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

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Abstract
Titanium dioxide nanoparticles (TiO2NPs) are widely produced and used nanoparticles. Yet, TiO2NP exposure may possess toxic effects to different cells and tissues, including the brain. Recent studies significantly expanded the understanding of the molecular mechanisms underlying TiO2NP neurotoxicity implicating a number of both direct and indirect mechanisms. In view of the significant recent progress in research on TiO2NP 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 demonstrate that although TiO2NP 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, TiO2NP exposure also results in reduced neurite outgrowth and impaired neurotransmitter metabolism, especially dopamine and glutamate. TiO2NP 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 TiO2NP neurotoxicity. Correspondingly, in vivo studies demonstrated that TiO2NPs induce a wide spectrum of adverse neurobehavioral effects, while epidemiological data are lacking. In addition, TiO2NPs were shown to promote neurotoxic effects of other toxic compounds. Here we show the contribution of a wide spectrum of molecular mechanisms to TiO2NP-induced neurotoxicity; yet, the role of TiO2NP 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 (TiO2) is considered one of the most produced nanoparticles [5] with a global production of approximately 4 million tons per year [6]. TiO2 nanoparticles (TiO2NPs) are found in three crystalline forms, including anatase, rutile, and brookite [7]. Anatase and rutile are the main forms of TiO2, 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, TiO2NPs 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 TiO2NP intake [10]. Medical applications...
of TiO$_2$NPs 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$_2$NPs accumulation in the organism and subsequent systemic toxicity [12].

Increased evidence demonstrates that overexposure to TiO$_2$NPs 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$_2$NPs exposure was shown to induce cytotoxicity due to oxidative stress, mitochondrial dysfunction and genotoxicity in ependimal 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$_2$NPs exposure may result in genotoxicity [20], altered lipid metabolism [21], cardiovascular damage [22], liver hepatotoxicity [23], intestinal inflammation [24]. TiO$_2$NPs 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$_2$NP exposure is lacking [27].

In view of its potential genotoxicity, the use of TiO$_2$NPs as a food additive has been limited in certain countries since 2020 [25] until European Food Safety Authority concluded that TiO$_2$NPs 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$_2$NP toxicity [29,30]. Several studies have shown that TiO$_2$NPs can accumulate in the brain upon exposure [31], while other studies studied failed to ascertain detectable TiO$_2$NP levels in the brain [32]. A number of excellent reviews published in 2015–2016 demonstrated that TiO$_2$NPs 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$_2$NPs neurotoxicity. Therefore, in view of the significant recent progress in research on TiO$_2$NP neurotoxicity, the objective of the present study is to provide a narrative review on the molecular mechanisms involved in TiO$_2$NP neurotoxicity with special emphasis on studies published in the last decade.


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$_2$NPs 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$_2$NPs accumulation in brain is inconsistent (Table 1, Ref. [17,31–33,38]). Specifically, prenatal exposure to TiO$_2$NPs 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$_2$NPs 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$_2$NP 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$_2$NPs 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$_2$NPs 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$_2$NPs more actively than did A549 and HepG2 cells. It has been also demonstrated that neuroblastoma cells, SH-SY5Y, were subjected to TiO$_2$NP-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$_2$NPs) in brain.

<table>
<thead>
<tr>
<th>Model</th>
<th>Nanoparticle</th>
<th>Dose</th>
<th>Exposure</th>
<th>Tissue level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c mice</td>
<td>Rutile-type TiO$_2$NPs, size—35 nm</td>
<td>0.8 mg per mouse</td>
<td>prenatal exposure through injection into maternal tail vein</td>
<td>Brain +(qualitative) Liver +(qualitative)</td>
<td>[31]</td>
</tr>
<tr>
<td>F344/DuCrlCrlj rats</td>
<td>Degussa P25 TiO$_2$NPs consisted of both anatase and rutile forms (70/30), size—21 nm</td>
<td>1 mg/kg b.w.</td>
<td>injected intravenously into the tail vein daily</td>
<td>After 24 h:</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Liver –230,000 mg/organ Spleen –4000 mg/organ Kidney –56 mg/organ Lungs –380 mg/organ Heart –14 mg/organ Brain –not detected</td>
<td>[32]</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>On day 30:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>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</td>
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<td>On day 1:</td>
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<td></td>
<td>Liver –133.8 µg/g Spleen –78.7 µg/g Lung –8.8 µg/g Kidney –0.67 µg/g Brain –not detected</td>
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<td>On day 14:</td>
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<td></td>
<td></td>
<td>Liver –99.5 µg/g Spleen –48.8 µg/g Lung –2.8 µg/g Kidney –0.2 µg/g Brain –not detected</td>
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<td>On day 28:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver –111.3 µg/g Spleen –33.3 µg/g Lung –2.3 µg/g Kidney –0.2 µg/g Brain –not detected</td>
<td></td>
</tr>
<tr>
<td>Wistar rats</td>
<td>TiO$_2$NPs consisted of both anatase and rutile forms (70/30), size—20–30 nm</td>
<td>5 mg/kg b.w.</td>
<td>injected intravenously into the tail vein daily</td>
<td>[33]</td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>Nanoparticle</td>
<td>Dose</td>
<td>Exposure</td>
<td>Tissue level</td>
<td>Ref.</td>
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<tr>
<td>Fisher F344 rats</td>
<td>Aeroxide TiO$_2$NPs P25 NPs consisted of both anatase and rutile forms (75/25)</td>
<td>1 mg/kg b.w.</td>
<td>single-dose intravenous administration</td>
<td>After 24 hours: Brain $\sim$70 ng/g, Liver $\sim$9500 ng/g, Lungs $\sim$7500 ng/g, Spleen $\sim$5500 ng/g, Kidneys $\sim$250 ng/g</td>
<td>[17]</td>
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<td></td>
<td>After 365 days: Brain $\sim$40 ng/g, Liver $\sim$3500 ng/g, Lungs $\sim$500 ng/g, Spleen $\sim$2000 ng/g, Kidneys $\sim$40 ng/g</td>
<td></td>
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<tr>
<td>Cell cultures</td>
<td>Degussa Aeroxide TiO$_2$NPs P25 consisted of both anatase and rutile forms (80/20), size—25 nm</td>
<td>10–200 $\mu$g/mL</td>
<td>24 h exposure in vitro</td>
<td>After 3 h of exposure to 200 $\mu$g/mL: A549 $\sim$52%, HepG2 $\sim$13%, A172 $\sim$33%, SH-SY5Y $\sim$8% cells with NPs</td>
<td>[38]</td>
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<td></td>
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<td></td>
<td>After 24 h of exposure to 200 $\mu$g/mL: A549 $\sim$83%, HepG2 $\sim$50%, A172 $\sim$77%, SH-SY5Y $\sim$82% cells with NPs</td>
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</table>
tinct differences in susceptibility to the toxic effects of TiO$_2$NPs were also observed even between different brain cell types. Specifically, comparative analysis demonstrated that neuronal (SH-SY5Y) cells were more sensitive to TiO$_2$NP 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$_2$NPs sized 10–20 nm significantly induced brain damage, BBB disruption, and glial cell damage, along with up-regulation of IL-1$\beta$, TNF$\alpha$, and IL-10 production in brain tissue, whereas large TiO$_2$ particles sized 200 nm did not possess such effect [44]. Small-sized TiO$_2$NPs were shown to penetrate BBB more effectively than the larger ones [45]. Another study demonstrated that large TiO$_2$NPs (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$_2$NPs were more profound than those of micro TiO$_2$ [46]. In contrast, exposure of human neural stem cells (hNSC) to both TiO$_2$NP (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 $HMGAl$ 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$_2$NPs did not cause neurotoxicity, and only chronic exposure induced modest neuronal dysfunction [48].

The neurotoxic effects of TiO$_2$NPs are dependent not only on the dose and duration of exposure but also on crystalline structure [49]. Following intranasal instillation, TiO$_2$NPs 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$_2$NPs on its neurotoxicity [50].

Although multiple studies demonstrate significant neurotoxic effects of TiO$_2$NPs, it is noteworthy that several studies have demonstrated that low-dose TiO$_2$NPs 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$_2$NPs.

3. Blood-Brain Barrier

Existing data demonstrate that TiO$_2$NPs not only cross the BBB but also impair its permeability. Specifically, TiO$_2$NPs 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, paracellu-

lar gap formation, and associated ROCKII activation [53]. Inhibition of claudin-5 expression can also mediate the adverse effects of TiO$_2$NPs on BBB permeability [54]. Shelly et al. (2021) [55] demonstrated that in a model of BBB, TiO$_2$NP 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$_2$NP-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 TGF$\beta$. 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$_2$NPs, the latter did not cause any significant effect on integrity of lipopolysaccharide (LPS)-treated BBB [45].

These findings demonstrate that TiO$_2$NP 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$_2$NPs [57] as well as other potentially hazardous substances to the brain.

4. Brain Cells as Targets for TiO$_2$NPs

4.1 Neurons

The role of TiO$_2$NPs 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$_2$NP 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$_2$NPs exposure as a compensatory mechanism to overcome TiO$_2$NP-induced oxidative stress in mouse brains [62]. Nalika et al. (2023) [63] demonstrated that both melatonin and quercetin ameliorated neurobehavioral deficiency in TiO$_2$NP 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$_2$ NPs in human neuroblastoma (SH-SY5Y) cell line [65]. In hippocampal neuron HT22 cells TiO$_2$ NPs resulted in a significant increase in ROS generation and intracellular Ca$^{2+}$ levels ultimately leading to ERS characterized by up-regulation of GRP78, IRE-1/α, ATF6, CHOP, and caspase-12 mRNA and protein expression and subsequent apoptosis [66]. It has been demonstrated that TiO$_2$NP-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$_2$NP exposure also affects neurogenesis [68]. Valentini et al. (2018) [69] demonstrated TiO$_2$NP-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$_2$NP-exposed rats [71].

4.2 Glia

Glial cells were shown to uptake TiO$_2$NPs 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$_2$NPs (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$_2$NPs (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$_2$NP neurotoxicity [77]. Furthermore, TiO$_2$NPs 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$_2$NPs in astrocytes, exposure of human D384 astrocytes to TiO$_2$NPs 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 mediating neuronal toxicity following TiO$_2$NP exposure.

5. Targets and Mechanisms of TiO$_2$NP 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$_2$NP 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$_2$NPs [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), phospholylated 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$_2$NP-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 α1-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$_2$NP-exposed *C. elegans*, which were characterized by altered locomotor activity [87].

Other studies also revealed significant adverse effects of TiO$_2$NPs 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-3/β 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 Neuritin-1, and up-regulation of growth inhibitors semaphorin type 3A and Nogo-A may underlie TiO$_2$NPs-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$_2$NPs were also shown to induce cytoskeletal disruption in neuronal cells that may significantly contribute to neurotoxicity. Specifically, SH-SY5Y cells exposed to TiO$_2$NPs 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$_2$NPs [92]. Correspondingly, TiO$_2$ exposure may affect microtubule formation by inhibiting tubulin polymerization [93].

Impaired neurite outgrowth due to TiO$_2$NPs exposure was also associated with impaired synaptic function. Prenatal TiO$_2$NP exposure was shown to affect synaptic plasticity in hippocampal dentate gyrus of rats [94]. TiO$_2$NP 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$_2$NPs were shown to impair the metabolism of neurotransmitters, especially glutamate/glutamine, and dopamine (Table 2, Ref. [80,96–104]). Exposure of mice to TiO$_2$NPs 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$_2$NPs 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$_2$NP exposure in rat primary cultured hippocampal neurons [97]. In addition, various forms of TiO$_2$NPs 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$_2$NP 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$_2$NPs 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$_2$NPs 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$_2$NPs 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 TiO2NP exposure on neurotransmitter metabolism.

<table>
<thead>
<tr>
<th>Model</th>
<th>Nanoparticle Description</th>
<th>Dose</th>
<th>Exposure</th>
<th>Effect (Neurotransmitter Metabolism)</th>
<th>Ref</th>
</tr>
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<tbody>
<tr>
<td>CD-1 (ICR) female mice</td>
<td>TiO(_2)NPs powder</td>
<td>1.25–5 mg/kg b.w. for 9 months</td>
<td>Nasal instillation</td>
<td>↑ Glu, PAG activity ↓ Gln, GS activity, NR1, NR2A, NR2B, mGluR2 mRNA and protein expression</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>Hydrophobic rutile-type TiO(_2)NPs and hydrophylic nano-sized particles with silica surface coating</td>
<td>500 g TiO(_2) per mouse for 30 days</td>
<td>Nasal instillation</td>
<td>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)</td>
<td>[98]</td>
</tr>
<tr>
<td>CD-1 (ICR) female mice</td>
<td>Anatase TiO(_2) particles</td>
<td>5, 10, and 50 mg/kg b.w. for 60 days</td>
<td>Oral exposure</td>
<td>↑ 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</td>
<td>[99]</td>
</tr>
<tr>
<td>CD female mice</td>
<td>TiO(_2) particles 25 nm, 80 nm and 155 nm</td>
<td>50 mg/kg b.w. 30 days</td>
<td>Intranasal instilled</td>
<td>↑ Brain Ti level (on days 10 and 20) ↓ NE and 5-HT ↓ DA, DOPAC, HVA and 5-HIAA</td>
<td>[100]</td>
</tr>
<tr>
<td>Balb/c mice</td>
<td>Anatase type TiO(_2)NPs sized 10 nm</td>
<td>10–50 mg/kg for 45 days</td>
<td>Oral exposure</td>
<td>↓ TH+ neurons in substantia nigra, hanging test time ↑ Pole test time</td>
<td>[102]</td>
</tr>
<tr>
<td>ICR mice</td>
<td>Anatase TiO(_2)NPs sized 25–70 nm</td>
<td>0.1 mL of TiO(_2)NPs 1 mg/mL subcutaneously injected to pregnant ICR mice at GD 6, 9, 12, 15, and 18</td>
<td>Prenatal exposure</td>
<td>Prefrontal cortex: ↑ DA, DOPAC, HVA, 3-MT Neostriatum: ↑ DA, DOPAC, HVA</td>
<td>[103]</td>
</tr>
<tr>
<td>Model</td>
<td>Nanoparticle</td>
<td>Dose</td>
<td>Exposure</td>
<td>Effect</td>
<td>Ref</td>
</tr>
<tr>
<td>-------------------------------------------</td>
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</tr>
<tr>
<td>Swiss albino male mice</td>
<td>TiO$_2$ NPs sized &lt;75 nm</td>
<td>500 mg/kg b.w. for 21 days</td>
<td>Oral exposure</td>
<td>Brain ▲ NE, DA, ROS, GST ▼ SOD, Catalase, GPX ↔ 5-HT</td>
<td>[104]</td>
</tr>
<tr>
<td>Primary hippocampal neurons from newborn</td>
<td>Nano anatase TiO$_2$</td>
<td>5–30 mg/mL</td>
<td>In vitro</td>
<td>▼ Neurite length ▼ Gln content ▲ Glu content ▲ PAG activity ▼ GS activity</td>
<td>[97]</td>
</tr>
<tr>
<td>Sprague Dawley rats</td>
<td></td>
<td></td>
<td></td>
<td>▲ NR1, NR2A, NR2B protein expression ▲ Intracellular Ca$^{2+}$ ▲ NO level ▲ NOS activity</td>
<td></td>
</tr>
<tr>
<td>Primary astrocytes from newborn Sprague</td>
<td>P25 TiO$_2$ NPs (70% anatase &amp; 30% rutile)</td>
<td>25–100 ppm</td>
<td>In vitro</td>
<td>▼ Cell viability ▼ Glutamate uptake ▲ MitoROS ▲ MMP ▲ Mfn1, Mfn2 and Drp1 expression</td>
<td>[80]</td>
</tr>
<tr>
<td>Dawley rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary olfactory bulb neurons</td>
<td>TiO$_2$ NPs sized &lt;20 nm</td>
<td>Different concentrations (1, 5, and 10 mg/mL) for various exposure times (24, 48 and 72 hours)</td>
<td>In vitro</td>
<td>▼ Cell viability ▲ DNA fragmentation ▼ BrdU ▲ Apoptosis ▲ OMP and TH mRNA expression</td>
<td>[101]</td>
</tr>
</tbody>
</table>

▲—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$_2$NP 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$_2$O$_3$ [104].

These findings demonstrate that TiO$_2$NP exposure may impair not only dopamine production but also its metabolism and signaling, resulting in dysregulation of dopaminergic neurotransmission. In addition, adrenergic and serotonergic signaling also appears to be significantly affected by TiO$_2$NP exposure. Taken together with the findings demonstrating altered neurite outgrowth, disruption of dopaminergic, adrenergic, and serotonergic 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 nuclear IkB kinase, NF-κB-inducible kinase, p52, and p65, as well as down-regulation of IkB and IL-2 expression [108]. Neuroinflammatory response to oral TiO$_2$NPs 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$_2$NPs-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$_2$NPs 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$_2$NP 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$_2$NPs 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$_2$NP-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$_2$NP induced ROS overproduction and hypothetical 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$_2$NPs, 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$_2$NPs were also shown to have a significant effect on β-amyloid production. Ribeiro et al. (2022) [124] demonstrated that direct interaction of TiO$_2$NPs with neuronal membrane prion protein (PrP$^C$) results in activation of NADPH-oxidase and 3-phosphoinositide-dependent kinase 1 (PKD1) 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$_2$NP 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$_2$NPs promote β-amyloid fibrillation [126]. Direct interaction between TiO$_2$NPs 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 TiO₂NPs and its aminated derivative TiO₂-NH₂NPs promoted early protein oligomerization [128].

Therefore, in vivo studies demonstrate that TiO₂NP 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 TiO₂NPs in dopaminergic neurotoxicity.

5.5 Epigenetics

Epigenetic mechanisms were shown to mediate toxic effects of TiO₂NPs 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 TiO₂NP neurotoxicity has been insufficiently studied.

It has been proposed that epigenetic effects may play a significant role in TiO₂NP toxicity [33]. Song et al. (2017) [130] demonstrated that exposure of PC12 cells to TiO₂NPs induced global DNA hypomethylation. Correspondingly, a later study demonstrated that prenatal TiO₂NP 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 TiO₂NPs in modulation of gut microbiota characteristics and intestinal health [135], it is reasonable to posit that this interplay may be involved in TiO₂NP-induced neurotoxicity. Indeed, it has been demonstrated that prenatal TiO₂NP 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 Campylobacterota was observed following prenatal TiO₂ exposure on postnatal day 49, but not at earlier periods. Taken together with the lack of influence of TiO₂ 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 TiO₂NPs [136]. TiO₂NP 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 TiO₂-exposed mice [35]. In adult female mice exposed to TiO₂NPs during pregnancy, the gut microbiota was characterized by reduced relative abundance of Verrucomicrobiota and Desulfofobacterota phyla, as well as increased abundance of Bacilli class. TiO₂-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 TiO₂NP 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 TiO₂NP-induced alterations in BBB, leading to its increased permeability.

6. Neurotoxic Effects of TiO₂NP Coexposure with Other Toxic Substances

Multiple studies aimed at assessing the effects of co-exposure to TiO₂NPs and neurotoxic pollutants including pesticides, flame retardants, antibiotics, and other persistent organic pollutants (POPs), as well as neurotoxic metals.

TiO₂ was shown to promote neurotoxic effects of POPs. Specifically, TiO₂ potentiated accumulation of BPA in zebrafish larvae and promoted adverse effects of Bisphenol A (BPA) on α-tubulin, mbp, and syn2a gene expression involved in neurodevelopment [138]. Adverse neurodevelopmental effects of BPA and TiO₂NP 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, TiO₂NPs 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 TiO₂NP co-exposure, while Ti and TPhP co-exposure significantly reduced serotonin levels [141]. Co-exposure of tetrabromobisphenol A (TBBPA) and TiO₂NPs 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$_2$NPs and various pesticides. TiO$_2$NP was shown to aggravate neurotoxic effects of a fungicide, difenoconazole, by promoting its adverse effects on neurodevelopment (down-regulation of elavl3, ngn1, gap43, glap and mbh gene expression) and axonal outgrowth, as well as oxidative stress and apoptosis in zebrafish larvae [143]. In a similar model, TiO$_2$NPs also promoted cypermethrin-induced down-regulation of glap, α1-tubulin, mbh mRNA expression, and up-regulation of neuro D expression, as well as reduction of serotonin, dopamine, and GABA levels [144].

Maternal exposure to TiO$_2$NPs 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, Glap, Fubh, Mobp, Chga, and Kcne2 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$_2$NP coexposure with pentachlorophenol significantly aggravated reduction of T3 levels through down-regulation of Ig 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$_2$NPs 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$_2$NP 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$_2$NP exposure also promoted Pb accumulation in zebrafish and aggravated Pb-induced inhibition of neurodevelopment-associated genes (α-tubulin, mbh, glap, 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$_2$NPs 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$_2$NPs with other neurotoxic substances can significantly potentiate adverse effects on brain functioning.

7. Behavioral Effects

Laboratory in vivo studies demonstrate that TiO$_2$NPs exposure possesses significant adverse neurobehavioral effects in different models. Specifically, prenatal TiO$_2$NP 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$_2$NPs 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$_2$NPs 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$_2$NP 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$_2$NP injection is associated with hippocampal oxidative stress and neuroinflammation [157]. Impaired spatial cognition and emotional reactivity following acute TiO$_2$NP 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$_2$NP 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$_2$NPs in various brain regions. The existing data demonstrated that TiO$_2$NP 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$_2$NP exposure. In addition, TiO$_2$NP toxicity was also associated with altered dopamine metabolism gene expression in striatum and substantia nigra.

Generally, both prenatal and postnatal exposures to TiO$_2$NPs 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$_2$NP-induced neurotoxicity. Following prenatal, intravenous, or oral exposure, TiO$_2$NPs 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$_2$NP exposure has been observed even in the absence of particle accumulation in brain, indicating the role of systemic proinflammatory and prooxidant effects of TiO$_2$NPs and its toxicity to BBB and increased permeability of the latter. In addition to neuronal damage, TiO$_2$NPs disrupt neurogenesis, neurite outgrowth, and affect synaptic plasticity. Taken together with TiO$_2$NP-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$_2$NPs 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$_2$NP-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$_2$NP neu-
rotoxicity. Due to the wide spectrum of molecular mechanisms involved in neurotoxicity, TiO$_2$NPs 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$_2$NPs in brain may be dependent but only on the dose of exposure, but also on the size of the particles. It also appears that TiO$_2$NPs possess higher toxicity as compared to non-nanosized TiO$_2$.

Taken together, the existing laboratory data demonstrate that TiO$_2$NPs causes a wide spectrum of neurotoxic effects, supporting the hypothesis of potential contribution of TiO$_2$NPs 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$_2$NPs exposure, indicating the potential contribution of TiO$_2$NPs 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$_2$NPs in impaired neurodevelopment, neurotransmission, 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 pathogenic 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$_2$NPs 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$_2$NPs exposure, are involved in pathogenesis of migraine and neuropathic pain [179]. Although no direct evidence supporting the role of TiO$_2$NPs 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$_2$NP 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$_2$NP exposure in human populations. Thus it is expected that certain titanium species and forms other than TiO$_2$NPs 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$_2$NPs 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$_2$NPs 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$_2$NP is capable of crossing the blood-brain barrier with subsequent accumulation in various brain regions. Neurotoxic effects of TiO$_2$NPs 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$_2$NPs and modulation of gut microbiota were also shown to contribute to its neurotoxicity. In vivo studies demonstrated that these neurotoxic effects of TiO$_2$NPs induce adverse neurobehavioral effects
in laboratory rodents and other model organisms. In contrast, direct evidence of adverse neuropsychiatric effects of TiO$_2$NP 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$_2$NPs 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$_2$NP neurotoxicity, as well as its relevance to human neuropsychiatric disorders should be carried out in the future, to better characterize the neurotoxicity of TiO$_2$NPs.

### 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 cycle 42
- 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
- GSK, glycogen synthase kinase
- HMGAI, 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 kinases
- 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$_2$NPs, 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

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### Conflict of Interest

The authors declare no conflict of interest.

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