Review

Potential Links between Cytoskeletal Disturbances and Electroneurophysiological Dysfunctions Induced in the Central Nervous System by Inorganic Nanoparticles

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Key Words

Cytoskeleton • Electrophysiology • CNS • Inorganic nanomaterial.

Abstract

Inorganic nanomaterials have been widely applied in biomedicine. However, several studies have noted that inorganic nanoparticles can enter the brain and induce cytoskeletal remodeling, as well as electrophysiological alterations, which are related to neurodevelopmental disorders and neurodegenerative diseases. The toxic effects of inorganic nanomaterials on the cytoskeleton and electrophysiology are summarized in this review. The relationships between inorganic NPs-induced cytoskeletal and electrophysiological alterations in the central nervous system remain obscure. We propose several potential relationships, including those involving N-methyl-D-aspartate receptor function, ion channels, transient receptor potential channels, and the Rho pathway.

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Introduction

Advances in nanotechnology have accelerated the application of nanomaterials in the biomedical field. Inorganic nanomaterials are nanoscale materials that are mainly composed of inorganic substances. An increasing number of studies have begun to focus on researching the toxicity of inorganic nanomaterials [1-3], some of these nanomaterials including TiO₂ nanoparticles (NPs), Ag NPs, and carbon nanotubes (CNTs), have shown great potential for applications in biomedical imaging, cancer therapy and targeted drug delivery because they have distinctive intrinsic physicochemical properties and good biocompatibility [4, 5]. Due to their small size, NPs can penetrate the body through various pathways as well as entering

the brain by crossing the blood-brain barrier (BBB), subsequently causing neurotoxicity, inflammation, and neurodegeneration in the central nervous system (CNS) [4].

The organization and dynamic remodeling of neuron cytoskeletal conformations are essential for morphological and functional changes to occur in nerve cells [6]. Recently, some genetic studies have suggested that various tubulin isotypes and microtubule (MT)-related proteins are mutated in many neurodevelopmental and neurodegenerative diseases [7, 8]. Moreover, some studies have indicated that nanomaterials exert toxic effects on cytoskeletal systems leading to altered neuron morphology and shorter neurite extensions. Electrophysiological functions are very important physiological mechanisms, especially for the heart and the CNS. For the CNS, almost all neurophysiological functions are developed via electrophysiology. Neuron electrophysiology is the main means of signal transmission; when a neuron is exposed to a certain stimulus, the cell membrane potential changes, and these electrical impulses will open some voltage-gated channels. Meanwhile, some neurotransmitters, e.g., dopamine (DA) and glutamic acid (Glu), are released and interact with receptors in the synaptic membrane. These potential-shift-induced changes are the epitome of electroneurophysiology. While nanomaterials have been used in therapeutic approaches for diseases of the nervous system, some studies have also indicated that NPs can induce electroneurophysiological dysfunctions in vitro and in vivo.

However, many of the molecular mechanisms by which inorganic NPs induce cytoskeletal and electroneurophysiological dysfunctions in nerve cells remain unclear. Here, we systematically review the neurotoxic effects induced by inorganic NPs on the cytoskeleton and electroneurophysiology of nerve cells, as well as the possible molecular mechanisms of these effects. More importantly, connections between the cytoskeleton and electrophysiology have been previously reported, and cytoskeletal disruptions can induce electroneurophysiological dysfunctions [9]. We will discuss the connections between the cytoskeleton and electrophysiology, which may reveal the potential nanotoxicological connections between them.

The entry of inorganic NPs into the CNS via the BBB

The BBB is composed of vascular endothelial cells and astrocytes and separates circulating blood from the cerebrospinal fluid. The BBB controls the microenvironment of the brain and is highly selective regarding particle penetration. This line of defense is largely due to brain capillary endothelial cells being closely connected by tight junctions (TJs) and a layer of astrocytes binding them to the basal membrane. However, due to their ultra-small size and unique physicochemical properties, NPs can escape host defenses, e.g., opsonophagocytosis, and pass through biological barriers to target specific organs, such as the brain. Some studies have reported that inorganic NPs could pass through the BBB through several pathways (Fig. 1) and accumulate in different regions of the brain [10-12] through different mechanisms and processes. For example, insulin-targeted gold NPs had been reported to cross the BBB by receptor-mediated endocytosis [13], and reduced graphene oxide was found to impair BBB integrity by downregulating the TJ protein occludin and the adherens junction protein β -catenin in vivo [14]. Due to these means of penetrating the BBB, some advanced nanomaterials conjugated with ligands or PEGylated NPs, among others, have been considered meaningful materials for cancer drug delivery and bioimaging applications [4], which is a significant advantage over other conventional medicine, which were incapable of penetrating the BBB. Furthermore, studies have found that these NPs could be translocated into the brain via the olfactory nerve after inhalation or intranasal instillation [15, 16], as demonstrated by high NPs concentrations in the olfactory bulbs [17].

In summary, although some studies have indicated that NPs can translocate into the brain via BBB and olfactory-brain pathways, the detailed mechanisms of these penetration processes remain obscure. Moreover, the translocation process of NPs in nerve cells along the soma and axon should be further studied, as well as whether the NP surface chemistry changes during translocation into the CNS.

Fig. 1. Schematic showing 5 possible mechanisms by which NPs could pass through the BBB. NOTE: 1. Diffusion pathway: hydrophobicity can enable lipid-based NPs to pass through the BBB endothelium. 2. Adsorptive transcytosis: positively charged NPs might cross the BBB due to contact with the negatively charged luminal membrane. 3. Carrier-mediated transport: NPs can encounter carrier proteins (e.g., choline, amino acids, glucose, purine bases, nucleosides) and be translocated through the BBB. 4. Receptor-mediated transcytosis: NPs can interact with specific receptors (e.g., insulin, transferrin), be transported into polarized endothelial cells via endocytosis and transcytosis, and then be secreted into the brain via exocytosis. 5. TJs: TJs are a paracellular pathway through which NPs less than 10 nm in size might pass, particularly when these TJs are disrupted [131].

The effects of inorganic NPs on the cytoskeleton

Inorganic NPs can range in size from 1–100 nm and have a high surface area-to-volume ratio; thus, they have marked reactivity and particle-to-cell contact and can be internalized by nerve cells, subsequently affecting the cytoskeleton system [18, 19], which is important for sustaining fundamental cellular morphology. The cytoskeleton is composed of MTs, microfilaments (MFs), and intermediate filaments (IFs). MTs play an indispensable role in the intracellular transport of cargos, such as organelles (e.g., lysosomes, mitochondria) and synaptic components (e.g., membrane receptors, neurotransmitters). MFs are highly versatile and play a functional role in both cytokinesis and cell morphology alterations. The structure of IFs is sufficiently stable, and they have been reported to have no association with molecular motors [20]. Studies have largely focused on the interaction between inorganic NPs and intracellular proteins, especially cytoskeletal proteins [21]. However this is the first review focusing on the effects of NPs on the cytoskeleton and systematically summarizing the underlying mechanisms.

Cell morphological changes

The organization and dynamic polymerization/depolymerization of the cytoskeleton direct the migration and morphogenesis of neurons [22]. These processes are involved in the establishment and maintenance of cell morphology and differentiation, the modulation of neurite outgrowth and extension, and the initiation and elongation of axons and dendrites.

There is plenty of evidence demonstrating that exposure to inorganic nanomaterials can alter the morphology of various types of nerve cells (Table 1), mainly characterized by neurite length and reduced numbers [23–26], as well as cell shape, e.g., rounded [26–28]. Fluorescent images have indicated that Ag NPs caused significant cellular structure degeneration by altering F-actin and β -tubulin proteins [23]. Additionally, C6 and U373 glial tumor cells exposed to TiO_2 NPs showed significant morphology changes and decreased F-actin organization [26]. When C17.2 neural progenitor cells were treated with gold NPs, cell spreading was reduced. Moreover, the cells displayed a clear loss of actin and tubulin network consistency when treated with higher concentrations of gold NPs [29]. In addition, inorganic NPs could alter synaptic structures and the expression of related proteins, including synaptophysin (a presynaptic vesicle protein) and the postsynaptic receptor density protein (PSD)-95, which is associated with the structure of the synapse [30].

Normal cell shape and neurite outgrowth enable the transmission of information among nerve cells, which is significant for neuronal functions. However, cell morphology changes

Table 1. Inorganic NPs induced various cytoskeleton-related neurotoxic effects *in vitro*. Note: PSD-95: post-synaptic density protein 95, FACS: focal adhesion complexes, PMA: polymethacrylate

Cell lines	Nanomaterial type	Particle size	Concentration/dose and exposure duration	Cytoskeleton-related neurotoxic effects	References
Primary rat cortical cells	Ag NPs	20 nm	1, 5, 10, 50 µg/mL for 3 days	Inhibition of neuronal extension and neurite overlap, dissolution of synaptic proteins (synaptophysin and PSD-95)	[23]
B35 neuroblastoma cells	Ag NPs	-	1.0 µg/mL	Reduction in neurite length	[132]
Primary rat SVZ-NSCs	Ag NPs	-	0.05, 0.1, 0.5, 1.0, 2.0 µg/mL	Formation of F-actin inclusions, reduction in neurite extension and neurite arborization	[132]
Human SK-N-SH cells	Si NPs	15 nm	2.5, 5.0, 10.0 µg/mL for 24h	Decrease in dendrite-like processes of cells	[51]
Mouse neuro2a neuroblastoma cells	Si NPs	15 nm	2.5, 5.0 and 10.0 µg/mL for 24h	Decrease in dendrite-like processes of cells	[51]
Primary cultured hippocampal neurons	TiO ₂ NPs	5-6 nm	0, 5, 15, 30 µg/mL for 24h	Reduction in neurite length	[25]
PC12 cells	Fe ₃ O ₄ NPs	30 nm	25, 50, 100, 200 µg/mL for 24h	G2/M arrest, increase in p53phosphorylation levels	[32]
C17.2 cells	PMA-coated gold NPs	13.9 ± 1.2 nm	50, 100 nM for 24h	Loss of actin and tubulin network coherence, reduction of cell spreading, decrease in FACS, increase in the amount of free vinculin, G2/M arrest, increase in cell doubling times	[29]
C17.2 cells	Four Fe ₂ O ₃ NPs with various coatings	4.0-14.0	500, 100 µg Fe mL ⁻¹	Reduction in cell proliferation, increase in cell doubling times, reduction of cell spreading, decrease in FACS	[37]
HBMECs	Al NPs	8-12 nm	1 µM - 10 mM for 24h	Shrinking of cells, rearrangement of F-actin with increased deposition at cell-cell borders, induced gap formation, alterations in TJ protein expression	[38]
hNSCs	Si NPs	30, 70 nm	≥0.1 mg/mL for 7 days	Shrinking or swelling of cells, increased nestin and N-FH expression	[40]
hNSCs	Ti NPs	80 nm	≥0.1 mg/mL for 7 days	Shrinking or swelling of cells, increased nestin and N-FH expression	[40]
SH-SY5Y cells	TiO ₂ NPs	20.90 ± 3.57 nm	0.1, 1, 10, and 100 µg/mL for 24 h	Disorder, disruption, retraction, and decreased intensity of MTs, increased soluble tubule levels, observed tau-TiO ₂ NPs interaction	[21]
PC12 cells	Fe ₃ O ₄ NPs	-	10, 50, 100, and 200 µg/mL for 72h	Altered cell morphology	[28]
PC12 cells	SiO ₂ NPs	15 ± 4	200 µg/mL for 24 h	Rounded cell shape and decreased cellular ability to generate neurites following NGF induction, G2/M arrest	[24]
PC12 cells	Fe-high and Fe-low NPs MWCNTs	2-50 nm in diameter, 50 nm in length	10, 30 µg/mL for 48 h	Altered cell morphology, formation of dot/nodular-like structures in the cell border, decreased neurite outgrowth and numbers, less organized microfilaments	[133]
U251 cells	Ag NPs	6-20 nm	0, 25, 50, 100, 200 and 400 µg/mL for 48h	Clustered cells, inhibited spreading patterns, G2/M arrest	[134]

are observed after treatment with inorganic nanomaterials, probably due to the cytoskeletal conformation changes in the nerve cells, which merits further investigation.

Cellular physiological activity changes

Cell mitosis. Many studies have indicated that cell proliferation can be inhibited by several types of NPs [19, 26, 28, 31]. Cell mitosis is the primary method by which mammalian cells proliferate, and the cytoskeleton plays an important role in this process. For example, MTs are involved in chromosome separation during the cell cycle, and they are important components of mitotic spindles, which are used to segregate chromosomes during cell division. Plenty of evidence has shown that NPs can induce cell cycle arrest at different phases of the cell cycle. Some studies have indicated that gold NPs treatments could induce significantly increased cell-doubling times and more cell cycle arrest at the sub-G0/G1 phase [18] or S phase [29] than the control treatments, indicating that not all cells could complete the whole cell cycle after being exposed to gold NPs. Cell cycle analyses have suggested that Fe₃O₄ NPs [32] and SiO₂ NPs [24] both resulted in G2/M arrest in PC12 cells, as well as an increase in the rate of apoptosis. PC12 cells showed an increase in the level of p53 phosphorylation, which is related to the G2/M DNA damage checkpoint and the promotion of apoptosis [32]. Furthermore, Fe₃O₄ NPs could induce the perturbation of PC12 cells growth by disturbing MT dynamics [28].

Neuronal migration. The coordinated migration of nerve cells is one of the most important steps for the development of brain architecture and function. Various morphological changes displayed by migrating neurons are based on cytoskeletal organization [33]. Studies have indicated that NPs could inhibit cell migration via cytoskeletal disruption [34, 35]. Decreases in the areas of focal adhesion complexes (FACS) and FAK activation (pY397-FAK) were observed in C17.2 cells after exposure to gold NPs [29]. FACS form a direct link between the intracellular actin network and the extracellular matrix. Studies have indicated that FACS and FAK play vital roles in cytoskeletal stabilization and cellular migration processes [36]. Furthermore, high intracellular levels of iron oxide NPs were observed to disrupt cytoskeletal components, e.g., MTs and MFs, as well as affect the expression of FAKs and the level of activated FAK [37].

Tight junctions. TJs are important components that can tightly bind cells to block molecules and ions from passing through the intercellular space. NPs can interact with the endothelial cytoskeleton and can thereby affect the TJs between endothelial cells. In this way,

NPs can change the permeability of epithelial barriers, especially the BBB by interacting with the cytoskeleton of endothelial cells. The rearrangement of F-actin, the altered expression of TJ-related proteins [38] and the formation of gaps between endothelial cells have been observed during this pathological process [3]. Moreover, Apopa et al. [39] indicated that Fe_2O_3 NPs can induce human microvascular endothelial cells (HMVECs) permeability via the polymerization and redistribution of MTs, which might disturb tightly linked inter-endothelial junctions.

IFs, which are also important cytoskeletal components that help maintain normal neural morphology, have not yet been sufficiently studied. Glial fibrillary acidic protein and neurofilaments are the most common IFs in nerve cells. Si NPs and TiO_2 NPs have been reported to increase the expression of neurofilament heavy polypeptide (N-FH) in human neural stem cells (hNSCs) [40]. A commonality of several neurodegenerative diseases, including Parkinson disease, Alzheimer disease, and amyotrophic lateral sclerosis (ALS), is the aberrant accumulation of IFs in cell bodies or along the axon of neurons, relating to axonal transport impairment and neuron degeneration [41]. Inorganic NPs may be capable of interacting with IFs, causing structural modifications; however, there is not enough evidence to support this argument. Further studies are needed to elucidate alterations in IFs after treatment with nanomaterials and the underlying mechanisms of those effects.

Related mechanisms

Reactive oxygen species (ROS)

NPs can clearly increase the levels of ROS in the brain, according to in vivo [15, 42] and in vitro [25, 29, 43, 44] studies, and this could be one of the main toxic effects of inorganic NPs on cell physiology. ROS comprise several reactive molecules containing oxygen constituents, including superoxide anions ($\text{O}_2^{\cdot-}$), hydroxyl ions ($\text{OH}^{\cdot}$), peroxy anions (ONOO^-) and hydrogen peroxide (H_2O_2). The occurrence of many harmful oxidation reactions in neurons will result in neuronal damage, including cell membrane lipid peroxidation, DNA fragmentation, mitochondrial capacity reduction, and cellular transport-related protein inactivation.

Although normal intracellular ROS production is vital for some cytoskeleton-dependent processes, e.g., proper cell migration, excessive oxidation under oxidative stress conditions tends to interrupt cytoskeletal polymerization and impair normal cytoskeletal function (Fig. 2) [45, 46]. Furthermore, biochemical analyses have shown that actin, tubulin and IFs were glutathionylated, suggesting that neurons and cytoskeletal proteins are prone to oxidation under basic conditions [47]. Landino et al. [48] suggested that the overproduction of ONOO^- , nitric oxide (NO), nitroxyl donors, could increase the oxidation of the thiol groups of tubulin monomers, as well as promote disulfide bond formation between α - and β -tubulin, consequently decreasing MT polymerization ability. Nevertheless, the overproduction of ROS and NO could reduce molecular motor expression and MICAL (a binding partner for the cytosolic domain of Plexin A) activity, which could then decrease neuronal axon length and impair neuronal cytoskeleton-dependent vesicle trafficking [49, 50].

According to an in vitro study, Fe_2O_3 NPs could promote MT polymerization and redistribution in HMVECs via ROS production [39]; in addition, the PI3K/Akt/GSK-3 β signaling pathways were involved in the ROS-mediated MT remodeling. Meanwhile, Si NPs were found to decrease the phosphorylation GSK-3 β overall and at Ser9 [51], while Ag NPs could also reduce GSK-3 α/β phosphorylation at Ser9 but increase GSK-3 α/β phosphorylation at Tyr216/279 [52]. Begum [52] also suggested that NPs-induced excess intracellular ROS production could overactivate GSK-3 β by down regulating the PI3K pathway and lead to the hyperphosphorylation of tau at GSK-3 β sites. It is possible that NPs activate GSK-3 α/β and induce hyperphosphorylation at various hotspots of tau through oxidative stress-mediated mechanisms, leading to MT instability. Akt/GSK-3 β signaling pathways are important for MT stabilization; GSK-3 β regulates MT depolymerization via the phosphorylation of several

Fig. 2. The potential connections between cytoskeletal system and various ROS levels. NOTE: physiological ROS concentrations play a key role in organizing MT and actin structures. Inorganic nanomaterials could increase intracellular ROS production, leading to the severing of F-actin and the disturbance of MT stabilization. The Cys 374 and Met 42 residues of F-actin are reported to be targets for oxidative molecules. The oxidation of Cys 374 results in actin glutathionylation, and the oxidation of Met 44 leads to the severing of actin filaments via Mical1 activation. Moreover, low ROS levels could reduce actin content due to NOX inhibition. Although α - and β -tubulin also contain several Cys residues, which can be affected by oxidation, no studies have reported the modification of tubulin monomer Cys residues due to oxidation [49].

MT-associated proteins, while Akt inhibits GSK-3 β phosphorylation at Ser9, leading to the inhibition of MT-associated protein phosphorylation and resulting in MT stabilization [53]. However, another study reported conflicting results indicating that the cytoskeleton-related morphological changes were not due to the NPs-induced ROS production [21]. Thus, further studies are needed to determine the underlying mechanism of how inorganic NPs modulate the cytoskeletal network by altering the intracellular redox balance in the brain.

Physical interactions

NPs can be transported into the cytoplasm and even into nuclei and organelles, e.g., mitochondria. Inevitably, NPs encounter cytoplasmic proteins, such as actin and tubulin. Some studies have demonstrated direct interactions between the cytoskeletal system and nanomaterials, [28, 54-56], and these interactions are probably the main reason for the resulting cytoskeletal dysfunction. Gheshlaghi et al. [54] have indicated that TiO₂ NPs could induce significant tubulin conformational changes and decreases in tubulin polymerization. Moreover, direct tau-TiO₂ NPs interactions were observed in another in vitro study [21]. Tau, as an MT-associated protein, is important for MT stabilization. Increased tau phosphorylation levels at Ser262 and Ser396 were detected in human SK-N-SH and mouse N2a cells after a Si NPs treatment [51]. Another study reported that NPs-induced morphological changes could be due to two possible reasons, one of which was alterations to the 3D tubulin structure, the other of which was direct interactions between tubulin dimers or tau proteins and the NPs, which would inhibit MT polymerization [28]. Shams et al. [57] reported that actin could bind to single-walled carbon nanotubes (SWCNTs) surfaces but allow the nanotubes to slide and rotate on the actin surface. Several 3D actin-SWCNT complexes were observed, and the results indicated that these alterations could possibly contribute to actin reorganization.

Intracellular energetic balance dysfunctions

Mitochondrial respiration. Many studies have reported that treatment with inorganic NPs could decrease the mitochondrial membrane potential (MMP) by varying degrees, which would indicate the degree of mitochondrial membrane damage [44, 58]. A stable MMP is required for maintaining oxidative phosphorylation and generating adenosine triphosphate (ATP) in mitochondria, and a decreased MMP leads to cellular physiological dysfunctions. Mitochondria produce ATP by transferring electrons along the mitochondrial electron transport chain (ETC) [59], resulting in intracellular ROS production. Long [60] detected

increasing intracellular release of H_2O_2 resulting from interference with the mitochondrial ETC after a TiO_2 NPs treatment, suggesting a disruption in the ETC. The underlying mechanism of this effect may be that the TiO_2 NPs inhibited the expression of ETC enzymatic complexes (complexes I and III). Graphene was reported to disturb electron transfer in iron-sulfur or iron-porphyrin centers in mitochondria by affecting the function of iron-sulfur centers [61]. The mechanisms of inorganic NPs-induced ETC disruption is different from that of cyanides, which can inhibit the enzyme cytochrome c oxidase in mitochondria, and donate electrons to ETC complex IV.

Mitochondria are the major sources of intracellular energy (i.e., ATP), which is necessary for sustaining the structural integrity and proper function of the cytoskeleton because each actin molecule is bound to an ATP or ADP molecule. Multiple studies have suggested that inorganic NPs can affect intracellular ATP synthesis by disrupting mitochondrial function [62, 63]. The conformational changes of the cytoskeleton are dependent on ATP, as well as motor proteins, such as myosin, dynein and kinesin, which move along MFs and MTs by interacting with actin and tubulin; the process utilizes energy obtained from ATP hydrolysis. It has been reported that mitochondrial depolarization may explain why cellular ATP levels decline [64], which might affect normal cytoskeletal functions, leading to dysfunctions of cytoskeleton-regulated cellular processes.

The Rho GTPase pathway / Rac1 / Cdc42. The Rho GTPases are a family of signaling G proteins belonging to the Ras superfamily. They serve as molecular switches in the process of cell signal transduction, and extracellular signals are transmitted among neurons by the rapid conversion between the active state with GTP binding and inactive state with GDP binding. The Rho GTPase pathway might be one significant factor of NPs-induced neural cytoskeleton alterations because it is known to be involved in the reorganization of the actin cytoskeleton. Studies have shown that nanofibers could alter the extracellular environment, leading to the activation of Rho GTPase pathway, which might be the mechanism underlying morphological consequences in cerebral cortical astrocytes [65].

Although there is not enough evidence to verify that nanomaterials influence the conformation and function of actin through the Rho GTPase pathway, there have been many in vitro experiments on other cell lines confirming that NPs can activate Rho GTPases and remodel actin structures [66], which eventually affects cellular migration. Future studies should focus on how the Rho GTPase pathway is involved in inorganic NPs-induced neural cytoskeleton alterations.

GTP binding sites. Fluorescence data have shown that TiO_2 NPs induced conformational changes in GTP binding sites, leading to inhibited tubulin polymerization, according to turbidimetric assays. The conformational changes in tubulin induced by NPs alter the position of tryptophan residues in MTs, moving them to GTP binding sites [54]. The major factors that regulate the MT growth rate are the intracellular levels of free GTP- and GDP-bound tubulin dimers near the MT extremities [67]; thus, both GTP and its binding site in tubulin are important for tubulin polymerization because GTP should be hydrolyzed to GDP when tubulin initiates polymerization. Moreover, the dynein motor domain has various ATP binding sites that are important for hydrolyzation, which produces the energy required for movement [67].

The effects of inorganic NPs on electrophysiology

Electrophysiology is the key element that reflects neuronal activity. Here, we focus on how inorganic NPs induce changes in neuronal activity, which are manifested by membrane potential changes and altered synaptic transmission-related neurotransmitter release in the brain. Membrane potential changes are always related to the opening or closing of the ion channels located on the cell membrane, resulting in altered intracellular ion levels.

Ion channels as targets for the actions of inorganic NPs

The properties of ion channels can be used as indicators of the status and activity of cells, because they can adjust neuronal activity, and alterations in neuronal activity can then impact both physiological and pathophysiological conditions [68]. NPs can influence the intracellular ionic homeostasis and alter the physiological functions of neurons, which may potentially influence the CNS [69]. In this review, we mainly focus on four kinds of ion channels that are largely studied in inorganic nanomaterials and bio-application, their main effects have been summarized in Table 2. Voltage-gated calcium channels (VGCCs) and voltage-gated potassium channels (VGKCs) both play a crucial role in neuronal excitation and neurotransmitter release. Additionally, the transient outward potassium current I_A and the delayed rectifier potassium current I_K are the two main neuronal voltage-gated K_1 currents, and they are essential components of neuronal excitability. Voltage-gated sodium channels (VGSCs) are also known to play a key role in regulating neuronal excitability and activity in the CNS. Transient receptor potential (TRP) channels are a group of ion channels that function as cellular sensors, and they regulate the Ca^{2+} and Na^+ levels via certain physical and chemical stimuli. TRP channels are divided into six subfamilies, known as TRP ankyrin, TRP canonical (TRPC), TRP mucolipin, TRP melastatin (TRPM), TRP polycystin and TRP vanilloid (TRPV) ion channels [70].

Some studies have indicated that NPs could induce Ca^{2+} influx [43, 71, 72] and increase intracellular Ca^{2+} levels by affecting the activity of VGCCs and other related ion channels. An in vitro study found that TiO_2 NPs could significantly increase the cytoplasmic Ca^{2+} concentration by inhibiting the activity of Na^+/K^+ -ATPase and Ca^{2+} -ATPase in primary cultured hippocampal neurons [25]. Other NPs, e.g., CdSe quantum dots [73] and ZnO NPs [74], have also been reported to increase intracellular Ca^{2+} levels rapidly and persistently in cells via Ca^{2+} influx from the extracellular spaces and/or intracellular calcium stores, while gold NPs did not induce this intracellular calcium response [43]. Jakubek et al. [75] demonstrated that CNTs could inhibit neuronal VGCCs with cationic yttrium, which was derived from the nanotube growth catalyst. Thus, cationic yttrium could potentially inhibit calcium ion channel function.

I_K and I_A current-voltage curve were reported to be influenced after treatment with TiO_2 NPs [25], and ZnO NPs [69], CuO NPs [76], and tungsten carbide NPs [77]. Ag NPs have been found to affect the VGKCs by producing a hyperpolarizing shift in the inactivation-voltage curve of I_A and the activation-voltage curve of I_K as well as by postponing the recovery of I_A from the inactivation state, thereby altering mouse primary CA1 neuron excitability [78]. Shan et al. [77] indicated that tungsten carbide NPs could decrease the amplitudes of the I_A and I_K currents by reducing the numbers of opened VGKCs and delaying the recovery of I_A from the inactivation state. NPs that inhibit the two main types of K^+ currents in neurons might also alter the intracellular K^+ concentration due to K^+ efflux [79]. Moreover, changes in K^+ currents could also alter Ca^{2+} levels and affect synaptic plasticity induction [80]. One possible mechanism for NPs-induced effects on K^+ currents is the activation of phosphorylation cascade effects. The VGKCs subunits in hippocampal neurons possess various phosphorylation sites [81, 82]. The diameters of Na^+ and K^+ channel pores are approximately 10 Å or smaller, which could be directly blocked by ultrafine NPs (~1.4 nm in diameter), leading to restricted ion movement through the channel [83]. Moreover, Park et al. [84] found that SWCNTs can block K^+ channel subunits and that this effect depends on their shapes and dimensions. Inflammatory effects [85] and oxidative stress [86] have also been considered to contribute to NPs-induced K^+ channel activation. However, Xu et al. [79] found that oxidative stress did not contribute to the process by which multi-walled carbon nanotubes (MWCNTs) induced the suppression of I_{to} , I_K and I_{K1} current densities.

Although gold NPs were found to not significantly affect I_{Na} [87], other inorganic NPs, e.g., Ag NPs have been found to affect the VGSCs by decreasing the amplitude of the I_{Na} current, and thus altering mouse primary CA1 neuron excitability [88]. Furthermore, treatment with CdSe quantum dots could enhance I_{Na} activation and inactivation by extending the I_{Na} activation process and delaying I_{Na} recovery, leading to a reduced fraction of available VGSCs

Table 2. Inorganic NPs induced various electrophysiology-related neurotoxic effects in vitro. Note: I_K : delayed rectifier potassium current, I_A : transient outward potassium current, I_{K1} : inward rectifier potassium current, I_{to} : transient outward current, I_{Na} : sodium current

Cell lines	Nanomaterial type	Concentration/dose and exposure duration	Electrophysiology-related neurotoxic effects	References
Primary cultured hippocampal neurons	TiO ₂ NPs	Acute 30 µg/mL	Cytoplasmic Ca ²⁺ concentration increased, current-voltage curve of I_K increased, current-voltage curve of I_{Na} decreased.	[25]
Mixed-cultured mouse frontal cortex neurons	Ag NPs	10 or 20 µg/mL for 24h	Cytoplasmic Ca ²⁺ concentration increased.	[43]
Rat Primary Cultured Hippocampal Neurons	CdSe quantum dots	acute 10- and 20-nM	Cytoplasmic Ca ²⁺ concentration increased, the activation and inactivation of the I_{Na} enhanced.	[73]
Rat hippocampal CA3 pyramidal neurons	ZnO NPs	10 ⁻⁴ g/ml	Current-voltage curve of I_K increased, the peak amplitudes of I_{Na} increased, the current-voltage curve of I_{Na} decreased, and peak amplitude and overshoot of the evoked single action potential increased and half-width diminished.	[69]
Rat hippocampus CA1 pyramidal neurons	CuO NPs	5×10 ⁻⁵ g/mL	I_K inhibited, the voltage-dependence of the activation of I_A and I_K were not affected.	[76]
Mouse primary CA1 neuron	Ag NPs	10 ⁻⁵ g/mL for 5min	I_A and I_K current amplitudes inhibited.	[78]
Mouse primary CA1 neuron	Ag NPs	10 ⁻⁵ g/mL for 5min	The amplitude of I_{Na} current decreased, but had no effects on the inactivation-voltage curve, the recovery of I_{Na} from inactivity delayed.	[88]
Rat hippocampal CA1 neurons	tungsten carbide NPs	10 ⁻⁶ , 10 ⁻⁷ , 10 ⁻⁸ g/mL	Current-voltage curve of I_K and I_A decreased, the evoked action potential duration prolonged and the firing rate lowered, the recovery of I_A from inactivation delayed.	[77]
PC12 cells	MWCNTs	5 µg/mL for 6, 12, 24h	The current densities of I_{to} , I_K and I_{K1} were suppressed in a time-dependent and irreversible manner.	[79]

[73]. One suggested mechanism related to the effect of inorganic NPs on I_{Na} is conformational changes of the channel, which would alter the gating of the channel. However, this compound has an equal probability of affecting ion channel function by combining with biological membranes via free radicals [88].

In an in vivo study, the level of TRPM2 gene expression was increased in mouse brain tissue after being treated with hydrated C₆₀ fullerenes, while no changes were observed in TRPM7 gene expression [89]. A probable reason could be that TRPM2 was required for the increased intracellular Ca²⁺ levels as a cellular redox potential sensor in oxidative stress which was induced by Si NPs. Moreover, Gilardino et al. [90] demonstrated that the TRPV4 channel was related to the SiO₂ NPs-induced calcium signaling alterations observed in two neuronal cell lines (GT1-7 and GN11).

These studies provide sufficient evidence to support that nanomaterials could alter the physiological functions of ion channels, leading to changes in intracellular ion concentrations and thus affecting the normal electrophysiology of neurons.

The effects of inorganic NPs on the release of neurotransmitters

Neurotransmitters including Glu, DA, γ -GABA and 5-HT, are chemical components released by neurons, that function as 'messengers' to transfer signals via synaptic transmissions and could be related to inorganic NPs-induced neuronal dysfunction and degeneration.

Studies have shown that inorganic NPs can affect the release of neurotransmitters in vivo and in vitro (Table 3). Treatment with TiO₂ NPs increased the Glu content and altered the Glu metabolism in primary hippocampal neurons [25]. Ag NPs have been shown to have no effect on presynaptic glutamate release, but they could change the postsynaptic receptor sensitivity to glutamate by reducing the amplitude of miniature excitatory post synaptic currents, leading to increased levels of glutamate in the brain [91]. However, an in vivo experiment suggested that the DA concentration in the brain was decreased after treatment with Ag NPs for 14 days but was followed by a compensatory increase by day 28 [92]; similar data were obtained in vitro using Ag NPs [93]. Moreover, increased concentrations of DA were observed in the prefrontal cortex and neostriatum of 6-week-old offspring after maternal exposure to TiO₂ NPs [94]. In contrast, an acute intravenous exposure to ZnO NPs was reported to have no effect on DA levels in the rat brain [95]. In addition, treatment with Ag NPs significantly activated the release of 5-HT [92].

Glutamate exerts its function by being released from the presynaptic terminals of neurons and binding to glutamate receptors such as the N-methyl-D-aspartate receptor (NMDAR). NPs could decrease glutamine synthetase (GS) activity, increase phosphate-activated glutaminase (PAG) activity, and reduce the production of ATP by causing

Table 3. Inorganic NPs altered neurotransmitter released *in vivo* and *in vitro*. Note: dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine hydrochloride (3-MT)

Animal models/Cell lines	Nanomaterial type	Particle size	Concentration/dose and exposure duration	Neurotransmitter release alterations	References
Female rats	Ag NPs	14 nm	2.25, 4.5 and 9 mg/kg b.w./day for 28 days	DA content decreased after 14 days, 5-HT content increased after 28 days	[92]
6-week male ICR mice	TiO ₂ NPs	25-70 nm	0.1mg/pregnant ICR mice at gestation days 6, 9, 12, 15 and 18	DA and its metabolites (DOPAC, HVA, 3-MT) increased in the prefrontal cortex, DA and its metabolites (DOPAC and HVA) increased in neostriatum of 6-week male offspring	[94]
Male Wistar rats	ZnO NPs	30-40 nm	25 mg/kg, intraperitoneal injection, 14 days	norepinephrine, epinephrine, DA and serotonin content no affected	[95]
PC12 cells	Ag NPs	15 nm	0, 5, 10, 50 µg/mL for 24h	DA and its metabolites (DOPAC and HVA) decreased	[93]
PC12 cells	Mn NPs	40 nm	0, 5, 10, 50 µg/mL for 24h	DA content decreased, and its metabolites, DOPAC and HVA decreased	[93]
Primary rat hippocampal neurons	TiO ₂ NPs	200-420 nm	0, 5, 15, 30 µg/mL	Glu content increased, Gln content decreased, PAG activity increased and GS activity decreased	[25]
Primary CA1 pyramidal neurons	Ag NPs	Mean 234.9 nm	10, 100 µg/mL	Glu released no affected, but glutamate content in brain increased	[91]

mitochondrial dysfunction [60] NPs such as Ag NPs have been shown to affect excitatory glutamatergic synaptic transmission and receptor function [43]. An *in vitro* study indicated that inorganic NPs could reduce the expression of NR1 and NR2 [25], weakening the ability of the NMDAR to interact with glutamate and glycine. Moreover, it has been suggested that *in vivo*, the upregulation of genes related to DA could be the mechanism causing the effects of inorganic NPs [96]. Furthermore, the administration of cerium oxide [97], Ag, Cu, and Mn NPs [98] could lead to altered mRNA expression levels of DA-related genes, e.g., TH, MAOA, and COMT.

Neurotransmitters play a crucial role in the synaptic signaling in the mammalian CNS and are considered to play a crucial role in neuronal synaptic plasticity, long-term memory, and brain development. However, abnormal neurotransmitter concentrations can lead to neuronal dysfunction and neurodegenerative diseases, such as ischemia, ALS and Parkinson's disease [99, 100]. The present study mostly focused on how inorganic nanomaterials can alter the concentrations of the above neurotransmitters in the brain by regulating their synthesis and release processes [24]. Therefore, the detailed mechanisms of how inorganic NPs-induced neuronal dysfunction, neurodegenerative diseases and affect the neurotransmitters require further investigation.

Electrophysiological effect-mediated signal transduction is the most important property of nerve cells, and this property can be used to directly assess neuron function. As expected, both ion channel properties and neurotransmitter release are significantly affected by NPs treatments, which necessitates further investigation into the electrophysiological effects of inorganic nanomaterials.

The potential relationship between the cytoskeleton and electrophysiology

As discussed above, NPs can interrupt the normal conformation and function of the cytoskeleton, as well as alter electrophysiological states, leading to an imbalance in the homeostasis of neurons. It is interesting that alterations in neuronal electrophysiology are related to cytoskeletal changes. Studying the interaction between the cytoskeleton and neuronal electrophysiology may provide a new approach for investigating the neurotoxicity induced by inorganic nanomaterials. We have postulated several potential relationships between the cytoskeleton and neuronal electrophysiology, as described below.

The NMDAR pathway

The NMDAR is very important for controlling synaptic plasticity and memory function [101]. The expression of genes such as activin β -A is upregulated during early-stage, long-term potentiation, and activin proteins can regulate actin dynamics in dendritic spines [102]. Inorganic NPs could alter cytoskeletal conformation in both the cell body and the dendritic spines, resulting in neuron branch shortening, thus causing a decrease in neuronal synaptic plasticity and impairments in both learning and memory *in vivo* [103]. Meanwhile, NP

treatments could open NMDARs by increasing the extracellular Glu concentration, leading to more Ca^{2+} influx [104, 105], which plays an important role in cytoskeletal remodeling and enables the lengthening of spines, resulting in an increased chance for synaptic contact with the axonal terminals of presynaptic cells [102]. Calcium induces F-actin depolymerization and leads decreased NMDAR function [106]. Conversely, increased F-actin stability, which exists in mice lacking Eps8 (an actin dynamics-related regulator), enhances NMDAR-mediated currents [107]. Calcium ion, the NMDAR and Glu all play crucial roles in synaptic plasticity and signal transmission.

Ion channels

The above text has summarized that the state of ion channels in neurons can be affected by being exposed to nanomaterials. Several studies have suggested that there is a connection between ion channels and the cytoskeleton that is related to physiological cellular activities in the cardiovascular system; however, few studies have directly demonstrated the close connection between the cytoskeleton and ion channels in neurons. Lascola et al. [108] suggested that actin modulates the open-state probability of Cl^- channels in neocortical astrocytes, thus controlling the degree of outward rectification. α -Actinin, the actin-binding protein, has been observed to bind to the central pore-forming $\text{Ca}_{v1.2}$ subunit and foster its surface expression, but when α -actinin is displaced by Ca^{2+} -calmodulin, it induces Ca^{2+} -mediated $\text{Ca}_{v1.2}$ endocytosis [109]. However, plenty of studies have demonstrated that in cardiomyocytes, the cytoskeleton modulates ion channels, e.g., L-type calcium channels [110]. In addition, K_{ATP} channel trafficking was activated by F-actin destabilization but inhibited by F-actin stabilization [111]. Many studies have reported how the cytoskeleton modulates the gating of voltage-gated channels, including $\text{K}_{v1.5}$ [112], $\text{Na}_{v1.5}$ [113], and L-type Ca^{2+} channels [114]. Research has shown that the interactions between these ion channels and actin filaments involve intermediary actin-binding molecules. Moreover, alterations in MT conformation and the interaction of MTs with dynein-dynactin complexes are reportedly involved with ion channel trafficking [9].

TRP channels

There is a good amount of evidence that TRP channels, e.g., TRPM2, TRPM7, and TRPV4, are associated with NPs-induced cytotoxicity by mediating ROS production and regulating calcium homeostasis [89, 115]. More interestingly, studies have provided evidence that many types of TRP channels, such as TRPV1 [116], TRPV4 [117], TRPC1 [118], TRPC5, and TRPC6 [119], can interact with the cytoskeleton. Different states of assembled MTs, such as tyrosinated tubulin, detyrosinated tubulin, acetylated tubulin, and polyglutamylated tubulin, can interact with TRPV1 [120]. Moreover, TRPV1 can also combine with polymerized MTs [116]. Long-term, low-level TRPV1 activation can lead to the shortening of neurites in primary neurons [121] and TRPC5 channels have also been suggested to play a well-established role in neuronal growth cone motility [122]. In addition, TRPV1 shows a significant stabilization effect on MTs, which has been demonstrated in MT-depolymerizing environments, such as those with nocodazole or high Ca^{2+} levels [116]. Furthermore, TRPV1 activation may increase the level of polymerized actin [123]. Nevertheless, another study has shown that the activation of TRPV1 can result in the rapid disassembly of MTs [124].

Smani et al. [125] systematically reviewed the bidirectional association of TRP channels with the cytoskeleton, including actin and MTs, and demonstrated the functional importance of this interaction for TRP channel function. The most likely factor connecting the cytoskeleton and TRP channels is the intracellular Ca^{2+} levels that are mediated by TRP channels.

The Rho pathway

As discussed above, Rho GTPases are important proteins in the cytoskeletal system. One major Rho GTPase related to spinal morphogenesis is RhoA, a protein that mediates the regulation and timing of cell division. Under physiological conditions in neurons, once Ca^{2+} enters cells via NMDARs, it can combine with calmodulin and activate CaMKII, leading

Fig. 3. Inorganic nanomaterial-induced toxic effects on the cytoskeletal system and electrophysiology. NOTE: Inorganic nanomaterials can induce cytoskeletal rearrangement, leading to alterations in some neural cytoskeleton-related processes, such as axon extension and neuron migration. Meanwhile, ion channels and neurotransmitter release are also affected by treatment with inorganic nanomaterials, which may lead to neuro-electrophysiological disturbances. Here, we have described these toxic effects in detail and have summarized the potential relations between the cytoskeleton and electrophysiology.

to RhoA activation [126]. The RhoA protein will activate ROCK, a RhoA kinase, which results in the stimulation of LIM kinase; in turn, LIM kinase inhibits the protein cofilin. Cofilin can reorganize the actin component of the cytoskeleton, i.e., it depolymerizes actin segments and thus suppresses the development of growth cones and the repair of axons [127]. Moreover, the increased intracellular Ca^{2+} suppresses the expression of two important proteins, nephrin, a component of the slit diaphragm, and synaptopodin, part of the cytoskeleton; increased intracellular Ca^{2+} also activates RhoA protein, which in turn induces F-actin rearrangement and foot process reduction [128]. Another small GTPase in the Rho family is Cdc42, which is involved in the regulation of cellular functions, including cell morphology, migration, and cell cycle progression [129]. Activated Cdc42 can phosphorylate p21-activated kinases (and then initiate actin reorganization [130]).

As discussed above, inorganic nanomaterial-induced neurotoxicity is involved in several potential relationships between the cytoskeleton and neuronal electrophysiology. NPs could change the activity of NMDARs, TRP channels or directly affect the properties of ion channels and Rho GTPase activation states, leading to disturbances in intracellular calcium homeostasis and alterations in the expression of cytoskeleton-related genes (e.g., actinin β -A) and proteins (e.g., cofilin). The alterations in Ca^{2+} levels are crucial for NP-induced cytoskeleton conformation changes and neuronal dysfunctions. Furthermore, NPs can remodel the cytoskeletal system, which regulates aspects of neuronal electrophysiology, such as ion channel properties and neuronal synaptic plasticity. However, few studies directly note the interactions between the cytoskeleton and neuronal electrophysiology. We are looking forward to obtaining more mechanistic information regarding the neuronal cytoskeletal system, as well as neuronal electrophysiology and related parameters, including NMDARs, ion channels, Rho GTPase activation and TRP channels, which could provide more novel nanotoxicological profiles for the CNS. These information will be beneficial for guiding inorganic NPs bioapplication guidance.

Conclusion

Inorganic nanomaterials have been widely applied in various biomedical applications, and many studies have indicated that inorganic nanomaterials can penetrate the BBB, causing neurotoxic effects. Moreover, the cytoskeleton, which is important for neuron morphology, the outgrowth and extension of neurites, and the initiation and elongation of axons and dendrites, can become degraded and reorganized after NPs exposure, leading to changes in cell morphology and physiological activity. The existing literature indicates that possible mechanisms for NPs-induced cytoskeletal dysfunction include ROS induction, direct physical interaction, and intracellular energetic imbalances. Electrophysiological alterations have also been observed both in vivo and in vitro after NPs exposure, especially in ion levels and neurotransmitter release. Most importantly, we summarized possible connections between the cytoskeleton and electrophysiology (Fig. 3), which are both vital for neurophysiological activities. In doing so, we hope to supplement current nanotoxicological data with novel potential mechanisms. This study was limited to cytoskeletal nanotoxicology, electrophysiology, and restrictions on the inorganic nanomaterial bioapplications; more information is required to ensure their safe use in the future.

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Disclosure Statement

The authors declare no competing financial interest.

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