1, p-LIMK1, activated Cdc42 kinase (ACK), and myotonic dystrophic kinase-associated Cdc42-binding kinase (MRCK), as well as down-regulation of N-methyl-D-aspartate receptor subunit (NR1, NR2A, NR2B) expression [85].

Similar findings were obtained in other *in vivo* models. Specifically, TiO_2NP-induced swimming speed and clockwise rotation times of zebrafish larvae were associated with reduced motor neuron axon length and down-regulation of early neurogenesis genes, *Nrd* and *Elavl3* mRNA expression, whereas that of α*1-tubulin*, *mbp*, and *gap43* was increased consistent with a compensatory mechanism to impaired axon outgrowth [86]. Reduced axonal

growth was also detected in neurons of TiO_2NP-exposed *C. elegans*, which were characterized by altered locomotor activity [87].

Other studies also revealed significant adverse effects of TiO_2NPs on neurite outgrowth. Such effects in cultured rat hippocampal neurons were shown to be mediated by inhibition of canonical Wnt signaling through down-regulation of Wnt3a, β-catenin, p-GSK-3β, and CyclinD1 and stimulation of GSK-3β expression, as well as decreasing non-canonical Wnt signaling through reduction of MKLP1, CRMP3, ErbB4, and KIF17 protein expression [88]. It has been also demonstrated that down-regulation of Netrin-1, growth-associated protein-43, and Neuropilin-1, and up-regulation of growth inhibitors semaphorin type 3A and Nogo-A may underlie TiO_2NPs-induced inhibition of axonal growth in primary cultured hippocampal neurons [89]. In addition, synaptic dysfunction characterized by reduced synapsin-1 and postsynaptic density 95 (PSD95) expression upon TiO_2 exposure was associated with down-regulated expression of BDNF and downstream p-CREB, p-Akt, and p-ERK in a dose-dependent manner, being indicative of the role of BDNF-TrkB pathway inhibition in TiO_2-induced inhibition of synaptic growth [90]. It is also noteworthy that TiO_2 exposure was capable of reducing neurite outgrowth in PC12 cells even in sub-cytotoxic concentrations [91].

TiO_2NPs were also shown to induce cytoskeletal disruption in neuronal cells that may significantly contribute to neurotoxicity. Specifically, SH-SY5Y cells exposed to TiO_2NPs were characterized by disruption, retraction, and disorder of microtubules, as well as increased microtubule solubility, and shortening, which may be associated with direct interaction between tubule heterodimers and tau proteins with TiO_2NPs [92]. Correspondingly, TiO_2 exposure may affect microtubule formation by inhibiting tubulin polymerization [93].

Impaired neurite outgrowth due to TiO_2NPs exposure was also associated with impaired synaptic function. Prenatal TiO_2NP exposure was shown to affect synaptic plasticity in hippocampal dentate gyrus of rats [94]. TiO_2NP exposure was also shown to alter axonal retrograde transport in addition to oxidative stress, apoptosis, and inflammatory response in dorsal root ganglion sensory neurons and glial cells [95].

5.2 Neurotransmission

In addition to impaired neurite outgrowth and synaptic plasticity, TiO_2NPs were shown to impair the metabolism of neurotransmitters, especially glutamate/glutamine, and dopamine (Table 2, Ref. [80,96–104]). Exposure of mice to TiO_2NPs resulted in a significant increase in hippocampal glutamate release and phosphate-activated glutaminase activity along with a decrease in glutamine levels and glutamine synthetase activity. At the same time, TiO_2NPs significantly down-regulated N-methyl-d-aspartate receptor subunit (NR1, NR2A, and NR2B) and metabotropic glutamate receptor 2 expression [96]. These effects were also shown to be associated with impaired neurite outgrowth upon TiO_2NP exposure in rat primary cultured hippocampal neurons [97]. In addition, various forms of TiO_2NPs were shown to induce impaired glutamate uptake by primary astrocytes in association with oxidative stress, mitochondrial dysfunction, and up-regulation of Mfn1, Mfn2 and Drp1 expression being markers of mitochondrial fission and fusion [80]. Taken together, these findings are indicative of impaired glutaminergic neurotransmission.

In addition, TiO_2NP exposure was shown to affect brain catecholamine and serotonin metabolism. Specifically, TiO_2 exposure significantly reduced noradrenaline and serotonin levels in hippocampus, cerebral cortex, cerebellum, and striatum, as well as dopamine content in cerebral cortex, cerebellum, and striatum, while increasing the levels of monoamine neurotransmitter metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid, and 5-hydroxyindoleacetic acid (5-HIAA), being indicative of increased dopamine catabolism [98]. Although Hu *et al*. (2010) [99] also demonstrated that intragastric gavage of TiO_2NPs also decreased brain noradrenaline, serotonin, and dopamine levels, it was shown to reduce the levels of DOPAC and 5-hydroxyindoleacetic acid (5-HIAA), while increasing glutamate and NO levels, and acetylcholine esterase activity, indicating significant alterations in adrenergic, cholinergic, dopaminergic, and serotoninergic neurotransmitter systems. Intranasal instillation of TiO_2 to CD female mice with its absorption by nasal mucosa significantly reduced brain levels of dopamine and its metabolites, while increasing norepinephrine and serotonin content [100]. The observed reduction in dopamine production is potentially mediated by inhibition of tyrosine hydroxylase along with activation of apoptotic cell death [101]. In addition, TiO_2 induced significant dopaminergic neurotoxicity as evidenced by a dose-dependent decrease in tyrosine hydroxylase-positive neurons in substantia nigra, leading to parkinsonian-like symptoms [102]. In addition, a detailed study by Umezawa demonstrated that fetal exposure to TiO_2NPs significantly affected genes associated with regions of the dopaminergic system including striatum and neostriatum, basal ganglia, and substantia nigra both in prenatal, as well as early and later postnatal periods [105]. Notably, gene expression profile analysis showed down-regulation of DA receptor D2, considered as one of the most differentially expressed genes in brain following nasal TiO_2NPs administration, along with altered cerebral expression of genes involved in regulation of cellular process, oxidative stress, immune response, apoptosis, DNA repair, brain development, signal transduction, memory and learning, as well as response to stimulus [106].

Takahashi *et al*. (2010) [103] demonstrated that prenatal TiO_2 exposure results in a significant increase in dopamine and its metabolites (DOPAC, HVA, 3-MT) levels

Table 2. A summary of *in vivo* and *in vitro* studies demonstrating the impact of TiO_2NP exposure on neurotransmitter metabolism.

Model	Nanoparticle	Dose	Exposure	Effect	Ref
CD-1 (ICR) female mice	TiO_2NPs powder	1.25–5 mg/kg b.w. for 9 months	Nasal instillation	↑ Glu, PAG activity ↓ Gln, GS activity, NR1, NR2A, NR2B, mGluR2 mRNA and protein expression	[96]
CD-1 (ICR) female mice	Hydrophobic rutile-type TiO_2NPs and hydrophylic nano-sized particles with silica surface coating	500 g TiO_2 per mouse for 30 days	Nasal instillation	Ti content: ↑ Cortex ↑↑ Striatum ↔ Hippocampus ↔ Cerebellum ↓ Norepinephrine (Hippocampus, Cerebral cortex, Cerebellum, Striatum) ↓ Dopamine (Cortex, cerebellum, striatum) ↑ DOPAC (hippocampus, Coretex, cerebellum, striatum) ↑ HVA (hippocampus) ↓ 5-HT (Hippocampus, Cerebral cortex, Cerebellum, Striatum) ↑ 5-HIAA (Hippocampus, Cerebral cortex, Cerebellum, Striatum)	[98]
CD-1 (ICR) female mice	Anatase TiO_2 particles	5, 10, and 50 mg/kg b.w. for 60 days	Oral exposure	↑ Brain Ti level, Ach, Glutamate, TNCS, NO, Ca, Na, AChE activity ↓ NE, DA, DOPAC, 5-HT, 5-HIAA, Mg, K, Zn, Fe, Na^+/K^+-ATPase, Ca^{2+}/Mg^{2+}-ATPase, Ca^{2+}-ATPase activity	[99]
CD female mice	TiO_2 particles 25 nm, 80 nm and 155 nm	50 mg/kg b.w. 30 days	Intranasal instilled	↑ Brain Ti level (on days 10 and 20) ↑ NE and 5-HT ↓ DA, DOPAC, HVA and 5-HIAA	[100]
Balb/c mice	Anatase type TiO_2NPs sized 10 nm	10–50 mg/kg for 45 days	Oral exposure	↓ TH+ neurons in substantia nigra, hanging test time ↑ Pole test time	[102]
ICR mice	Anatase TiO_2NPs sized 25–70 mn	0.1 mL of TiO_2NPs 1 mg/mL subcutaneously injected to pregnant ICR mice at GD 6, 9, 12, 15, and 18	Prenatal exposure	Prefrontal cortex: ↑ DA, DOPAC, HVA, 3-MT Neostriatum: ↑ DA, DOPAC, HVA	[103]

Table 2. Continued.

Model	Nanoparticle	Dose	Exposure	Effect	Ref
Swiss albino male mice	TiO_2NPs sized <75 nm	500 mg/kg b.w. for 21 days	Oral exposure	Brain ↑ NE, DA, ROS, GST ↓ SOD, Catalase, GPX ↔ 5-HT	[104]
Primary hippocampal neurons from newborn Sprague Dawley rats	Nano anatase TiO_2	5–30 mg/mL	*In vitro*	↓ Neurite length ↓ Gln content ↑ Glu content ↑ PAG activity ↓ GS activity ↓ NR1, NR2A, NR2B protein expression ↑ Intracellular Ca^{2+} ↑ NO level ↑ NOS activity	[97]
Primary astrocytes from newborn Sprague Dawley rats	P25 TiO_2NPs (70% anatase & 30% rutile)	25–100 ppm	*In vitro*	↓ Cell viability ↓ Glutamate uptake ↑ MitoROS ↓ MMP ↑ *Mfn1*, *Mfn2* and *Drp1* expression	[80]
Primary olfactory bulb neurons	TiO_2NPs sized <20 nm	Different concentrations (1, 5, and 10 mg/mL) for various exposure times (24, 48 and 72 hours)	*In vitro*	↓ Cell viability ↑ DNA fragmentation ↓ BrdU ↑ Apoptosis ↓ OMP and TH mRNA expression	[101]

↓–down regulation; ↑–up-regulation; ↔–no significant changes.

in prefrontal cortex and neostriatum, whereas that in other brain regions was not affected. In another study, oral TiO_2NP administration was also shown to increase brain NE and DA levels and inhibit antioxidant enzymes in mouse brains, although this effect was less pronounced as compared to exposures to ZnO and Al_2O_3 [104].

These findings demonstrate that TiO_2NP exposure may impair not only dopamine production but also its metabolism and signaling, resulting in dysregulation of dopaminergic neurotransmission. In addition, adrenergic and serotoninergic signaling also appears to be significantly affected by TiO_2NP exposure. Taken together with the findings demonstrating altered neurite outgrowth, disruption of dopaminergic, adrenergic, and serotoninergic neurotransmitter systems likely contributes to loss of neuronal connectivity, playing a significant role in the development of neuropsychiatric disorders [107].

5.3 Neuroinflammation

TiO_2 was shown to induce hippocampal neuroinflammation associated with overproduction of proinflammatory Toll-like receptors (TLR2, TLR4) and tumor necrosis factor-α (TNFα) through up-regulation of NF-κB and NF-κB-associated nucleic IκB kinase, NF-κB-inducible kinase, p52, and p65, as well as down-regulation of IκB and IL-2 expression [108]. Neuroinflammatory response to oral TiO_2NPs exposure was also shown to be associated with increased cerebral IL-6 levels [109]. In primary rat astrocytes TiO_2 exposure also induced IkB degradation leading to increased NF-κB translocation, although NLRP3 expression was found to be reduced at least partially due to the activation of autophagy [79]. It has been also demonstrated that TiO_2NPs-induced neurotoxicity and neuroinflammation was shown to be mediated by receptor-interacting protein kinase 1 (RIP1) as evidenced by protective effects of necrostatin-1 in SH-SY5Y cells [110].

It is also noteworthy that TiO_2 did not induce neuroinflammation in healthy brains, whereas in brain of LPS-induced septic rats, TiO_2 exposure promoted IL-1β and TNFα mRNA expression. Concomitantly, in LPS-stimulated BV2 microglial cells, TiO_2 significantly up-regulated TNFα through an increase in NF-κB binding activity [111]. Proinflammatory effects of TiO_2 exposure in microglia were also shown to be mediated by inhibition of TGF-β1 and SMAD1/2/3 expression [112]. Transcriptomic analysis of exposed T98G human glioblastoma cells also demonstrated that TiO_2-induced alterations in BBB integrity may be mediated by neuroinflammation, corroborative by the increased IL-8 production [113]. It has been also demonstrated that TiO_2-induced IL-6-mediated neuroinflammation and inhibition of hippocampal BDNF expression and may be dependent on iNOS activation, as evidenced by experiment demonstrating neuroprotective effect of iNOS inhibitor, aminoguanidine [114]. Neuroinflammatory response to intravenous injection of TiO_2NPs was shown to be associated with profound alterations in brain renin-angiotensin system characterized by reduced angiotensinogen and renin gene and protein expression and up-regulated gene and protein expressions of angiotensin I converting enzyme 1 and 2 [115], consistent with the role of the brain renin-angiotensin system in modulation of neuroinflammation [116].

5.4 Protein Aggregation and Neurodegeneration

In addition to direct toxicity through induction of oxidative stress, endoplasmic reticulum stress, and apoptosis, TiO_2NP exposure was also shown to promote protein aggregation associated with neurodegenerative disorders such as in Parkinson's disease and Alzheimer's disease. Specifically, α-synuclein [117] and β-amyloid [118] oligomerization was shown to induce neurodegeneration by binding to biological membranes, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, dysregulation of proteostasis, neuroinflammation, and synaptic dysfunction.

In dopaminergic PC12 neurons, TiO_2NPs exposure significantly up-regulated α-Syn expression with subsequent α-Syn aggregation, which was partially reversed by NAC pretreatment, being indicative of the role of ROS in this process. Another mechanism of TiO_2NP-induced α-Syn aggregation may involve inhibition of ubiquitin-proteasome system through down-regulation of ubiquitin C-terminal hydrolase protein expression [119]. In zebrafish larvae TiO_2NP induced ROS overproduction and hypothalamic neuronal death, as well as loss of dopaminergic neurons in parallel with up-regulation of *pink1*, *parkin*, *α-syn*, and *uchl1* gene expression, being indicative of the potential role of Ti in PD pathogenesis [120]. Moreover, TiO_2 but not SiO_2 or SnO_2NPs, induced α-synuclein fibril formation through an increase in α-synuclein nucleation [121]. Moreover, TiO_2 was shown to promote formation of amorphous tau aggregates increasing nanoparticle neurotoxicity [122]. At the same time, despite significant neurotoxicity of TiO_2 exposure in normal (fetal) brain cells, it did not aggravate neurotoxicity in neurons derived from a PD model [123].

In addition to aggregation of α-synuclein and tau protein, TiO_2NPs were also shown to have a significant effect on β-amyloid production. Ribeiro *et al.* (2022) [124] demonstrated that direct interaction of TiO_2NPs with neuronal membrane prion protein (PrP^C) results in activation of NADPH-oxidase and 3-phosphoinositide-dependent kinase 1 (PDK1) with subsequent internalization of TACE α-secretase, resulting in increased sensitivity to TNFα and accumulation of amyloid precursor protein with amyloid Aβ40/42 overproduction. In addition, genotoxic effects of oral TiO_2NP exposure evidenced by DNA fragmentation were also associated with a point mutation at exon 5 of *PSEN1* gene that is known to be associated with inherited forms of Alzheimer's disease [125]. *In vitro* studies also demonstrate that TiO_2NPs promote β-amyloid fibrillation [126]. Direct interaction between TiO_2NPs and β-

amyloid significantly increased amyloid aggregation and fibrillation, as well as induced conformational changes in α-synuclein molecule when incubated at 37 °C [127]. Correspondingly, absorption of Aβ42 peptide on TiO_2NPs and its aminated derivative TiO_2-NH_2NPs promoted early protein oligomerization [128].

Therefore, *in vivo* studies demonstrate that TiO_2NP exposure significantly increases β-amyloid and α-synuclein accumulation, whereas both *in vivo* and *in vitro* data indicate promotion of β-amyloid, α-synuclein, and tau-protein aggregation, which may significantly contribute to pathogenesis of Alzheimer's and Parkinson's disease, especially in view of the earlier discussed role of TiO_2NPs in dopaminergic neurotoxicity.

5.5 Epigenetics

Epigenetic mechanisms were shown to mediate toxic effects of TiO_2NPs in colonic, liver, lung, skin [34], and endothelial [129] cells. The potential mechanisms involve modulation of activity of DNA methyltransferases, histone deacetylases, and ten-eleven translocation (TET) methylcytosine dioxygenases [129]. However, the particular role of epigenetic mechanisms in TiO_2NP neurotoxicity has been insufficiently studied.

It has been proposed that epigenetic effects may play a significant role in TiO_2NP toxicity [33]. Song *et al*. (2017) [130] demonstrated that exposure of PC12 cells to TiO_2NPs induced global DNA hypomethylation. Correspondingly, a later study demonstrated that prenatal TiO_2NP exposure significantly reduced DNA methylation of 6220 and 6477 genes, whereas DNA methylation rate was increased in 614 and 2924 genes in brains of male and female offspring, respectively [131].

5.6 Gut Microbiota

The role of gut microbiota as a mediator of toxic effects was demonstrated for a number of environmental pollutants [132] including metals like manganese [133] and lead [134]. Given the role of TiO_2NPs in modulation of gut microbiota characteristics and intestinal health [135], it is reasonable to posit that this interplay may be involved in TiO_2NP-induced neurotoxicity. Indeed, it has been demonstrated that prenatal TiO_2NP exposure did not affect early postnatal neurodevelopment (postnatal day 21), but affected locomotor activity, learning, and memory, as well as caused anxiety-like behaviors on postnatal day 49 along with significant alteration of gut microbiota. Specifically, a significant reduction in *Bacteroidetes* and *Cyanobacteria* relative abundance in parallel with an increased relative abundance of *Campilobacterota* was observed following prenatal TiO_2 exposure on postnatal day 49, but not at earlier periods. Taken together with the lack of influence of TiO_2 exposure on gut-derived neuropeptides and gut-brain peptides, these findings demonstrate that alterations in taxonomic characteristics of gut microbiota may at least partially mediate adverse neurobehavioral effects of TiO_2NPs [136]. TiO_2NP exposure through intragastric gavage significantly reduced gut microbiota biodiversity with a decrease in relative abundance of Bacteroidetes, whereas that of *Proteobacteria* and *Actinobacteria* was significantly increased. These alterations along with excitement on enteric neurons were associated with inhibition of locomotor activity, whereas no alterations in gut-brain peptides, brain 5-HT, or neuroinflammation were observed in TiO_2-exposed mice [35]. In adult female mice exposed to TiO_2NPs during pregnancy, the gut microbiota was characterized by reduced relative abundance of *Verrucomicrobiota* and *Desulfobacterota* phyla, as well as increased abundance of *Bacilli* class. TiO_2-induced alterations in taxonomic characteristics of gut microbiota were also accompanied by increased intestinal permeability due to down-regulation of tight junction protein expression, as well as gut-brain axis dysregulation, altogether being associated with brain damage and neurobehavioral alterations [137].

Although scant, the existing data support the potential role of gut microbiota and intestinal health as potential players in TiO_2NP neurotoxicity. It has been proposed that alterations in gut microbiota biodiversity and its taxonomic characteristics combined with increased gut permeability may increase translocation of neuroactive bacterial metabolites like lipopolysaccharide to the bloodstream and further to the brain. The latter may be further aggravated by TiO_2NP-induced alterations in BBB, leading to its increased permeability.

6. Neurotoxic Effects of TiO_2NP Coexposure with Other Toxic Substances

Multiple studies aimed at assessing the effects of co-exposure to TiO_2NPs and neurotoxic pollutants including pesticides, flame retardants, antibiotics, and other persistent organic pollutants (POPs), as well as neurotoxic metals.

TiO_2 was shown to promote neurotoxic effects of POPs. Specifically, TiO_2 potentiated accumulation of BPA in zebrafish larvae and promoted adverse effects of Bisphenol A (BPA) on *α1-tubulin*, *mbp*, and *syn2a* gene expression involved in neurodevelopment [138]. Adverse neurodevelopmental effects of BPA and TiO_2NP exposure were shown to be mediated by aggravation of BPA-induced reduction in T4 levels and its transfer to the eggs in zebrafish, resulting in lethargic swimming behavior [139]. Analogous to BPA, TiO_2NPs increased the bioavailability and neurotoxicity of another POP, polybrominated diphenyl ether congener (BDE-209), as well as promoted its adverse effect on hypothalamic-pituitary-thyroid axis in in zebrafish larvae [140]. Triphenyl phosphate-induced neuronal damage and axonal growth inhibition in secondary motor neurons were all aggravated by TiO_2NP co-exposure, while Ti and TPhP co-exposure significantly reduced serotonin levels [141]. Co-exposure of tetrabromobisphenol A (TBBPA) and TiO_2NPs significantly promoted accumula-

tion of both agents in zebrafish larvae leading to oxidative stress, neuronal apoptosis, and behavioral deficits [142].

Synergistic neurotoxic effects were demonstrated for TiO_2NP and various pesticides. TiO_2NP was shown to aggravate neurotoxic effects of a fungicide, difenoconazole, by promoting its adverse effects on neurodevelopment (down-regulation of *elavl3*, *ngn1*, *gap43*, *gfap* and *mbp* gene expression) and axonal outgrowth, as well as oxidative stress and apoptosis in zebrafish larvae [143]. In a similar model, TiO_2NPs also promoted cypermethrin-induced down-regulation of *gfap*, *α1-tubulin*, *mbp* mRNA expression, and up-regulation of neuro D expression, as well as reduction of serotonin, dopamine, and GABA levels [144].

Maternal exposure to TiO_2NPs did not promote adverse effects induced by a herbicide, paraquat, in the offspring. TiO_2 exposure decreased paraquat-induced elevation in plasma CXCL concentrations and striatal up-regulation of *Nefl*, *Nefh*, *Gfap*, *Fa2h*, *Mobp*, *Chga*, and *Kcnc2* expression. Furthermore, Gene Set Enrichment Analysis demonstrated that combined paraquat and TiO_2 exposure had a significant impact on regulation of neurotransmitters, neurons, axons extension, and voltage potassium channels pathways [145], thus increasing the risk of adverse neurological outcome. Although TiO_2NP coexposure with pentachlorophenol significantly aggravated reduction of T3 levels through down-regulation of *tg* and *dio2* gene transcription, no significant additive or potentiating effect of coexposure on neurodevelopment was observed [146].

Antibiotic, tetracycline-induced neurotoxicity was shown to be potentiated by TiO_2NPs exposure, resulting in adverse neurodevelopmental and neurobehavioral effects through alteration of development-associated genes and an increase in 5-hydroxytryptamine, dopamine, acetylcholinesterase, and γ-aminobutyric acid levels [147].

TiO_2NP coexposure with acrylamide significantly increased cerebral ROS generation and single- and double-stranded DNA breaks along with a more profound up-regulation of p53, TNFα, IL-6, and Presenilin-1 gene expression as compared to single exposures in mice [148].

In addition to organic pollutants, TiO_2NP exposure also promoted Pb accumulation in zebrafish and aggravated Pb-induced inhibition of neurodevelopment-associated genes (*α-tubulin*, *mbp*, *gfap*, and *shha*) expression, as well as modulated the impact of Pb on hypothalamic-pituitary-thyroid axis [149]. Aggravation of adverse effects of Pb on neurodevelopment by TiO_2 due to Pb adsorption on TiO_2NPs and its increased bioavailability was also associated with increased metallothionein content and reduction in fish locomotor activity [150].

Taken together, these findings demonstrate that coexposure of TiO_2NPs with other neurotoxic substances can significantly potentiate adverse effects on brain functioning.

7. Behavioral Effects

Laboratory *in vivo* studies demonstrate that TiO_2NPs exposure possesses significant adverse neurobehavioral effects in different models. Specifically, prenatal TiO_2NP exposure was shown to induce depressive-like behaviors in an adult rat model [151], whereas maternal exposure to TiO_2 during lactation also resulted in impaired memory and learning in rat offspring [152]. Maternal exposure to TiO_2NPs was also shown to impair respiratory center development as evidenced by tachypnoea in the offspring [153]. Early postnatal TiO_2 exposure was also shown to affect behavior depending on the sex and time of exposure. Specifically, female rats exposed to TiO_2NPs at postnatal days 2–5 and 7–10 were characterized by decreased acoustic startle response and motor dyscoordination with increased locomotor activity, respectively. In contrast, decreased locomotor activity was observed in male rats exposed at postnatal days 17–20. These effects were associated with a significant increase in brain dopamine levels in rats exposed to TiO_2 at postnatal days 2–5 (males and females) and 7–10 (females), whereas females were also characterized by a significant reduction in brain NE levels when exposed at postnatal days 17–20, altogether being accompanied by alterations in altered amino acid metabolism and biosynthesis, aminoacyl-tRNA biosynthesis, and lipid metabolism pathways [154]. Notter *et al*. (2018) [155] demonstrated that maternal TiO_2NP exposure induced neurobehavioral alterations characteristic for murine models of autism spectrum disorder (ASD) including impaired neonatal vocal communication, altered juvenile sociability, and an increase in prepulse inhibition of the acoustic startle reflex, although no effect on pregnancy outcome or postnatal offspring growth was observed.

Adverse effects of TiO_2 exposure were also demonstrated following postnatal exposure through various routes. Intraperitoneal injection of TiO_2 significantly increased anxiety along with overall toxicity including liver damage in rats [156]. Correspondingly, anxiety-like behavior and cognitive dysfunction following i.p. TiO_2NP injection is associated with hippocampal oxidative stress and neuroinflammation [157]. Impaired spatial cognition and emotional reactivity following acute TiO_2NP exposure were associated with severe morphological alterations in brain tissue including edema, capillary dilations, vascular congestion, and increased abundance of lymphocytic clusters [158]. In addition, alteration of spatial memory in mice was shown to be associated with inhibition of CREB-target gene transcription due to down-regulation of CaMKIV [159]. Intratracheal instillation of TiO_2NP also reduced grip strength and cortical evoked potential latency in rats [160].

TiO_2 exposure was also shown to affect motor and social behaviors in zebrafish larvae due to ROS overproduction, lipid peroxidation, apoptosis, and altered neurodevelopmental gene expression [161].

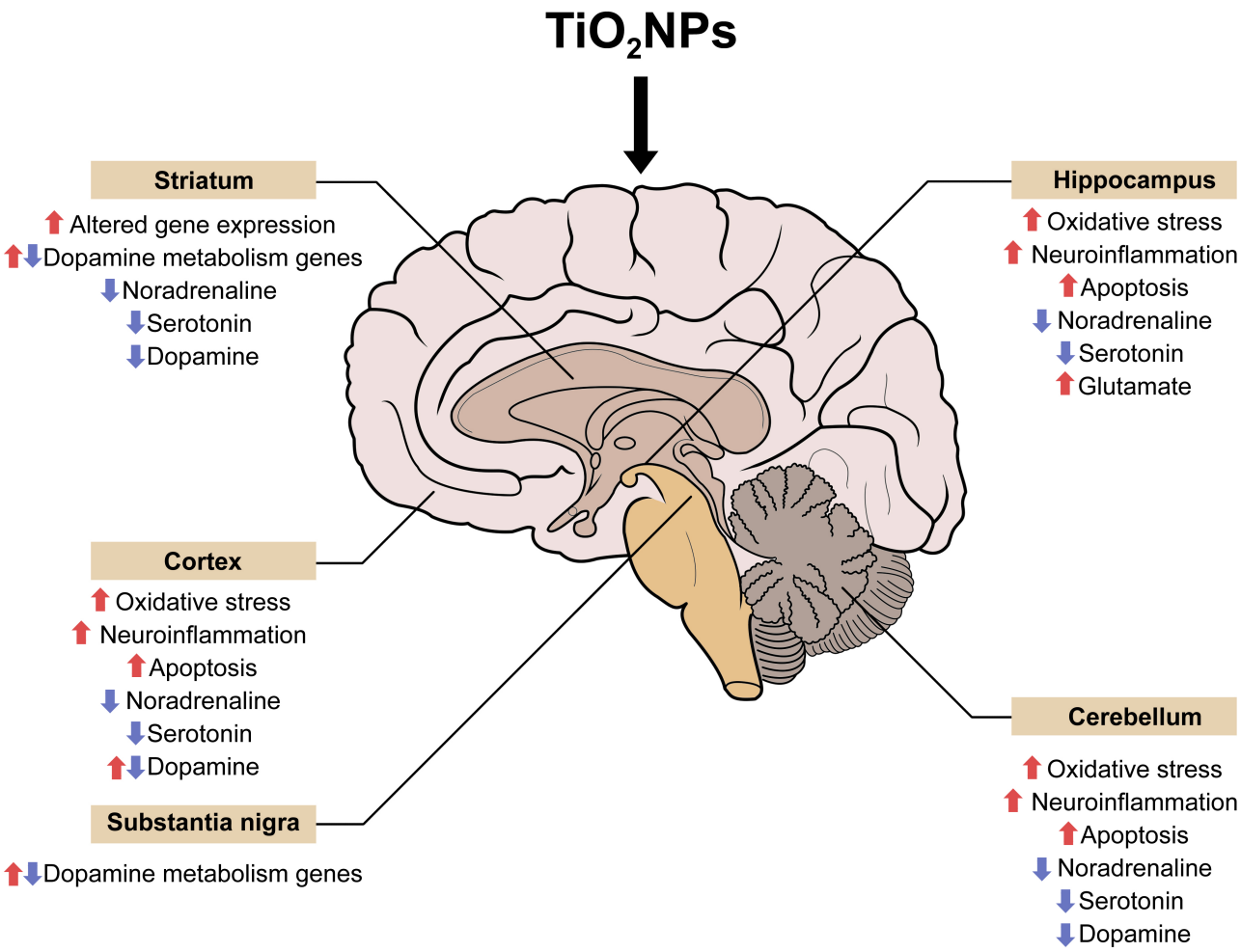

Fig. 1. Neurotoxic effects of TiO_2NPs in various brain regions. The existing data demonstrated that TiO_2NP exposure induces oxidative stress, apoptosis, and neuroinflammation in hippocampus, cortex, and cerebellum. Reduction of noradrenaline and serotonin levels was observed in hippocampus, striatum, and cerebellum, whereas alterations of dopamine and its metabolites levels were revealed in striatum, cerebellum, and cortex following TiO_2NP exposure. In addition, TiO_2NP toxicity was also associated with altered dopamine metabolism gene expression in striatum and substantia nigra.

Generally, both prenatal and postnatal exposures to TiO_2NPs through various routes result in a wide spectrum of adverse neurobehavioral effects, including reduced locomotor activity, impaired memory, and learning, autistic-like behaviors, as well as anxiety and depressive-like behaviors.

8. Discussion

As noted in the preceding sections, most recent findings broaden our understanding on the mechanisms of TiO_2NP-induced neurotoxicity. Following prenatal, intravenous, or oral exposure, TiO_2NPs are translocated across the BBB and accumulate in the brain, inducing mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, inflammatory response, and apoptosis in different types of neural cells, including neurons and astrocytes. Brain damage following TiO_2NP exposure has been observed even in the absence of particle accumulation in brain, indicating the role of systemic proinflammatory and prooxidant effects of TiO_2NPs and its toxicity to BBB and increased permeability of the latter. In addition to neuronal damage, TiO_2NPs disrupt neurogenesis, neurite outgrowth, and affect synaptic plasticity. Taken together with TiO_2NP-induced impairment in synthesis and metabolism of dopamine, serotonin, noradrenaline, glutamate, and glutamine, these effects result in altered neurotransmission (Fig. 1).

The neurotoxic effects of TiO_2NPs also likely involve increased production, accumulation, and aggregation of β-amyloid, α-synuclein, and tau proteins, leading to neurodegenerative changes inherent to Alzheimer's disease and Parkinson's disease. Recent findings also demonstrated that TiO_2NP-induced epigenetic effects, as well as alteration of the gut-brain axis due to impaired taxonomic characteristics of gut microbiota and increased gut wall permeability, effects which may significantly contribute to TiO_2NP neu-

rotoxicity. Due to the wide spectrum of molecular mechanisms involved in neurotoxicity, TiO_2NPs significantly promote the adverse effects of pesticides, flame retardants, antibiotics, and other persistent organic pollutants (POPs), as well as Pb on neuronal health. It is also notable that the effects of TiO_2NPs in brain may be dependent bot only on the dose of exposure, but also on the size of the particles. It also appears that TiO_2NPs possess higher toxicity as compared to non-nanosized TiO_2.

Taken together, the existing laboratory data demonstrate that TiO_2NPs causes a wide spectrum of neurotoxic effects, supporting the hypothesis of potential contribution of TiO_2NPs exposure to human neuropsychiatric diseases [162]. Specifically, neuronal oxidative stress, apoptosis, neuroinflammation, α-synuclein aggregation and toxicity, dopaminergic dysregulation, hallmarks of the pathogenesis of Parkinson's disease [163], as well as amyloid β and phosphorylated tau protein neurotoxicity, hallmarks in the development of Alzheimer's disease [164], were shown to be modulated by TiO_2NPs exposure, indicating the potential contribution of TiO_2NPs in the development of these neurodegenerative diseases associated with cognitive decline. Nonetheless, it is noteworthy that results of 2879 older adults demonstrated that blood Ti levels were inversely associated with cognitive function only in a single-metal model, while in a model considering multiple metal exposures no significant relationship was observed [165].

In addition, contemporary studies demonstrate the role of TiO_2NPs in impaired neurodevelopment, neurotransmitter, excitotoxicity, neuroinflammation, neuronal oxidative stress and mitochondrial disfunction with subsequent apoptosis, epigenetic effects, and modulation of gut microbiota, all being involved in development of neurodevelopmental disorders including ASD [166,167] and attention-deficit/hyperactivity disorder (ADHD) [168]. The results of a computational study also demonstrated that TiO_2 exposure targeted 50 of 3449 autism susceptibility genes [169]. However, epidemiological studies linking titanium exposure to adverse neurodevelopmental effects are scarce. A longitudinal prospective birth cohort study by Jiang *et al.* (2023) [170] demonstrated that prenatal Ti exposure may affect neurodevelopment. Specifically, increased urinary Ti levels in the third trimester were associated with significantly lower developmental quotient scores in the language domain as well as increased risk of language development delay, but no other adverse neurodevelopmental outcomes. Despite the lack of difference in whole blood and urinary Ti levels between autistic and neurotypical children, blood Ti levels correlated positively with Autism Behavior Checklist (ABC) total scores [171]. Although water Ti levels were found to be associated with the risk of ADHD in certain models [172], no significant difference in hair Ti accumulation in children with ADHD and neurotypical controls was observed [173].

While the pathogenesis of depression and anxiety is quite complex [174], key pathogenetic mechanisms including neuroinflammation, altered neurogenesis, synaptic dysfunction, altered neuromediator metabolism, and increased BBB permeability [175] as well as alterations in gut microbiome [176] should be considered as potential effects of TiO_2NPs neurotoxicity. Yet, blood Ti levels were not associated with depression risk in elderly women from Lu'an (Anhui, China) [177]. Correspondingly, no significant association between the risk of depression and anxiety with Ti levels in particular matter was observed in Beijing-Tianjin-Hebei region of China [178].

Excitotoxicity, microglia activation, neuroinflammation that were all induced by TiO_2NPs exposure, are involved in pathogenesis of migraine and neuropathic pain [179]. Although no direct evidence supporting the role of TiO_2NPs exposure in development of migraine exist, a recent case-control study demonstrated that systemic Ti toxicity after total hip arthroplasty polyethylene failure was characterized by weakness, fatigue, headache, as well as vision problems [180].

Therefore, in contrast to clear laboratory indications of TiO_2NP neurotoxicity, epidemiological findings demonstrate only indirect evidence of its potential contribution to human neuropsychiatric diseases. Moreover, a significant limitation of this association raises from the lack of titanium speciation in epidemiological studies and evaluation of only total Ti levels, as well as the absence of specific biomarkers of TiO_2NP exposure in human populations. Thus it is expected that certain titanium species and forms other than TiO_2NPs may contribute to total Ti levels in the studied biosamples from patients with neuropsychiatric diseases in the reviewed studies.

Therefore, the perspectives for further studies in the field of TiO_2NPs neurotoxicity include not only investigation of intimate mechanisms in laboratory *in vivo* and *in vitro* models, but also assessment of the potential risk of adverse neurological effects upon TiO_2NPs exposure with a special focus on dose-dependence and the impact of exposure duration.

9. Conclusions

Taken together, the existing *in vivo* and *in vitro* laboratory studies demonstrated that TiO_2NP is capable of crossing the blood-brain barrier with subsequent accumulation in various brain regions. Neurotoxic effects of TiO_2NPs were shown to be mediated by neuronal and glial oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, neuroinflammation, alteration of neurite outgrowth and neurotransmission, as well as induction of β-amyloid, α-synuclein and phosphorylated tau accumulation. Epigenetic effects of TiO_2NPs and modulation of gut microbiota were also shown to contribute to its neurotoxicity. *In vivo* studies demonstrated that these neurotoxic effects of TiO_2NPs induce adverse neurobehavioral effects

in laboratory rodents and other model organisms. In contrast, direct evidence of adverse neuropsychiatric effects of TiO_2NP exposure in human subjects are lacking. Although certain studies demonstrated the association between titanium accumulation in human biosamples and neuropsychiatric disorders, the particular contribution of TiO_2NPs into this relationship is questionable as the latter may result from exposure to other titanium species. Therefore, further studies aimed at investigation of both molecular mechanisms of TiO_2NP neurotoxicity, as well as its relevance to human neuropsychiatric disorders should be carried out in the future, to better characterize the neurotoxicity of TiO_2NPs.

Abbreviations

3-MT, 3-Methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; Ach, acetylcholine; AChE, acetylcholine esterase; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; ATF6, activating transcription factor 6; Aβ, amyloid β; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; BPA, bisphenol A; CCL2, chemokine ligand 2; Cdc42, cell division cycle42; CHOP, C/EBP Homologous Protein; CREB, cAMP response element binding protein; CRMP3, collapsin Response mediator Protein 3; CXCL1, CXC motif chemokine ligand 1; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; Drp1, dynamin-related protein 1; ERK1/2, extracellular signal-regulated kinases; GABA, gamma-aminobutyric acid; GRP78, 78-kDa glucose-regulated protein; GS, glutamine synthetase; GSK, glycogen synthase kinase; HMGA1, high Mobility Group AT-Hook 1; HVA, homovanillic acid; IL-1β, interleukin 1β; IRE-1α, inositol-requiring transmembrane kinase/endoribonuclease 1α; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; LIMK, LIM kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; Mfn1, mitofusin-1; mGluR2, metabotropic glutamate receptor 2; MKLP1, mitotic kinesin-like protein 1; MRCK, myotonic dystrophic kinase-associated Cdc42-binding kinase; NAC, N-acetylcysteine; NE, norepinephrine; NF-κB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; NMDAR, N-methyl-D-aspartate receptor; NO, nitric oxide; NOS, nitric oxide synthase; NQO1, NAD(P)H dehydrogenase [quinone] 1; Nrf2, nuclear factor erythroid 2-related factor 2; PAG, phosphate activated glutaminase; POPs, persistent organic pollutants; Rac1, Ras-related C1 botulinum toxin substrate; RIP1, receptor-interacting protein kinase 1; ROCKII, Rho-associated kinase II; ROS, reactive oxygen species; SOD, superoxide dismutase; TBBPA, tetrabromobisphenol A; TGFβ, Transforming growth factor β; TiO_2NPs, titanium dioxide nanoparticles; TLR, Toll-like receptor; TNFα, tumor necrosis factor-α; TrkB, Tropomyosin receptor kinase B; ZO-1, zonula occludens-1.

Author Contributions

These should be presented as follows: MA, AVS, and AAT designed the study. AS, ABD, YT, YJ, RL, MBV, and AAT performed the literature search. MA, AVS, AS, ABD, YT, YJ, RL, MBV, and AAT wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research work was supported by the Academic leadership program Priority 2030 proposed by Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Santos CS, Gabriel B, Blanchy M, Menes O, García D, Blanco M, *et al*. Industrial applications of nanoparticles–a prospective overview. Materials Today: Proceedings. 2015; 2: 456–465.

[2] Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry. 2019; 12: 908–931.

[3] McNamara K, Tofail SA. Nanoparticles in biomedical applications. Advances in Physics: X. 2017; 2: 54–88.

[4] Najahi-Missaoui W, Arnold RD, Cummings BS. Safe Nanoparticles: Are We There Yet? International Journal of Molecular Sciences. 2020; 22: 385.

[5] Ziental D, Czarczynska-Goslinska B, Mlynarczyk DT, Glowacka-Sobotta A, Stanisz B, Goslinski T, *et al*. Titanium Dioxide Nanoparticles: Prospects and Applications in Medicine. Nanomaterials. 2020; 10: 387.

[6] Piccinno F, Gottschalk F, Seeger S, Nowack B. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. Journal of Nanoparticle Research. 2012; 14: 1109.

[7] Irshad MA, Nawaz R, Rehman MZU, Adrees M, Rizwan M, Ali S, *et al*. Synthesis, characterization and advanced sustainable applications of titanium dioxide nanoparticles: A review. Ecotoxicology and Environmental Safety. 2021; 212: 111978.

[8] Shah SNA, Shah Z, Hussain M, Khan M. Hazardous Effects of Titanium Dioxide Nanoparticles in Ecosystem. Bioinorganic Chemistry and Applications. 2017; 2017: 4101735.

[9] Zielińska A, Costa B, Ferreira MV, Miguéis D, Louros JMS, Durazzo A, *et al*. Nanotoxicology and Nanosafety: Safety-By-Design and Testing at a Glance. International Journal of Environmental Research and Public Health. 2020; 17: 4657.

[10] He L, Wang H, Duan S, Gao Y, Lyu L, Ou X, *et al*. Characterization of titanium dioxide nanoparticles in confectionary products and estimation of dietary exposure level among the Chinese population. NanoImpact. 2022; 28: 100435.
[11] Dréno B, Alexis A, Chuberre B, Marinovich M. Safety of titanium dioxide nanoparticles in cosmetics. Journal of the European Academy of Dermatology and Venereology. 2019; 33: 34–46.
[12] Wang J, Zhou G, Chen C, Yu H, Wang T, Ma Y, *et al*. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicology Letters. 2007; 168: 176–185.
[13] Baranowska-Wójcik E, Szwajgier D, Oleszczuk P, Winiarska-Mieczan A. Effects of Titanium Dioxide Nanoparticles Exposure on Human Health-a Review. Biological Trace Element Research. 2020; 193: 118–129.
[14] Hong F, Yu X, Wu N, Zhang YQ. Progress of *in vivo* studies on the systemic toxicities induced by titanium dioxide nanoparticles. Toxicology Research. 2017; 6: 115–133.
[15] Shukla RK, Sharma V, Pandey AK, Singh S, Sultana S, Dhawan A. ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. Toxicology in Vitro. 2011; 25: 231–241.
[16] Botelho MC, Costa C, Silva S, Costa S, Dhawan A, Oliveira PA, *et al*. Effects of titanium dioxide nanoparticles in human gastric epithelial cells in vitro. Biomedicine & Pharmacotherapy. 2014; 68: 59–64.
[17] Natarajan V, Wilson CL, Hayward SL, Kidambi S. Titanium Dioxide Nanoparticles Trigger Loss of Function and Perturbation of Mitochondrial Dynamics in Primary Hepatocytes. PLoS ONE. 2015; 10: e0134541.
[18] Liu R, Zhang X, Pu Y, Yin L, Li Y, Zhang X, *et al*. Small-sized titanium dioxide nanoparticles mediate immune toxicity in rat pulmonary alveolar macrophages in vivo. Journal of Nanoscience and Nanotechnology. 2010; 10: 5161–5169.
[19] Liao F, Chen L, Liu Y, Zhao D, Peng W, Wang W, *et al*. The size-dependent genotoxic potentials of titanium dioxide nanoparticles to endothelial cells. Environmental Toxicology. 2019; 34: 1199–1207.
[20] Li Y, Yan J, Ding W, Chen Y, Pack LM, Chen T. Genotoxicity and gene expression analyses of liver and lung tissues of mice treated with titanium dioxide nanoparticles. Mutagenesis. 2017; 32: 33–46.
[21] Chen Z, Han S, Zheng P, Zhou D, Zhou S, Jia G. Effect of oral exposure to titanium dioxide nanoparticles on lipid metabolism in Sprague-Dawley rats. Nanoscale. 2020; 12: 5973–5986.
[22] Chen Z, Wang Y, Zhuo L, Chen S, Zhao L, Luan X, *et al*. Effect of titanium dioxide nanoparticles on the cardiovascular system after oral administration. Toxicology Letters. 2015; 239: 123–130.
[23] Alarifi S, Ali D, Al-Doaiss AA, Ali BA, Ahmed M, Al-Khedhairy AA. Histologic and apoptotic changes induced by titanium dioxide nanoparticles in the livers of rats. International Journal of Nanomedicine. 2013; 8: 3937–3943.
[24] Mu W, Wang Y, Huang C, Fu Y, Li J, Wang H, *et al*. Effect of Long-Term Intake of Dietary Titanium Dioxide Nanoparticles on Intestine Inflammation in Mice. Journal of Agricultural and Food Chemistry. 2019; 67: 9382–9389.
[25] Cornu R, Béduneau A, Martin H. Ingestion of titanium dioxide nanoparticles: a definite health risk for consumers and their progeny. Archives of Toxicology. 2022; 96: 2655–2686.
[26] Wang Y, Chen Z, Ba T, Pu J, Chen T, Song Y, *et al*. Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. Small. 2013; 9: 1742–1752.
[27] Shabbir S, Kulyar MFEA, Bhutta ZA, Boruah P, Asif M. Toxicological Consequences of Titanium Dioxide Nanoparticles (TiO_2NPs) and Their Jeopardy to Human Population. BioNanoScience. 2021; 11: 621–632.
[28] Blaznik U, Krušič S, Hribar M, Kušar A, Žmitek K, Pravst I. Use of Food Additive Titanium Dioxide (E171) before the Introduction of Regulatory Restrictions Due to Concern for Genotoxicity. Foods. 2021; 10: 1910.
[29] Song B, Liu J, Feng X, Wei L, Shao L. A review on potential neurotoxicity of titanium dioxide nanoparticles. Nanoscale Research Letters. 2015; 10: 1042.
[30] Czajka M, Sawicki K, Sikorska K, Popek S, Kruszewski M, Kapka-Skrzypczak L. Toxicity of titanium dioxide nanoparticles in central nervous system. Toxicology in Vitro. 2015; 29: 1042–1052.
[31] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, *et al*. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nature Nanotechnology. 2011; 6: 321–328.
[32] Shinohara N, Danno N, Ichinose T, Sasaki T, Fukui H, Honda K, *et al*. Tissue distribution and clearance of intravenously administered titanium dioxide (TiO2) nanoparticles. Nanotoxicology. 2014; 8: 132–141.
[33] Song B, Zhang Y, Liu J, Feng X, Zhou T, Shao L. Unraveling the neurotoxicity of titanium dioxide nanoparticles: focusing on molecular mechanisms. Beilstein Journal of Nanotechnology. 2016; 7: 645–654.
[34] Pogribna M, Koonce NA, Mathew A, Word B, Patri AK, Lyn-Cook B, *et al*. Effect of titanium dioxide nanoparticles on DNA methylation in multiple human cell lines. Nanotoxicology. 2020; 14: 534–553.
[35] Zhang S, Jiang X, Cheng S, Fan J, Qin X, Wang T, *et al*. Titanium dioxide nanoparticles via oral exposure leads to adverse disturbance of gut microecology and locomotor activity in adult mice. Archives of Toxicology. 2020; 94: 1173–1190.
[36] Grissa I, ElGhoul J, Mrimi R, Mir LE, Cheikh HB, Horcajada P. In deep evaluation of the neurotoxicity of orally administered TiO_2 nanoparticles. Brain Research Bulletin. 2020; 155: 119–128.
[37] Halawa A, Elshopakey G, El-Adl M, Lashen S, Shalaby N, Eldomany E, *et al*. Chitosan attenuated the neurotoxicity-induced titanium dioxide nanoparticles in brain of adult rats. Environmental Toxicology. 2022; 37: 612–626.
[38] Brandão F, Fernández-Bertólez N, Rosário F, Bessa MJ, Fraga S, Pásaro E, *et al*. Genotoxicity of TiO_2 Nanoparticles in Four Different Human Cell Lines (A549, HEPG2, A172 and SH-SY5Y). Nanomaterials. 2020; 10: 412.
[39] Fabian E, Landsiedel R, Ma-Hock L, Wiench K, Wohlleben W, van Ravenzwaay B. Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. Archives of Toxicology. 2008; 82: 151–157.
[40] Disdier C, Chalansonnet M, Gagnaire F, Gaté L, Cosnier F, Devoy J, *et al*. Brain Inflammation, Blood Brain Barrier dysfunction and Neuronal Synaptophysin Decrease after Inhalation Exposure to Titanium Dioxide Nano-aerosol in Aging Rats. Scientific Reports. 2017; 7: 12196.
[41] Disdier C, Devoy J, Cosnefroy A, Chalansonnet M, Herlin-Boime N, Brun E, *et al*. Tissue biodistribution of intravenously administrated titanium dioxide nanoparticles revealed blood-brain barrier clearance and brain inflammation in rat. Particle and Fibre Toxicology. 2015; 12: 27.
[42] Rosário F, Costa C, Lopes CB, Estrada AC, Tavares DS, Pereira E, *et al*. In Vitro Hepatotoxic and Neurotoxic Effects of Titanium and Cerium Dioxide Nanoparticles, Arsenic and Mercury Co-Exposure. International Journal of Molecular Sciences. 2022; 23: 2737.
[43] Coccini T, Grandi S, Lonati D, Locatelli C, De Simone U. Comparative cellular toxicity of titanium dioxide nanoparticles on

human astrocyte and neuronal cells after acute and prolonged exposure. Neurotoxicology. 2015; 48: 77–89.
[44] Liu Y, Xu Z, Li X. Cytotoxicity of titanium dioxide nanoparticles in rat neuroglia cells. Brain Injury. 2013; 27: 934–939.
[45] Chen IC, Hsiao IL, Lin HC, Wu CH, Chuang CY, Huang YJ. Influence of silver and titanium dioxide nanoparticles on in vitro blood-brain barrier permeability. Environmental Toxicology and Pharmacology. 2016; 47: 108–118.
[46] Jia X, Wang S, Zhou L, Sun L. The Potential Liver, Brain, and Embryo Toxicity of Titanium Dioxide Nanoparticles on Mice. Nanoscale Research Letters. 2017; 12: 478.
[47] Fujioka K, Hanada S, Inoue Y, Sato K, Hirakuri K, Shiraishi K, *et al*. Effects of silica and titanium oxide particles on a human neural stem cell line: morphology, mitochondrial activity, and gene expression of differentiation markers. International Journal of Molecular Sciences. 2014; 15: 11742–11759.
[48] Gerber LS, Heusinkveld HJ, Langendoen C, Stahlmecke B, Schins RP, Westerink RH. Acute, sub-chronic and chronic exposures to TiO_2 and Ag nanoparticles differentially affects neuronal function in vitro. Neurotoxicology. 2022; 93: 311–323.
[49] Valdiglesias V, Costa C, Sharma V, Kiliç G, Pásaro E, Teixeira JP, *et al*. Comparative study on effects of two different types of titanium dioxide nanoparticles on human neuronal cells. Food and Chemical Toxicology. 2013; 57: 352–361.
[50] Wang J, Liu Y, Jiao F, Lao F, Li W, Gu Y, *et al*. Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO(2) nanoparticles. Toxicology. 2008; 254: 82–90.
[51] Wang YJ, He ZZ, Fang YW, Xu Y, Chen YN, Wang GQ, *et al*. Effect of titanium dioxide nanoparticles on zebrafish embryos and developing retina. International Journal of Ophthalmology. 2014; 7: 917–923.
[52] Sofranko A, Wahle T, Heusinkveld HJ, Stahlmecke B, Dronov M, Pijnenburg D, *et al*. Evaluation of the neurotoxic effects of engineered nanomaterials in C57BL/6J mice in 28-day oral exposure studies. Neurotoxicology. 2021; 84: 155–171.
[53] Liu X, , Sui B, , Sun J, . Size- and shape-dependent effects of titanium dioxide nanoparticles on the permeabilization of the blood-brain barrier. Journal of Materials Chemistry. B. 2017; 5: 9558–9570.
[54] Hong F, Mu X, Ze Y, Li W, Zhou Y, Ji J. Damage to the Blood Brain Barrier Structure and Function from Nano Titanium Dioxide Exposure Involves the Destruction of Key Tight Junction Proteins in the Mouse Brain. Journal of Biomedical Nanotechnology. 2021; 17: 1068–1078.
[55] Shelly S, Liraz Zaltsman S, Ben-Gal O, Dayan A, Ganmore I, Shemesh C, *et al*. Potential neurotoxicity of titanium implants: Prospective, in-vivo and in-vitro study. Biomaterials. 2021; 276: 121039.
[56] Brun E, Carrière M, Mabondzo A. In vitro evidence of dysregulation of blood-brain barrier function after acute and repeated/long-term exposure to TiO(2) nanoparticles. Biomaterials. 2012; 33: 886–896.
[57] Zeman T, Loh EW, Čierný D, Šerý O. Penetration, distribution and brain toxicity of titanium nanoparticles in rodents' body: a review. IET Nanobiotechnology. 2018; 12: 695–700.
[58] Liu S, Xu L, Zhang T, Ren G, Yang Z. Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. Toxicology. 2010; 267: 172–177.
[59] Sobhani S, Tehrani AA, Sobhani G, Fatima S, Ulloa L, Motaghinejad M, *et al*. Melatonin Protects Against Titanium Oxide-Induced Neurotoxicity: Neurochemical, Neurobehavioral, and Histopathological Evidences. Biological Trace Element Research. 2023; 201: 3861–3881.
[60] Nalika N, Parvez S. Mitochondrial dysfunction in titanium dioxide nanoparticle-induced neurotoxicity. Toxicology Mechanisms and Methods. 2015; 25: 355–363.
[61] Kandeil MA, Mohammed ET, Hashem KS, Aleya L, Abdel-Daim MM. Moringa seed extract alleviates titanium oxide nanoparticles (TiO2-NPs)-induced cerebral oxidative damage, and increases cerebral mitochondrial viability. Environmental Science and Pollution Research International. 2020; 27: 19169–19184.
[62] Ze Y, Zheng L, Zhao X, Gui S, Sang X, Su J, *et al*. Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice. Chemosphere. 2013; 92: 1183–1189.
[63] Nalika N, Waseem M, Kaushik P, Salman M, Andrabi SS, Jamal A, *et al*. Role of melatonin and quercetin as countermeasures to the mitochondrial dysfunction induced by titanium dioxide nanoparticles. Life Sciences. 2023; 328: 121403.
[64] Wu J, Sun J, Xue Y. Involvement of JNK and P53 activation in G2/M cell cycle arrest and apoptosis induced by titanium dioxide nanoparticles in neuron cells. Toxicology Letters. 2010; 199: 269–276.
[65] Ferraro SA, Domingo MG, Etcheverrito A, Olmedo DG, Tasat DR. Neurotoxicity mediated by oxidative stress caused by titanium dioxide nanoparticles in human neuroblastoma (SH-SY5Y) cells. Journal of Trace Elements in Medicine and Biology. 2020; 57: 126413.
[66] He Q, Zhou X, Liu Y, Gou W, Cui J, Li Z, *et al*. Titanium dioxide nanoparticles induce mouse hippocampal neuron apoptosis via oxidative stress- and calcium imbalance-mediated endoplasmic reticulum stress. Environmental Toxicology and Pharmacology. 2018; 63: 6–15.
[67] Sheng L, Ze Y, Wang L, Yu X, Hong J, Zhao X, *et al*. Mechanisms of TiO2 nanoparticle-induced neuronal apoptosis in rat primary cultured hippocampal neurons. Journal of Biomedical Materials Research. Part a. 2015; 103: 1141–1149.
[68] Ebrahimzadeh Bideskan A, Mohammadipour A, Fazel A, Haghir H, Rafatpanah H, Hosseini M, *et al*. Maternal exposure to titanium dioxide nanoparticles during pregnancy and lactation alters offspring hippocampal mRNA BAX and Bcl-2 levels, induces apoptosis and decreases neurogenesis. Experimental and Toxicologic Pathology. 2017; 69: 329–337.
[69] Valentini X, Deneufbourg P, Paci P, Rugira P, Laurent S, Frau A, *et al*. Morphological alterations induced by the exposure to TiO_2 nanoparticles in primary cortical neuron cultures and in the brain of rats. Toxicology Reports. 2018; 5: 878–889.
[70] Sun Y, Wang S, Zheng J. Biosynthesis of TiO_2 nanoparticles and their application for treatment of brain injury-An in-vitro toxicity study towards central nervous system. Journal of Photochemistry and Photobiology. B, Biology. 2019; 194: 1–5.
[71] Mohammadipour A, Fazel A, Haghir H, Motejaded F, Rafatpanah H, Zabihi H, *et al*. Maternal exposure to titanium dioxide nanoparticles during pregnancy; impaired memory and decreased hippocampal cell proliferation in rat offspring. Environmental Toxicology and Pharmacology. 2014; 37: 617–625.
[72] Huerta-García E, Márquez-Ramírez SG, Ramos-Godinez MDP, López-Saavedra A, Herrera LA, Parra A, *et al*. Internalization of titanium dioxide nanoparticles by glial cells is given at short times and is mainly mediated by actin reorganization-dependent endocytosis. Neurotoxicology. 2015; 51: 27–37.
[73] Huerta-García E, Pérez-Arizti JA, Márquez-Ramírez SG, Delgado-Buenrostro NL, Chirino YI, Iglesias GG, *et al*. Titanium dioxide nanoparticles induce strong oxidative stress and mitochondrial damage in glial cells. Free Radical Biology & Medicine. 2014; 73: 84–94.
[74] Rihane N, Nury T, M'rad I, El Mir L, Sakly M, Amara S, *et al*. Microglial cells (BV-2) internalize titanium dioxide (TiO2) nanoparticles: toxicity and cellular responses. Environmental Science and Pollution Research International. 2016; 23: 9690–9699.

[75] Márquez-Ramírez SG, Delgado-Buenrostro NL, Chirino YI, Iglesias GG, López-Marure R. Titanium dioxide nanoparticles inhibit proliferation and induce morphological changes and apoptosis in glial cells. Toxicology. 2012; 302: 146–156.
[76] Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environmental Science & Technology. 2006; 40: 4346–4352.
[77] Long TC, Tajuba J, Sama P, Saleh N, Swartz C, Parker J, *et al*. Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons in vitro. Environmental Health Perspectives. 2007; 115: 1631–1637.
[78] Hsiao IL, Chang CC, Wu CY, Hsieh YK, Chuang CY, Wang CF, *et al*. Indirect effects of TiO2 nanoparticle on neuron-glial cell interactions. Chemico-Biological Interactions. 2016; 254: 34–44.
[79] Pérez-Arizti JA, Ventura-Gallegos JL, Galván Juárez RE, Ramos-Godinez MDP, Colín-Val Z, López-Marure R. Titanium dioxide nanoparticles promote oxidative stress, autophagy and reduce NLRP3 in primary rat astrocytes. Chemico-biological Interactions. 2020; 317: 108966.
[80] Wilson CL, Natarajan V, Hayward SL, Khalimonchuk O, Kidambi S. Mitochondrial dysfunction and loss of glutamate uptake in primary astrocytes exposed to titanium dioxide nanoparticles. Nanoscale. 2015; 7: 18477–18488.
[81] De Simone U, Lonati D, Ronchi A, Coccini T. Brief exposure to nanosized and bulk titanium dioxide forms induces subtle changes in human D384 astrocytes. Toxicology Letters. 2016; 254: 8–21.
[82] Zhou Y, Ji J, Chen C, Hong F. Retardation of Axonal and Dendritic Outgrowth Is Associated with the MAPK Signaling Pathway in Offspring Mice Following Maternal Exposure to Nanosized Titanium Dioxide. Journal of Agricultural and Food Chemistry. 2019; 67: 2709–2715.
[83] Zhou Y, Hong F, Tian Y, Zhao X, Hong J, Ze Y, *et al*. Nanoparticulate titanium dioxide-inhibited dendritic development is involved in apoptosis and autophagy of hippocampal neurons in offspring mice. Toxicology Research. 2017; 6: 889–901.
[84] Hong F, Zhou Y, Ji J, Zhuang J, Sheng L, Wang L. Nano-TiO_2 Inhibits Development of the Central Nervous System and Its Mechanism in Offspring Mice. Journal of Agricultural and Food Chemistry. 2018; 66: 11767–11774.
[85] Zhou Y, Ji J, Hong F, Zhuang J, Wang L. Maternal Exposure to Nanoparticulate Titanium Dioxide Causes Inhibition of Hippocampal Development Involving Dysfunction of the Rho/NMDAR Signaling Pathway in Offspring. Journal of Biomedical Nanotechnology. 2019; 15: 839–847.
[86] Gu J, Guo M, Huang C, Wang X, Zhu Y, Wang L, *et al*. Titanium dioxide nanoparticle affects motor behavior, neurodevelopment and axonal growth in zebrafish (Danio rerio) larvae. The Science of the Total Environment. 2021; 754: 142315.
[87] Hu CC, Wu GH, Hua TE, Wagner OI, Yen TJ. Uptake of TiO_2 Nanoparticles into C. elegans Neurons Negatively Affects Axonal Growth and Worm Locomotion Behavior. ACS Applied Materials & Interfaces. 2018; 10: 8485–8495.
[88] Hong F, Ze Y, Zhou Y, Hong J, Yu X, Sheng L, *et al*. Nanoparticulate TiO_2 -mediated inhibition of the Wnt signaling pathway causes dendritic development disorder in cultured rat hippocampal neurons. Journal of Biomedical Materials Research. Part a. 2017; 105: 2139–2149.
[89] Mu X, Li W, Ze X, Li L, Wang G, Hong F, *et al*. Molecular mechanism of nanoparticulate TiO_2 induction of axonal development inhibition in rat primary cultured hippocampal neurons. Environmental Toxicology. 2020; 35: 895–905.
[90] Hong F, Ze X, Mu X, Ze Y. Titanium Dioxide Inhibits Hippocampal Neuronal Synapse Growth Through the Brain-Derived Neurotrophic Factor-Tyrosine Kinase Receptor B Signaling Pathway. Journal of Biomedical Nanotechnology. 2021; 17: 37–52.
[91] Irie T, Kawakami T, Sato K, Usami M. Sub-toxic concentrations of nano-ZnO and nano-TiO_2 suppress neurite outgrowth in differentiated PC12 cells. The Journal of Toxicological Sciences. 2017; 42: 723–729.
[92] Mao Z, Xu B, Ji X, Zhou K, Zhang X, Chen M, *et al*. Titanium dioxide nanoparticles alter cellular morphology via disturbing the microtubule dynamics. Nanoscale. 2015; 7: 8466–8475.
[93] Gheshlaghi ZN, Riazi GH, Ahmadian S, Ghafari M, Mahinpour R. Toxicity and interaction of titanium dioxide nanoparticles with microtubule protein. Acta Biochimica et Biophysica Sinica. 2008; 40: 777–782.
[94] Gao X, Yin S, Tang M, Chen J, Yang Z, Zhang W, *et al*. Effects of developmental exposure to TiO2 nanoparticles on synaptic plasticity in hippocampal dentate gyrus area: an in vivo study in anesthetized rats. Biological Trace Element Research. 2011; 143: 1616–1628.
[95] Erriquez J, Bolis V, Morel S, Fenoglio I, Fubini B, Quagliotto P, *et al*. Nanosized TiO2 is internalized by dorsal root ganglion cells and causes damage via apoptosis. Nanomedicine. 2015; 11: 1309–1319.
[96] Ze X, Su M, Zhao X, Jiang H, Hong J, Yu X, *et al*. TiO2 nanoparticle-induced neurotoxicity may be involved in dysfunction of glutamate metabolism and its receptor expression in mice. Environmental Toxicology. 2016; 31: 655–662.
[97] Hong F, Sheng L, Ze Y, Hong J, Zhou Y, Wang L, *et al*. Suppression of neurite outgrowth of primary cultured hippocampal neurons is involved in impairment of glutamate metabolism and NMDA receptor function caused by nanoparticulate TiO2. Biomaterials. 2015; 53: 76–85.
[98] Zhang L, Bai R, Li B, Ge C, Du J, Liu Y, *et al*. Rutile TiO_2 particles exert size and surface coating dependent retention and lesions on the murine brain. Toxicology Letters. 2011; 207: 73–81.
[99] Hu R, Gong X, Duan Y, Li N, Che Y, Cui Y, *et al*. Neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO2 nanoparticles. Biomaterials. 2010; 31: 8043–8050.
[100] Wang JX, Li YF, Zhou GQ, Li B, Jiao F, Chen CY, *et al*. Influence of intranasal instilled titanium dioxide nanoparticles on monoaminergic neurotransmitters of female mice at different exposure time. Chinese Journal of Preventive Medicine. 2007; 41: 91–95. (In Chinese)
[101] Yu Y, Ren W, Ren B. Nanosize titanium dioxide cause neuronal apoptosis: a potential linkage between nanoparticle exposure and neural disorder. Neurological Research. 2008; 30: 1115–1120.
[102] Heidari Z, Mohammadipour A, Haeri P, Ebrahimzadeh-Bideskan A. The effect of titanium dioxide nanoparticles on mice midbrain substantia nigra. Iranian Journal of Basic Medical Sciences. 2019; 22: 745–751.
[103] Takahashi Y, Mizuo K, Shinkai Y, Oshio S, Takeda K. Prenatal exposure to titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and neostriatum of mice. The Journal of Toxicological Sciences. 2010; 35: 749–756.
[104] Shrivastava R, Raza S, Yadav A, Kushwaha P, Flora SJS. Effects of sub-acute exposure to TiO2, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. Drug and Chemical Toxicology. 2014; 37: 336–347.
[105] Umezawa M, Tainaka H, Kawashima N, Shimizu M, Takeda K. Effect of fetal exposure to titanium dioxide nanoparticle on brain development - brain region information. The Journal of Toxicological Sciences. 2012; 37: 1247–1252.

[106] Ze Y, Hu R, Wang X, Sang X, Ze X, Li B, *et al*. Neurotoxicity and gene-expressed profile in brain-injured mice caused by exposure to titanium dioxide nanoparticles. Journal of Biomedical Materials Research. Part A. 2014; 102: 470–478.
[107] Di Gregorio F, Battaglia S. Advances in EEG-based functional connectivity approaches to the study of the central nervous system in health and disease. Advances in Clinical and Experimental Medicine. 2023; 32: 607–612.
[108] Ze Y, Sheng L, Zhao X, Hong J, Ze X, Yu X, *et al*. TiO2 nanoparticles induced hippocampal neuroinflammation in mice. PLoS ONE. 2014; 9: e92230.
[109] Grissa I, Guezguez S, Ezzi L, Chakroun S, Sallem A, Kerkeni E, *et al*. The effect of titanium dioxide nanoparticles on neuroinflammation response in rat brain. Environmental Science and Pollution Research International. 2016; 23: 20205–20213.
[110] Zhou T, Huang WK, Xu QY, Zhou X, Wang Y, Yue ZH, *et al*. Nec-1 Attenuates Neurotoxicity Induced by Titanium Dioxide Nanomaterials on Sh-Sy5y Cells Through RIP1. Nanoscale Research Letters. 2020; 15: 65.
[111] Shin JA, Lee EJ, Seo SM, Kim HS, Kang JL, Park EM. Nanosized titanium dioxide enhanced inflammatory responses in the septic brain of mouse. Neuroscience. 2010; 165: 445–454.
[112] Huang W, Tao Y, Zhang X, Zhang X. TGF-β1/SMADs signaling involved in alleviating inflammation induced by nanoparticulate titanium dioxide in BV2 cells. Toxicology in Vitro. 2022; 80: 105303.
[113] Fuster E, Candela H, Estévez J, Vilanova E, Sogorb MA. Titanium Dioxide, but Not Zinc Oxide, Nanoparticles Cause Severe Transcriptomic Alterations in T98G Human Glioblastoma Cells. International Journal of Molecular Sciences. 2021; 22: 2084.
[114] Asghari A, Hosseini M, Beheshti F, Shafei MN, Mehri S. Inducible nitric oxide inhibitor aminoguanidine, ameliorated oxidative stress, interleukin-6 concentration and improved brain-derived neurotrophic factor in the brain tissues of neonates born from titanium dioxide nanoparticles exposed rats. The Journal of Maternal-Fetal & Neonatal Medicine. 2019; 32: 3962–3973.
[115] Krawczyńska A, Dziendzikowska K, Gromadzka-Ostrowska J, Lankoff A, Herman AP, Oczkowski M, *et al*. Silver and titanium dioxide nanoparticles alter oxidative/inflammatory response and renin-angiotensin system in brain. Food and Chemical Toxicology. 2015; 85: 96–105.
[116] Cosarderelioglu C, Nidadavolu LS, George CJ, Oh ES, Bennett DA, Walston JD, *et al*. Brain Renin-Angiotensin System at the Intersect of Physical and Cognitive Frailty. Frontiers in Neuroscience. 2020; 14: 586314.
[117] Du XY, Xie XX, Liu RT. The Role of α-Synuclein Oligomers in Parkinson's Disease. International Journal of Molecular Sciences. 2020; 21: 8645.
[118] Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. Medical Principles and Practice. 2015; 24: 1–10.
[119] Wu J, Xie H. Effects of titanium dioxide nanoparticles on α-synuclein aggregation and the ubiquitin-proteasome system in dopaminergic neurons. Artificial Cells, Nanomedicine, and Biotechnology. 2016; 44: 690–694.
[120] Hu Q, Guo F, Zhao F, Fu Z. Effects of titanium dioxide nanoparticles exposure on parkinsonism in zebrafish larvae and PC12. Chemosphere. 2017; 173: 373–379.
[121] Mohammadi S, Nikkhah M. TiO2 Nanoparticles as Potential Promoting Agents of Fibrillation of α-Synuclein, a Parkinson's Disease-Related Protein. Iranian Journal of Biotechnology. 2017; 15: 87–94.
[122] Fardanesh A, Zibaie S, Shariati B, Attar F, Rouhollah F, Akhtari K, *et al*. Amorphous aggregation of tau in the presence of titanium dioxide nanoparticles: biophysical, computational, and cellular studies. International Journal of Nanomedicine. 2019; 14: 901–911.
[123] Jamali B, Entezari M, Babaei N, Hashemi M. The Evaluation of Vitamin E and TiO_2 Nanoparticles Administration in Parkinson's Rat Model. Cell Journal. 2023; 25: 102–109.
[124] Ribeiro LW, Pietri M, Ardila-Osorio H, Baudry A, Boudet-Devaud F, Bizingre C, *et al*. Titanium dioxide and carbon black nanoparticles disrupt neuronal homeostasis via excessive activation of cellular prion protein signaling. Particle and Fibre Toxicology. 2022; 19: 48.
[125] Mohamed HRH, Hussien NA. Genotoxicity Studies of Titanium Dioxide Nanoparticles (TiO2NPs) in the Brain of Mice. Scientifica. 2016; 2016: 6710840.
[126] Wu WH, Sun X, Yu YP, Hu J, Zhao L, Liu Q, *et al*. TiO2 nanoparticles promote beta-amyloid fibrillation in vitro. Biochemical and Biophysical Research Communications. 2008; 373: 315–318.
[127] Slekiene N, Snitka V, Bruzaite I, Ramanavicius A. Influence of TiO_2 and ZnO Nanoparticles on α-Synuclein and β-Amyloid Aggregation and Formation of Protein Fibrils. Materials. 2022; 15: 7664.
[128] Li Q, Wen J, Yan Z, Sun H, Song E, Song Y. Mechanistic Insights of TiO_2 Nanoparticles with Different Surface Charges on Aβ_42 Peptide Early Aggregation: An In Vitro and In Silico Study. Langmuir. 2023; 39: 1997–2007.
[129] Fernandes CJDC, da Silva RAF, Wood PF, Ferreira MR, de Almeida GS, de Moraes JF, *et al*. Titanium-Enriched Medium Promotes Environment-Induced Epigenetic Machinery Changes in Human Endothelial Cells. Journal of Functional Biomaterials. 2023; 14: 131.
[130] Song B, Zhou T, Liu J, Shao L. Measuring global DNA methylation to assess neurotoxicity of titanium dioxide nanoparticles. Science of Advanced Materials. 2017; 9: 1051–1056.
[131] Tachibana K, Kawazoe S, Onoda A, Umezawa M, Takeda K. Effects of Prenatal Exposure to Titanium Dioxide Nanoparticles on DNA Methylation and Gene Expression Profile in the Mouse Brain. Frontiers in Toxicology. 2021; 3: 705910.
[132] Claus SP, Guillou H, Ellero-Simatos S. The gut microbiota: a major player in the toxicity of environmental pollutants? NPJ Biofilms and Microbiomes. 2016; 2: 16003.
[133] Tinkov AA, Martins AC, Avila DS, Gritsenko VA, Skalny AV, Santamaria A, *et al*. Gut Microbiota as a Potential Player in Mn-Induced Neurotoxicity. Biomolecules. 2021; 11: 1292.
[134] Sun L, Zou Y, Su P, Xue C, Wang D, Zhao F, *et al*. Lead Exposure Induced Neural Stem Cells Death via Notch Signaling Pathway and Gut-Brain Axis. Oxidative Medicine and Cellular Longevity. 2022; 2022: 7676872.
[135] Rinninella E, Cintoni M, Raoul P, Mora V, Gasbarrini A, Mele MC. Impact of Food Additive Titanium Dioxide on Gut Microbiota Composition, Microbiota-Associated Functions, and Gut Barrier: A Systematic Review of In Vivo Animal Studies. International Journal of Environmental Research and Public Health. 2021; 18: 2008.
[136] Su J, Duan X, Qiu Y, Zhou L, Zhang H, Gao M, *et al*. Pregnancy exposure of titanium dioxide nanoparticles causes intestinal dysbiosis and neurobehavioral impairments that are not significant postnatally but emerge in adulthood of offspring. Journal of Nanobiotechnology. 2021; 19: 234.
[137] Yang C, Xue J, Qin Q, Xia Y, Cheng S, Jiang X, *et al*. Prenatal exposure to titanium dioxide nanoparticles induces persistent neurobehavioral impairments in maternal mice that is associated with microbiota-gut-brain axis. Food and Chemical Toxicology. 2022; 169: 113402.
[138] Fu J, Guo Y, Yang L, Han J, Zhou B. Nano-TiO_2 enhanced bioaccumulation and developmental neurotoxicity of bisphenol a in zebrafish larvae. Environmental Research. 2020; 187:

109682.
[139] Guo Y, Chen L, Wu J, Hua J, Yang L, Wang Q, *et al*. Parental co-exposure to bisphenol A and nano-TiO_2 causes thyroid endocrine disruption and developmental neurotoxicity in zebrafish offspring. The Science of the Total Environment. 2019; 650: 557–565.
[140] Wang Q, Chen Q, Zhou P, Li W, Wang J, Huang C, *et al*. Bioconcentration and metabolism of BDE-209 in the presence of titanium dioxide nanoparticles and impact on the thyroid endocrine system and neuronal development in zebrafish larvae. Nanotoxicology. 2014; 8: 196–207.
[141] Fan B, Dai L, Liu C, Sun Q, Yu L. Nano-TiO_2 aggravates bioaccumulation and developmental neurotoxicity of triphenyl phosphate in zebrafish larvae. Chemosphere. 2022; 287: 132161.
[142] Chen J, Li J, Jiang H, Yu J, Wang H, Wang N, *et al*. Developmental co-exposure of TBBPA and titanium dioxide nanoparticle induced behavioral deficits in larval zebrafish. Ecotoxicology and Environmental Safety. 2021; 215: 112176.
[143] Zhu R, Liu C, Wang J, Zou L, Yang F, Chi X, *et al*. Nano-TiO_2 aggravates bioaccumulation and developmental neurotoxicity of difenoconazole in zebrafish larvae via oxidative stress and apoptosis: Protective role of vitamin C. Ecotoxicology and Environmental Safety. 2023; 251: 114554.
[144] Li M, Wu Q, Wang Q, Xiang D, Zhu G. Effect of titanium dioxide nanoparticles on the bioavailability and neurotoxicity of cypermethrin in zebrafish larvae. Aquatic Toxicology. 2018; 199: 212–219.
[145] Hamdaoui Q, Zekri Y, Richard S, Aubert D, Guyot R, Markossian S, *et al*. Prenatal exposure to paraquat and nanoscaled TiO_2 aerosols alters the gene expression of the developing brain. Chemosphere. 2022; 287: 132253.
[146] Lei L, Qiao K, Guo Y, Han J, Zhou B. Titanium dioxide nanoparticles enhanced thyroid endocrine disruption of pentachlorophenol rather than neurobehavioral defects in zebrafish larvae. Chemosphere. 2020; 249: 126536.
[147] Xu L, Yang X, He Y, Hu Q, Fu Z. Combined exposure to titanium dioxide and tetracycline induces neurotoxicity in zebrafish. Comparative Biochemistry and Physiology. Toxicology & Pharmacology. 2023; 267: 109562.
[148] Safwat G, Mohamed AA, Mohamed HRH. Estimation of genotoxicity, apoptosis and oxidative stress induction by TiO_2 nanoparticles and acrylamide subacute oral coadministration in mice. Scientific Reports. 2022; 12: 18648.
[149] Miao W, Zhu B, Xiao X, Li Y, Dirbaba NB, Zhou B, *et al*. Effects of titanium dioxide nanoparticles on lead bioconcentration and toxicity on thyroid endocrine system and neuronal development in zebrafish larvae. Aquatic Toxicology. 2015; 161: 117–126.
[150] Hu S, Han J, Yang L, Li S, Guo Y, Zhou B, *et al*. Impact of co-exposure to titanium dioxide nanoparticles and Pb on zebrafish embryos. Chemosphere. 2019; 233: 579–589.
[151] Cui Y, Chen X, Zhou Z, Lei Y, Ma M, Cao R, *et al*. Prenatal exposure to nanoparticulate titanium dioxide enhances depressive-like behaviors in adult rats. Chemosphere. 2014; 96: 99–104.
[152] Mohammadipour A, Hosseini M, Fazel A, Haghir H, Rafatpanah H, Pourganji M, *et al*. The effects of exposure to titanium dioxide nanoparticles during lactation period on learning and memory of rat offspring. Toxicology and Industrial Health. 2016; 32: 221–228.
[153] Colnot E, Cardoit L, Cabirol MJ, Roudier L, Delville MH, Fayoux A, *et al*. Chronic maternal exposure to titanium dioxide nanoparticles alters breathing in newborn offspring. Particle and Fibre Toxicology. 2022; 19: 57.
[154] Mortensen NP, Pathmasiri W, Snyder RW, Caffaro MM, Watson SL, Patel PR, *et al*. Oral administration of TiO_2 nanoparticles during early life impacts cardiac and neurobehavioral performance and metabolite profile in an age- and sex-related manner. Particle and Fibre Toxicology. 2022; 19: 3.
[155] Notter T, Aengenheister L, Weber-Stadlbauer U, Naegeli H, Wick P, Meyer U, *et al*. Prenatal exposure to TiO_2 nanoparticles in mice causes behavioral deficits with relevance to autism spectrum disorder and beyond. Translational Psychiatry. 2018; 8: 193.
[156] Younes NRB, Amara S, Mrad I, Ben-Slama I, Jeljeli M, Omri K, *et al*. Subacute toxicity of titanium dioxide (TiO2) nanoparticles in male rats: emotional behavior and pathophysiological examination. Environmental Science and Pollution Research International. 2015; 22: 8728–8737.
[157] Cui Y, Che Y, Wang H. Nono-titanium dioxide exposure during the adolescent period induces neurotoxicities in rats: Ameliorative potential of bergamot essential oil. Brain and Behavior. 2021; 11: e02099.
[158] Naima R, Imen M, Mustapha J, Hafedh A, Kamel K, Mohsen S, *et al*. Acute titanium dioxide nanoparticles exposure impaired spatial cognitive performance through neurotoxic and oxidative mechanisms in Wistar rats. Biomarkers. 2021; 26: 760–769.
[159] Ze Y, Sheng L, Zhao X, Ze X, Wang X, Zhou Q, *et al*. Neurotoxic characteristics of spatial recognition damage of the hippocampus in mice following subchronic peroral exposure to TiO2 nanoparticles. Journal of Hazardous Materials. 2014; 264: 219–229.
[160] Horváth T, Vezér T, Kozma G, Papp A. Functional neurotoxicity and tissue metal levels in rats exposed subacutely to titanium dioxide nanoparticles via the airways. Ideggyogyaszati Szemle. 2018; 71: 35–42.
[161] Chen J, Lei L, Mo W, Dong H, Li J, Bai C, *et al*. Developmental titanium dioxide nanoparticle exposure induces oxidative stress and neurobehavioral changes in zebrafish. Aquatic Toxicology. 2021; 240: 105990.
[162] Grande F, Tucci P. Titanium Dioxide Nanoparticles: a Risk for Human Health? Mini Reviews in Medicinal Chemistry. 2016; 16: 762–769.
[163] Kwakye GF, McMinimy RA, Aschner M. Disease-Toxicant Interactions in Parkinson's Disease Neuropathology. Neurochemical Research. 2017; 42: 1772–1786.
[164] Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Molecular Neurodegeneration. 2020; 15: 40.
[165] Xiao L, Zan G, Qin J, Wei X, Lu G, Li X, *et al*. Combined exposure to multiple metals and cognitive function in older adults. Ecotoxicology and Environmental Safety. 2021; 222: 112465.
[166] Panisi C, Guerini FR, Abruzzo PM, Balzola F, Biava PM, Bolotta A, *et al*. Autism spectrum disorder from the womb to adulthood: Suggestions for a paradigm shift. Journal of Personalized Medicine. 2021; 11: 70.
[167] Bjørklund G, Skalny AV, Rahman MM, Dadar M, Yassa HA, Aaseth J, *et al*. Toxic metal(loid)-based pollutants and their possible role in autism spectrum disorder. Environmental Research. 2018; 166: 234–250.
[168] Purper-Ouakil D, Ramoz N, Lepagnol-Bestel AM, Gorwood P, Simonneau M. Neurobiology of attention deficit/hyperactivity disorder. Pediatric Research. 2011; 69: 69R–76R.
[169] Carter CJ, Blizard RA. Autism genes are selectively targeted by environmental pollutants including pesticides, heavy metals, bisphenol A, phthalates and many others in food, cosmetics or household products. Neurochemistry International. 2016; 101: 83–109.
[170] Jiang Y, Wei Y, Guo W, Du J, Jiang T, Ma H, *et al*. Prenatal titanium exposure and child neurodevelopment at 1 year of age: A longitudinal prospective birth cohort study. Chemosphere. 2023;

311: 137034.

[171] Zhao G, Liu SJ, Gan XY, Li JR, Wu XX, Liu SY, *et al*. Analysis of Whole Blood and Urine Trace Elements in Children with Autism Spectrum Disorders and Autistic Behaviors. Biological Trace Element Research. 2023; 201: 627–635.

[172] Thygesen M, Schullehner J, Hansen B, Sigsgaard T, Voutchkova DD, Kristiansen SM, *et al*. Trace elements in drinking water and the incidence of attention-deficit hyperactivity disorder. Journal of Trace Elements in Medicine and Biology. 2021; 68: 126828.

[173] Tabatadze T, Kherkheulidze M, Kandelaki E, Kavlashvili N, Ivanashvili T. Attention deficit hyperactivity disorder and hair heavy metal and essential trace element concentrations. is there a link? Georgian Medical News. 2018; 88–92.

[174] Tanaka M, Szabó Á, Vécsei L. Preclinical modeling in depression and anxiety: Current challenges and future research directions. Advances in Clinical and Experimental Medicine. 2023; 32: 505–509.

[175] Ménard C, Hodes GE, Russo SJ. Pathogenesis of depression: Insights from human and rodent studies. Neuroscience. 2016; 321: 138–162.

[176] Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The gut microbiota in anxiety and depression - A systematic review. Clinical Psychology Review. 2021; 83: 101943.

[177] Lv J, Li YL, Ren WQ, Li R, Chen JR, Bao C, *et al*. Increased depression risk for elderly women with high blood levels of strontium and barium. Environmental Chemistry Letters. 2021; 19: 1787–1796.

[178] Shi W, Li T, Zhang Y, Sun Q, Chen C, Wang J, *et al*. Depression and Anxiety Associated with Exposure to Fine Particulate Matter Constituents: A Cross-Sectional Study in North China. Environmental Science & Technology. 2020; 54: 16006–16016.

[179] Tajti J, Szok D, Csáti A, Szabó Á, Tanaka M, Vécsei L. Exploring Novel Therapeutic Targets in the Common Pathogenic Factors in Migraine and Neuropathic Pain. International Journal of Molecular Sciences. 2023; 24: 4114.

[180] Tarpada SP, Loloi J, Schwechter EM. A Case of Titanium Pseudotumor and Systemic Toxicity After Total Hip Arthroplasty Polyethylene Failure. Arthroplasty Today. 2020; 6: 710–715.