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Review

Does Nanosilver Have a Pronounced Toxic Effect on Humans?

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Abstract: Due to the development of high-tech industries, the modern world is characterized by the increased production and consumption of nanoparticles (NPs) and nanomaterials. Among produced metal nanoparticles, silver nanoparticles are widely used in everyday life products, cosmetics, and medicine. It has already been established that, in nanoscale form, many even inert materials become toxic. Therefore, the study of the toxicity of various substances in nanoscale form is an urgent scientific task. There is now a body of experience on the toxic effect of AgNPs. In the present review, the most well-known results obtained over the 2009–2021 period, including the own performance on the toxicity of silver NPs, are collected and analyzed. Along with the data reporting a certain level of toxicity of silver NPs, experiments that did not reveal any obvious toxicity of nanosized forms of silver are discussed. According to the performed studies, the toxicity of silver NPs is often caused not by NPs themselves but by silver ions, compounds used for nanoparticle stabilization, and other reasons. Based on the analysis of the collected data, it can be concluded that at actual levels of silver NPs used in everyday life, workplace, and medicine, they will not have strong toxic effects on a healthy adult body.

Keywords: nanoparticles; silver; toxicity; occupational; brain; behavior; development

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1. Introduction

At present, nanoparticles are found in numerous spheres of life. Their application in medicine, food and cosmetic industries, and everyday life is expanding [1]. Due to their unique electrical, optical, chemical, and antimicrobial properties, silver nanoparticles (AgNPs) are the most common type of NPs used in consumer products [2]. At the same time, according to publications in toxicology, a wealth of information has already been accumulated confirming that contact with NPs entails serious negative consequences for cells, tissues, and organs, such as the development of oxidative stress, the accumulation of DNA disorders, the induction of apoptosis and inflammation, and the disturbance of the structure and functions of tissues and organs [3–5].

Almost every person in the modern world is constantly in contact with NPs, through products or objects containing nanoparticles, or through air, water, and soil. The environment begins to suffer greatly from pollution by various nano-sized substances as a result of emissions from enterprises and vehicle exhaust, sewage discharges, and waste. Due to the widespread use of NPs, including AgNPs, in various industries, employees in the workplace face an increased risk of health problems owing to prolonged (chronic) contact with small doses of NPs that enter the body from the air in contaminated work areas, as well as with water and/or food. Occupational pathology has already accumulated bodies of evidence on the development of diseases caused by NPs in industrial workers, which

cannot be explained by other reasons [6,7]. In women of reproductive age employed in industrial fields, contact with NPs is associated with risks for their unborn children due to the ability of NPs to penetrate the placental barrier, which leads to the transfer of NPs from mother to child [8,9]. Therefore, the study of the toxicity of AgNPs, including the modeling of chronic contact close to industrial conditions, is a critical area of research.

2. Results of Investigations Performed by Other Researchers

2.1. General Toxicity of AgNPs for Cells, Tissues, and Organs

According to numerous publications, the main mechanism of the cellular and molecular toxicity of AgNPs is oxidative stress [10,11]. Thus, 20 nm spherical AgNPs reduced mRNA levels of sodium dismutase 1 and glutathione reductase in male Wistar rats [9]. In the presence of AgNPs, the formation of reactive oxygen species [10], the level of gene expression and the content of antioxidant proteins decrease, and DNA damage [11] increases. The prolonged exposure of adult rats to citrate-stabilized silver NPs at a low dose of 0.2 mg/kg resulted in a decrease in the level of expression of structural proteins, in particular, myelin in myelin sheath cells. AgNPs were also found to increase the body weight and body temperature of animals [12]. The level of developmental regulators (for example, neuronal) also decreases; however, the levels of expression of apoptosis regulators increase; therefore, ultimately, the cell often undergoes apoptosis. At the tissue level, inflammation, swelling, and, as an extreme outcome, necrosis, can develop [13]. In zebrafish, AgNPs caused a decrease in brain and muscle acetylcholinesterase activity, as well as in liver and gill catalase activity. Contact with AgNPs also led to morphological changes such as the fusion of secondary lamellae, curvature, dilated marginal channel, and epithelial lifting [14]. The administration of male CD-1 mice with AgNPs of 10 nm size, compared with NPs with a size of 40 and 100 nm, resulted in overt hepatobiliary toxicity [15].

Some reports indicate that AgNPs are genotoxic and mutagenic, and the degree of their impact directly depends on the dose of NPs and inversely depends on their size [16–18]. After exposure of BEAS-2B cells to AgNPs of different primary particle sizes (10, 40, and 75 nm), a cytotoxic effect was observed only at a particle size of 10 nm [19]. The same finding was reported in [20]. Coating agents used to stabilize NPs in a solution can also affect general toxicity, including genotoxicity. For example, AgNPs coated with citrate had more noticeable toxic effects on L5718Y cells than those coated with polyvinylpyrrolidone [21]. The study by Recordati et al. [15] showed that the coating of NPs had no relevant impact on animals. Citrate-coated silver nanopowder was toxic to human skin HaCaT keratinocyte cells, whereas polyvinylpyrrolidone-coated silver nanoprism or nanoparticle powder did not exhibit any toxic effect [22]. In a study by Gliga et al. [19], the coating-dependent difference in the cytotoxicity of AgNPs was not detected. El Badawy and al. [23] demonstrated a direct correlation between the toxicity of AgNPs and their surface charge. Thus, negatively charged citrate-coated AgNPs were less toxic to microorganisms than positively charged branched polyethyleneimine-coated AgNPs. The low toxicity of citrate-coated AgNPs was observed in comparison with polyvinylpyrrolidone- and gum arabic-coated AgNPs [24].

It should be noted that the results of evaluating the mutagenicity of AgNPs may also depend on the chosen object of study; for example, Prokhorova et al. [25] did not find chromosomal abnormalities in plant cells after exposure to AgNPs. Studying the effect of AgNPs on freshwater invertebrates with different life strategies—namely, *Hydra vulgaris*, *Daphnia carinata*, and *Paratya australiensis*—the authors described *Daphnia carinata* as the most sensitive species, followed by *Paratya australiensis* and *Hydra vulgaris* [26]. Toxic effects of AgNPs on different organisms in the study by Ivask et al. [20] varied by about two orders of magnitude, with the lowest observed for crustaceans and algae and the highest for mammalian cells.

The results of studies of the effects of NPs on the reproductive function and development of animal organisms are also ambiguous and often contradictory. The bulk of studies demonstrated that AgNPs could cause defects in the neurological, cardiovascular, reproductive, and immune systems of the embryos and fetus; however, in some studies, no

adverse effect on the development of AgNPs was found, even at high doses [27]. The development of oxidative stress, the occurrence of morphological defects, slowing heart rate, the appearance of pericardial edema, delayed hatching from eggs, and increased mortality were observed in studies on zebrafish embryos exposed to AgNPs [28–30]. Morphological changes in the ovary and testis were observed in rat offspring exposed to AgNPs [31]. Developmental exposure to AgNPs results in long-term gut dysbiosis, body fat increase, and neurobehavioral alterations in mouse offspring [32]. At the same time, it should be mentioned that silver ions (Ag^+) provoke similar toxic effects at concentrations hundreds of times lower than those applied for AgNPs [29,30]. It was suggested by some authors that the compounds used for NP coating significantly affected the level of AgNP toxicity. Thus, after contact with polyvinylpyrrolidone-coated AgNPs, zebrafish larva did not differ in morphology and behavior from control ones; however, when citrate was used as a coating agent, some disturbances were detected [33]. In a study by González et al. [34], in which the level of AgNPs in solution was close to their environmental concentrations in water (0.03–3 ppm), no negative changes in the survival, hatching, or morphology of *Danio rerio* were found. According to a number of studies, after a single oral administration, reproductive dysfunctions in males and females (inhibition of spermatogenesis, histopathological disorders in ovaries, etc.) were observed in rodents exposed to AgNPs. It is noteworthy that the smaller the dose and size of AgNPs were, the less pronounced were the effects [35]. Undoubtedly, the dose–response principle also works in nanotoxicology.

2.2. Effect of AgNPs on the Brain and Behavior

AgNPs along with other metal NPs are able to cross not just the placental barrier but the blood–brain barrier as well [8,9]. Tang et al. [36] proposed two mechanisms of AgNP penetration across the blood–brain barrier, i.e., the transcytosis of endothelial cells of the brain–blood capillary and the reduction in the close connection between endothelial cells, or dissolution of endothelial cell membranes. The main mechanisms of nanoparticles for crossing the blood–brain barrier according to the literature are summarized in Figure 1.

Therefore, upon contact with NPs, there is a risk of biochemical, histological, and functional disorders in the brain, including cognitive dysfunctions. Studies of the effect of NPs on brain functions have received much attention in recent years. After a single dose injection of AgNPs, the parameters of oxidative stress and the antioxidant potential of the brain on gene expression and the level of protein (superoxide dismutase and glutathione reductase) activity were altered [37]. The shape of astrocytes was disturbed, cerebral capillaries were deformed, and edema was developed in adjacent areas [10,13,38]. In addition, the permeability of the blood–brain barrier increased in direct proportion to the dose of AgNPs received by animals [10].

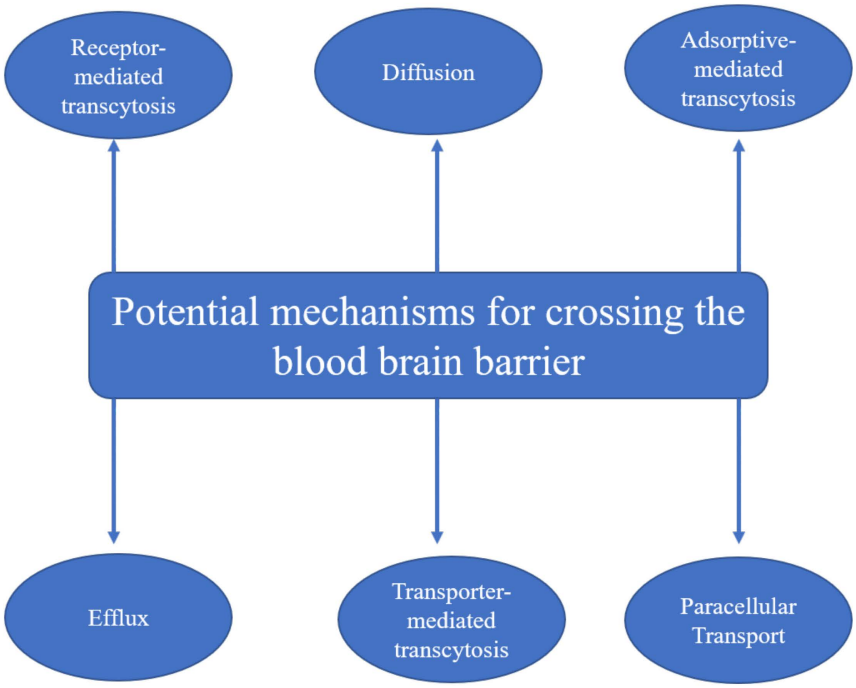

Figure 1. Possible mechanisms of AgNPs for crossing the blood–brain barrier [39–41].

After seven daily injections, impairments of working memory were noted in rats: The animals meaningfully more often made mistakes by repeatedly looking into the arm of the maze they had just examined; however, referential memory and the long-term understanding of the structure of space were not disturbed [38]. After three weeks of injections, the social behavior of the mice differed significantly from the normal behavior in the study by Greish et al. [42] The mice administrated with AgNPs preferred to stay in an empty chamber rather than to familiarize themselves with new animals. In experimental animals, motor coordination and balance were impaired, but the swimming speed in the Morris test was preserved, which revealed the absence of spatial memory formation in individuals treated with AgNPs. The emotional state, the level of anxiety, and the ability to conditioned-reflex learning, in which fear is the motivation, were considered in the study by Antsiferova et al. [43]. After long-term (1–6 months) oral contact with small doses of AgNPs (50 μg) through drinking water, the authors recorded and interpreted the test results as two attempts of the brain to adapt to the effects of AgNPs. Thus, after 2 months of contact, anxiety and fear increased, and after 4 months, they decreased with a simultaneous increase in exploratory behavior. After continued contact with AgNPs (up to 6 months), the impairment of long-term memory and conditioned-reflex learning was observed. The fading effect was noted in the research by González et al. [34]. Although several days after exposure to AgNPs, zebrafish larvae were hyperactive at changes in illumination, no fluctuations in their activity were detected after 5 days.

The influence of prenatal contact with AgNPs on the brain and behavior is described in a limited number of studies. After regular injections of AgNPs to future mothers during pregnancy, the spatial memory of their offspring was impaired, while conditioned-reflex learning and the emotional state of young rats did not differ from the offspring of the control group [44]. The experimental offspring demonstrated signs of depressive-like behavior, i.e., passivity and lack of interest in food [45]. After zebrafish embryos were exposed to AgNP solution, their avoidance motor response to the touch was disrupted; the membrane potentials of their motoneurons were decreased, and the expression of many genes connected with neurogenesis was low [46].

In studies of the effect of AgNP coating material (BSA, polyethylene glycol, and citrate) on cognition, spatial memory, and neurotransmitter levels in the rat hippocampus, only rats administrated with citrate-coated NPs maintained long-term spatial memory. For other

NPs and Ag^+, the induction of peripheral inflammation, which was reflected by alterations in the level of serum inflammatory mediators, was observed [47]. Wu et al. [48] showed a significant reduction in GAP-43 mRNA and protein expression in the hippocampus of offspring exposed to uncoated AgNPs, suggesting cognitive impairments in rats.

However, in some studies on adult animals, after subchronic contact with AgNPs, no behavioral disturbances were noticed. In the study by Liu et al. [49], no significant differences between experimental and control mice in the formation of spatial and working memory were found, and secondary neurogenesis in the hippocampus was not impaired. In the research by Dabrowska-Bouta et al. [12], pathological changes in the structure of myelin sheaths and a decrease in the level of three myelin-specific proteins were detected in the brain of experimental rats treated with AgNPs or Ag^+. However, neither motion nor exploratory behavior or memory was impaired in animals treated with silver nanoparticles present in the two forms. After an intranasal introduction of AgNPs, experimental mice coped with the recognition of a new object in the same way as the control ones; however, their spatial memory was presumably impaired, while the ability for spatial learning itself did not decrease [50]. After AgNPs were injected into lactating mice, their grown offspring, which contacted with AgNPs through milk, did not differ from control animals in terms of their emotional state, social interactions, and locomotion [51]. A summary of the experiments on the influence of AgNPs on different model organisms is given in Table 1.

Table 1. Summary of the experiments on the influence of AgNPs on different model organisms.

Animals	Study Goal	Coating	Dose of AgNPs and Method of Admission	Amount of Silver in the Brain	Effect	Reference
Adult rats (age 12 to 14 weeks)	Influence of long-term sleep deprivation on the blood–brain barrier function and brain pathology	Tween 80	50 mg/kg; intraperitoneal injection once per day for 7 days	Not measured	The permeability of the blood–brain barrier increased in direct proportion to the dose of AgNPs received by animals.	[10]
Adult rats	Effects of the oral administration of AgNPs or silver ions on behavior and cerebral myelin	Citrate	0.2 mg/kg per day for 14 days; through a gastric tube	below the detection limit of the inductively coupled plasma mass spectrometry in brain homogenates	Increased body weight and body temperature of AgNP-treated animals. Both nano-Ag and Ag+ induce pathological changes in the structure of myelin sheaths and a decrease in the level of three myelin-specific proteins.	[12]
Zebrafish (embryos, larvae)	Assessment of the potential toxicity of environmentally relevant concentrations of AgNPs	Alginate	0.03, 0.1, 0.3, 1, 3 ppm (mg/L); from 4 to 120 h post fertilization	Not measured	The hyperactivity of larvae in response to illumination changes faded after 5 days of exposure.	[34]

Table 1. *Cont.*

Animals	Study Goal	Coating	Dose of AgNPs and Method of Admission	Amount of Silver in the Brain	Effect	Reference
Adult rats (age 6–8 months)	Mechanisms underlying the effects of AgNPs on brain functions	Polyamide-hydroxy-urethane	5 µg/kg or 10 µg/kg; intraperitoneal injection once per day for 7 days	Not measured	Impairments in working memory; significant histopathological changes in the brain; oxidative stress markers	[38]
Adult mice (age 8–10 weeks)	Effects of AgNPs on memory, learning, social behavior, and the motor function	Citrate	2 µg per animal; intravenous injection once per week for 1, 2, or 3 weeks	Not measured	Reduction in social interaction and exploratory activity along with the impairment in memory, learning, and motor functions for all treated groups	[42]
Adult mice (age 8 weeks)	Accumulation of silver in the brain and its influence on the emotional state and spatial cognition and memory	Polyvinyl-pyrrolidone	50 µg per day for 30, 60, 120, or 180 days; solution in drinking water	Not measured	After 2 months of contact, anxiety and fear increased. After 4 months, they decreased with a simultaneous increase in exploratory behavior. After 6 months, long-term memory and conditioned-reflex learning were impaired.	[43]
Adult mice	Assessment of AgNPs' influence on spatial cognition and adult hippocampal neurogenesis	None	10, 25, or 50 mg/kg; intraperitoneal injection once per day for 7 days	0.3, 0.4 and 0.5 µg/g wet weight, respectively	No changes in spatial and working memory; secondary neurogenesis in the hippocampus was not impaired.	[49]
Adult mice	Toxic effect and cognitive functions after AgNP inhalation	None	50 mg/kg; intranasal, once per day for 7 days	150 ng/g	Signs of oxidative stress in the hippocampus but not in the cortex; impaired spatial memory	[50]
Offspring of treated female mice	Assessment of the effects of AgNPs after exposure during pregnancy on the neurobehav-ioral development of adult offspring	Citrate	0.2 or 2 mg/kg; subcutaneous injection to mothers every 3 days during pregnancy	Not measured	Impaired spatial memory	[44]

Table 1. *Cont.*

Animals	Study Goal	Coating	Dose of AgNPs and Method of Admission	Amount of Silver in the Brain	Effect	Reference
Offspring of treated female mice	Estimation of the safety of AgNPs' use during lactation	Citrate	10 mg/kg; single dose via a gastral tube to mothers on day 3 after giving birth	2–6 ng/sample	No changes in social interactions, anxiety levels, motor activity	[51]
Offspring of treated female mice	Effects of exposure to AgNPs during the gestational period on offspring's depression behavior	Citrate	0.2 or 2 mg/kg; subcutaneous injection to mothers every 3 days during pregnancy	Not measured	Depressive-like behavior, possibly gender-specific	[45]
Zebrafish (embryos)	Biological effects of AgNPs on fish embryogenesis and the underlying molecular mechanisms	Citrate	0.4 mg/L AgNPs solution, 0.024 mg/L silver ion solution or citrate solution	Not measured	Abnormal motor response to touch, decreased membrane potentials of motoneurons, disrupted neurogenesis	[46]

3. Experimental Research Conducted by the Authors of the Review

3.1. Main Direction of Research and General Idea of Industrial Nanotoxicology

Since NPs can penetrate the blood–brain barrier, for production workers and employees of other organizations where long-term contact with NPs is possible (scientific laboratories, medical organizations), there is a potential danger of the development of previously unknown nosological forms of neurological and mental diseases [52]. Inhalation and oral administration of NPs into animals are suitable for modeling chronic daily contact with NPs. However, it is difficult to conduct a long-term controlled study when choosing an inhalation contact, as it is necessary to constantly maintain the required content of NPs in the air of the inhalation chamber, preventing them from settling. To date, there are practically no reliable methods for monitoring the concentration of NPs in the air; however, when administrated orally, the concentration of NPs in a solution can be controlled with greater precision. These studies are simpler, cheaper, safer for researchers; therefore, in our studies, prolonged chronic contact with NPs by replacing drinking water to animals with a colloidal solution of AgNPs was chosen as the main mode of introducing AgNPs.

Experimental mice drank a solution of AgNPs of a selected concentration for 1–4 months, which, at an average mouse life expectancy of 1.5–2 years, corresponds to approximately 3–12 years of the daily human consumption of NPs. Further, the accumulation of AgNPs in the brain and other organs of the animals (for comparison) was evaluated using neutron activation analysis (NAA). The presence and nature of cognitive impairments in experimental animals, compared with control ones, were evaluated using behavioral tests. The methodology of the experiments and the conditions for keeping the animals in the vivarium of M.F. Vladimirskiy Moscow Regional Research and Clinical Institute were approved by the institutional ethics committee and are consistent with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The methodology of cognitive tests in the Morris water maze was published in our earlier study [52]. A short description of the performed experiments and the effect of AgNPs on the objects of study are summarized in Table 2.

Table 2. Summary of the experiments on the impact of AgNPs on mice: purpose and experimental conditions.

Age	Study Goal	Coating	Dose of AgNPs	Amount of Silver in the Brain	Effect	Reference
3–4 weeks	Influence on health and behavior, blood composition, and reproduction	None	1.8, 3.7 or 7.1 mg/kg for 1 month	Not measured	Signs of oxidative stress in blood composition	[52]
1.5 years	Influence on health and behavior, blood composition	None	1.8 mg/kg for 1 month	Not measured	Signs of oxidative stress in blood composition	[53]
2 months	Influence on cognitive abilities in adults, estimation of accumulation in the brain	Polyvinylpyrrolidone	4.5 and 5 mg/kg for males and females for 2 or 4 months	319 ± 155 ng after 2 months; 870 ± 200 ng after 4 months	Spatial orientation, memory, and retrieval are undisturbed in adults.	[54–56]
2 months	Influence on cognitive abilities in offspring, estimation of accumulation in the brain	Polyvinylpyrrolidone	5 mg/kg for 2 months	373 ± 75 ng in mothers; 385 ± 57 ng in offspring	No spatial memory formation in offspring	[57,58]

Besides water, NPs can enter the body with food. In one of our studies, rats were administrated with unmodified and functionalized *Spirulina platensis* biomass AgNPs for 28 days. As in the above-described experiments, the silver content in the organs of control and experimental animals was assessed by NAA. In animals administrated with both types of NPs (unmodified and modified), the highest content of silver was determined in the brain. At the same time, the hematological and biochemical indices of the rats did not change significantly under the action of AgNPs, except for the content of reticulocytes and eosinophils, which increased considerably [59].

3.2. First Results with Uncoated AgNPs

The study of the impact of AgNPs on animals began with the use of uncoated AgNPs in order to exclude the possible effect of the coating substance on the results. A colloidal solution "Silver Shield" (LLC "Fractal-M", Russia) with an average nanoparticle size of 15 nm was used. In the first experiments performed in 2009–2010, the task was to determine the maximum allowable dose of AgNPs, for example, LD10 for groups of mice, to explore it in further experiments. According to the design of the experiments on the effect of AgNPs on cognitive functions, mice should have received a strong toxic effect from AgNPs but not died from the general intoxication of the body. Therefore, it was necessary to select such a dose experimentally. For that purpose, two similar experiments were carried out on mice of different ages at the beginning of NP administration: young (3–4 weeks) in the first experiment and old (1.5 years) in the second one [52,53]. The duration of NP intake was one month, and their concentrations in solutions were 25, 50, or 100 mg/L. A concentration of 100 mg/L is the maximum possible for a colloidal solution of uncoated AgNPs, at which NPs are suspended in the solution throughout the experiment. At concentrations above 100 mg/L, they conglomerate (aggregate) and settle to the bottom.

Even according to the obtained results, small biochemical disturbances occurred in the blood of the mice, indicating the development of oxidative stress and, possibly, renal disorders [52,53], which were more pronounced in adult mice than in young ones; all experimental animals to all appearances and behaviors in all groups did not differ from the control group. There were no deaths of mice in the groups. At average daily doses of the consumption of AgNPs per mouse 1.8; 3.7 or 7.1 mg/kg of body weight, the condition of internal organs in all experimental mice also did not differ in appearance from that of control animals. Analyzing histological preparations of the heart, liver, kidneys, and spleen, no obvious pathological changes were noted in any group of mice. In the first experiment, at the end of the intake of AgNPs, the birth rate in young females was also assessed. According to the obtained results, it did not differ from the other groups of animals used in the experiment. In general, both experimental and control individuals gave birth to 7–8 healthy infants [52]. Thus, no obvious toxic effects of AgNPs without coating were revealed, even at a concentration of AgNPs in a drinking solution of 100 mg/L. It should be noted that one month of the experiment on mice corresponds to approximately 3–4 years of daily human contact with NPs.

3.3. AgNPs Effect on the Brain and Behavior

To study the effect of AgNPs on the brain and behavior, experiments were performed on mice exposed to AgNPs in adulthood [55]. The animals received a solution of AgNPs (size 25 nm; polyvinylpyrrolidone as coating agent) at a concentration of 25 μg/mL for 2 or 4 months, while control individuals drank pure water. When consuming compound animal feedstuff, one mouse drank an average of 5.38 mL of liquid per day; consequently, the daily dose of AgNPs per mouse was 4.5 and 5 mg/kg of body weight for males and females, respectively. At the end of the experiment, the learning ability and spatial memory of the animals were assessed in the Morris water maze (MWM) [60]. In this standard behavioral test, an underwater platform on which the animal can climb to come out of the water is placed in a round white pool filled with water colored and turbid with milk powder. The task of the animal is to find this platform and memorize its location relative to the out-of-labyrinth landmarks [60] in order to use its spatial memory next time to find the platform quicker. The methodology for conducting tests is described in detail in [55].

To establish a correlation between possible cognitive impairments in the brain of animals exposed to NPs and the level of AgNPs accumulated in the brain, the silver content in the brain, as well as in other tissues and organs, was determined after the animals' slaughter using NAA [56]. NAA, due to the possibility of determining a wide number of elements in biological samples, allows assessing the content of silver and iron in organs and blood samples, estimating the passage of nanoparticles through the blood–brain barrier, and quantifying its accumulation in the neuronal tissues of the brain. The technique is described in detail in [55,56].

According to the NAA results, the relative mass of silver in the brain of experimental animals was 319 $\pm$ 155 ng after 2 months of nanoparticle intake and 870 $\pm$ 200 ng after 4 months, which is significantly higher, almost an order of magnitude, than the silver content in the control group [55]. Notwithstanding the accumulation of silver in the brain, within groups of individuals with a preference for different behavioral strategies in the MWM (for details, see [61]), adult experimental animals learned to find the platform just as successfully as control ones. In experiments in which the period of AgNP intake passed after the initial testing, which made it possible to identify groups of individuals with a preference for different behavioral strategies in the test [55], all mice retained learned information about the test for 2 and 4 months. Thus, it was concluded that in adult animals, the long-term oral consumption of AgNPs did not impair the ability to form spatial memory, as well as the ability to store and apply information learned before exposure to AgNPs.

Following this series of experiments, a study was carried out that simulated the effect of AgNPs on children born by women employed in the production industry or who were in close contact with AgNPs during pregnancy and lactation at home [57]. During the entire

period of pregnancy and lactation (2 months in total), female mice were given to drink a solution of AgNPs (size 25 nm; polyvinylpyrrolidone as coating agent) with a concentration of 25 μg/mL. Thus, their offspring were exposed to AgNPs during prenatal development and early postnatal development. When the pups reached the age of 2 months, their learning ability and spatial memory were assessed in the MWM, while the silver content in their brain and the mother's brain was determined by NAA. It was found that within groups of individuals with a preference for different behavioral strategies in the MWM, experimental mice did not learn to find the platform, while control individuals coped with the task successfully. Silver accumulated in the brain of both mothers and pups was at a level comparable to that in adult mice after 2 months of silver NP intake (373 ± 75 ng and 385 ± 57 ng, respectively), which exceeded the silver content in the control group approximately 20 times [58]. This experiment clearly demonstrated that, firstly, AgNPs easily overcame various biological barriers (blood–brain, placental) and migrated from the mother's body to the offspring's body; secondly, the study revealed that the fragile body of a developing child, including its brain, in general, was more sensitive to the toxic effect of AgNPs than the body of an adult, even an elderly one.

3.4. Supplementary Experiments with Microorganisms

Since there is a significant body of literature on the bactericidal and/or bacteriostatic effect of silver [2], in particular, silver ions (Ag^+), as well as the study of the toxicity of AgNPs for animals, in 2010–2011, research was conducted to elucidate the toxic effect of uncoated AgNPs on Gram-negative and Gram-positive bacteria, and on yeast-like fungi [62]. The effect of concentrated solutions of AgNPs (50 or 100 mg/L) or drugs based on Ag^+ (in the form of nitrate or proteinate) on the growth of microorganisms in Petri dishes was assessed. According to the obtained results, AgNPs did not affect the growth of microorganisms, as opposed to the pronounced antiseptic effect of solutions of known drugs based on Ag^+ and antibiotics. When studying the effect of AgNPs coated with polyethylene glycol at a concentration range of 0.025–0.5 μM, it was shown that NPs stimulated spirulina biomass, and the content of proteins, carbohydrates, and auxiliary pigments was slightly influenced by the presence of NPs in the cultivation medium [63].

These results, despite the fact that they contrast with the common descriptions of AgNPs as an antibacterial agent, can be easily explained. Silver itself is practically insoluble in water and does not create a toxic level of Ag^+ in solutions. The formation of Ag^+ is possible only from a silver salt, for example, from $AgNO_3$ used in medicine (silver nitrate, Stilus Lapidis). At the same time, both Ag^+ and the acidic residue of the NO_3^- solution act as antiseptics, which was demonstrated in the described experiments when nitrates of other metals were used.

4. Discussion

Along with reports on the toxicity of AgNPs, there are contradicting studies in which toxic effects of NPs are (1) not disclosed, (2) not detected during the behavioral testing, and (3) not detected in certain types of behavioral tests. At the same time, it is known in medicine that the excessive use of silver preparations, even in the usual, non-nano-scale form, can lead to toxic effects and systemic diseases, for example, at doses of more than 70 mg of silver per kg of body weight, to argyria [64].

Several aspects may be related to the occurrence of such discrepancies. Firstly, it has not yet been determined what exactly causes the supposed toxic effect of AgNPs. Some studies suggest that the observed toxicity (including the antibacterial effect) may be caused by Ag^+ present in the solution and not by NPs themselves [29,65]. The assumption was made on the basis of the similarity and, at the same time, significantly greater severity of toxic effects provoked by animals' contact with a solution containing Ag^+ in comparison with NPs. However, silver practically does not dissociate in aqueous solutions; therefore, the content of Ag^+ in solutions of AgNPs is very low, hundreds of times lower than its concentrations used in experiments with Ag^+. Secondly, AgNP toxicity can be determined

by the compounds used to cover nanoparticles, i.e., biopolymers that form a protein crown and polymers of stabilizing coating. The structure and interaction of NPs coated by the protein crown with cell structures are poorly studied in vivo, especially in terms of the toxic effect of NPs [66,67]. There is evidence that toxic effects of AgNPs were expressed to varying degrees in experimental animals administrated with NPs with different coatings, and only in several studies, the effect of the coating substance on animals was assessed [12,45,68]. Precise mechanisms of how coating materials can impact toxicity remain unknown and need to be studied. However, for example, El Badawy et al. [23] associated the low toxicity of citrate-coated AgNPs for microorganisms with their negative charge. Since functional groups on the cellular membrane of bacteria provide organisms with a negative charge, there is a high degree of repulsion between negative NPs and bacterial cells, which forms an electrostatic barrier that limits cell–particle interactions, thereby reducing toxicity. Thirdly, the age and state of experimental animals are also important factors—namely, healthy, mobile, sexually mature young animals in the first half of their life are more resistant to any adverse effects than aged animals or juveniles.

If we discuss toxic effects observed in the experiments with animals through the prism of a possible analogy with humans, then it is noteworthy that in many experiments, the doses of AgNPs were so high in terms of the animal's body weight that it is difficult to imagine such a situation for a human being. For example, in our experiments simulating chronic occupational or household contact with NPs, the doses of silver received by an animal in terms of 1 kg of body weight significantly exceed the amount of silver that a person can actually receive if the individual does not consume silver forcibly. For instance, for a person weighing 70 kg to receive an amount of silver comparable to that given to a mouse in [55], the person needs to drink 12.6 L of a concentrated solution of silver NPs per day for 6–12 years. Apart from cases of the intentional consumption of AgNP solutions, it is difficult to imagine such a situation. However, even in this case, the revealed toxic effects of AgNPs are not fatal for the organism. An adult healthy individual is able to cope with such an impact with minimal damage. The question of the impact of AgNPs on children, the elderly, or people with any chronic diseases remains open. Few such experiments were carried out, but it is reasonable to suggest that the elderly and children are more sensitive to the effects of NPs.

5. Conclusions

AgNPs are one of the most extensively studied nanomaterials due to their unique optical, catalytic, sensing, and antimicrobial properties. The toxicity of AgNPs is dependent on various factors such as particle size, dose, and coating agent. The toxicity of AgNPs is mainly associated with oxidative stress, inflammation, swelling, cognitive disorders, etc. Despite a plethora of studies describing AgNP toxicity, there is still a high level of uncertainty with regard to their true toxicity.

According to the results obtained by the authors of the present review, it can be assumed that, at real dosages that a healthy adult can accidentally encounter at home or at work (without taking into account cases of the presence of any diseases, the forced consumption of large doses of NPs with water and food), toxic effects provoked by AgNPs are very weak and not critical for a healthy adult. Their effects on children, the elderly, or people with chronic diseases are currently poorly studied, but it is possible that they may be more pronounced.

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Abbreviations

AgNPs	Silver nanoparticles
NPs	Nanoparticles
NAA	Neutron activation analysis
MWM	Morris water maze

References

1. Burdușel, A.C.; Gherasim, O.; Grumezescu, A.M.; Mogoantă, L.; Ficai, A.; Andronescu, E. Biomedical applications of silver nanoparticles: An up-to-date overview. *Nanomaterials* **2018**, *8*, 681. [CrossRef] [PubMed]
2. Temizel-Sekeryan, S.; Hicks, A.L. Global environmental impacts of silver nanoparticle production methods supported by life cycle assessment. *Resour. Conserv. Recycl.* **2020**, *156*, 104676. [CrossRef]
3. Bertrand, C.; Zalouk-Vergnoux, A.; Giambérini, L.; Poirier, L.; Devin, S.; Labille, J.; Perrein-Ettajani, H.; Pagnout, C.; Châtel, A.; Levard, C.; et al. The influence of salinity on the fate and behavior of silver standardized nanomaterial and toxicity effects in the estuarine bivalve *Scrobicularia plana*. *Environ. Toxicol. Chem.* **2016**, *35*, 2550–2561. [CrossRef] [PubMed]
4. Chien, H.W.; Kuo, C.J.; Kao, L.H.; Lin, G.Y.; Chen, P.Y. Polysaccharidic spent coffee grounds for silver nanoparticle immobilization as a green and highly efficient biocide. *Int. J. Biol. Macromol.* **2019**, *140*, 168–176. [CrossRef] [PubMed]
5. Zhou, Y.; Hong, F.; Tian, Y.; Zhao, X.; Hong, J.; Ze, Y.; Wang, L. Nanoparticulate titanium dioxide-inhibited dendritic development is involved in apoptosis and autophagy of hippocampal neurons in offspring mice. *Toxicol. Res.* **2017**, *6*, 889–901. [CrossRef] [PubMed]
6. Bergamaschi, E.; Garzaro, G.; Jones, G.W.; Buglisi, M.; Caniglia, M.; Godono, A.; Bosio, D.; Fenoglio, I.; Canu, I.G. Occupational exposure to carbon nanotubes and carbon nanofibres: More than a cobweb. *Nanomaterials* **2021**, *11*, 745. [CrossRef]
7. McCormick, S.; Niang, M.; Dahm, M.M. Occupational Exposures to Engineered Nanomaterials: A Review of Workplace Exposure Assessment Methods. *Curr. Environ. Health Rep.* **2021**, *8*, 223–234. [CrossRef]
8. Meng, Q.; Meng, H.; Pan, Y.; Liu, J.; Li, J.; Qi, Y.; Huang, Y. Influence of nanoparticle size on blood-brain barrier penetration and the accumulation of anti-seizure medicines in the brain. *J. Mater. Chem. B* **2022**, *10*, 271–281. [CrossRef]
9. Ceña, V.; Játiva, P. Nanoparticle crossing of blood-brain barrier: A road to new therapeutic approaches to central nervous system diseases. *Nanomedicine* **2018**, *13*, 1513–1516. [CrossRef]
10. Sharma, A.; Muresanu, D.F.; Lafuente, J.V.; Patnaik, R.; Tian, Z.R.; Buzoianu, A.D.; Sharma, H.S. Sleep Deprivation-Induced Blood-Brain Barrier Breakdown and Brain Dysfunction are Exacerbated by Size-Related Exposure to Ag and Cu Nanoparticles. Neuroprotective Effects of a 5-HT3 Receptor Antagonist Ondansetron. *Mol. Neurobiol.* **2015**, *52*, 867–881. [CrossRef]
11. Flores-López, L.Z.; Espinoza-Gómez, H.; Somanathan, R. Silver nanoparticles: Electron transfer, reactive oxygen species, oxidative stress, beneficial and toxicological effects. Mini review. *J. Appl. Toxicol.* **2019**, *39*, 16–26. [CrossRef] [PubMed]
12. Dąbrowska-Bouta, B.; Zięba, M.; Orzelska-Górka, J.; Skalska, J.; Sulkowski, G.; Frontczak-Baniewicz, M.; Talarek, S.; Listos, J.; Strużyńska, L. Influence of a low dose of silver nanoparticles on cerebral myelin and behavior of adult rats. *Toxicology* **2016**, *363–364*, 29–36. [CrossRef] [PubMed]
13. Moradi-Sardareh, H.; Basir, H.R.G.; Hassan, Z.M.; Davoudi, M.; Amidi, F.; Paknejad, M. Toxicity of silver nanoparticles on different tissues of Balb/C mice. *Life Sci.* **2018**, *211*, 81–90. [CrossRef] [PubMed]
14. Marinho, C.S.; Matias, M.V.F.; Toledo, E.K.M.; Smaniotto, S.; Ximenes-da-Silva, A.; Tonholo, J.; Santos, E.L.; Machado, S.S.; Zanta, C.L. Toxicity of silver nanoparticles on different tissues in adult Danio rerio. *Fish Physiol. Biochem.* **2021**, *47*, 239–249. [CrossRef] [PubMed]
15. Recordati, C.; De Maglie, M.; Bianchessi, S.; Argentiere, S.; Cella, C.; Mattiello, S.; Cubadda, F.; Aureli, F.; D'Amato, M.; Raggi, A.; et al. Tissue distribution and acute toxicity of silver after single intravenous administration in mice: Nano-specific and size-dependent effects. *Part. Fibre Toxicol.* **2016**, *13*, 12. [CrossRef] [PubMed]
16. Rodriguez-Garraus, A.; Azqueta, A.; Vettorazzi, A.; de Cerain, A.L. Genotoxicity of silver nanoparticles. *Nanomaterials* **2020**, *10*, 251. [CrossRef]
17. Lebedová, J.; Hedberg, Y.S.; Odnevall Wallinder, I.; Karlsson, H.L. Size-dependent genotoxicity of silver, gold and platinum nanoparticles studied using the mini-gel comet assay and micronucleus scoring with flow cytometry. *Mutagenesis* **2018**, *33*, 77–85. [CrossRef] [PubMed]
18. Guo, X.; Li, Y.; Yan, J.; Ingle, T.; Jones, M.Y.; Mei, N.; Boudreau, M.D.; Cunningham, C.K.; Abbas, M.; Paredes, A.M.; et al. Size- and coating-dependent cytotoxicity and genotoxicity of silver nanoparticles evaluated using in vitro standard assays. *Nanotoxicology* **2016**, *10*, 1373–1384. [CrossRef]
19. Gliga, A.R.; Skoglund, S.; Odnevall Wallinder, I.; Fadeel, B.; Karlsson, H.L. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: The role of cellular uptake, agglomeration and Ag release. *Part. Fibre Toxicol.* **2014**, *11*, 11. [CrossRef]

20. Ivask, A.; Kurvet, I.; Kasemets, K.; Blinova, I.; Aruoja, V.; Suppi, S.; Vija, H.; Kakïnen, A.; Titma, T.; Heinlaan, M.; et al. Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells in vitro. *PLoS ONE* **2014**, *9*, e102108. [CrossRef]
21. Butler, K.S.; Peeler, D.J.; Casey, B.J.; Dair, B.J.; Elespuru, R.K. Silver nanoparticles: Correlating nanoparticle size and cellular uptake with genotoxicity. *Mutagenesis* **2015**, *30*, 577–591. [CrossRef] [PubMed]
22. Lu, W.; Senapati, D.; Wang, S.; Tovmachenko, O.; Singh, A.K.; Yu, H.; Ray, P.C. Effect of surface coating on the toxicity of silver nanomaterials on human skin keratinocytes. *Chem. Phys. Lett.* **2010**, *487*, 92–96. [CrossRef] [PubMed]
23. El Badawy, A.M.; Silva, R.G.; Morris, B.; Scheckel, K.G.; Suidan, M.T.; Tolaymat, T.M. Surface charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* **2011**, *45*, 283–287. [CrossRef] [PubMed]
24. Yang, X.; Gondikas, A.P.; Marinakos, S.M.; Auffan, M.; Liu, J.; Hsu-Kim, H.; Meyer, J.N. Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in caenorhabditis elegans. *Environ. Sci. Technol.* **2012**, *46*, 1119–1127. [CrossRef]
25. Prokhorova, I.M.; Kibrik, B.S.; Pavlov, A.V.; Pesnya, D.S. Estimation of mutagenic effect and modifications of mitosis by silver nanoparticles. *Bull. Exp. Biol. Med.* **2013**, *156*, 255–259. [CrossRef]
26. Lekamge, S.; Miranda, A.F.; Abraham, A.; Li, V.; Shukla, R.; Bansal, V.; Nugegoda, D. The toxicity of silver nanoparticles (AgNPs) to three freshwater invertebrates with different life strategies: *Hydra vulgaris*, *Daphnia carinata*, and *Paratya australiensis*. *Front. Environ. Sci.* **2018**, *6*, 152. [CrossRef]
27. Zhang, J.; Liu, S.; Han, J.; Wang, Z.; Zhang, S. On the developmental toxicity of silver nanoparticles. *Mater. Des.* **2021**, *203*, 109611. [CrossRef]
28. Park, K.; Tuttle, G.; Sinche, F.; Harper, S.L. Stability of citrate-capped silver nanoparticles in exposure media and their effects on the development of embryonic zebrafish (*Danio rerio*). *Arch. Pharm. Res.* **2013**, *36*, 125–133. [CrossRef]
29. Massarsky, A.; Dupuis, L.; Taylor, J.; Eisa-Beygi, S.; Strek, L.; Trudeau, V.L.; Moon, T.W. Assessment of nanosilver toxicity during zebrafish (*Danio rerio*) development. *Chemosphere* **2013**, *92*, 59–66. [CrossRef]
30. Xin, Q.; Rotchell, J.M.; Cheng, J.; Yi, J.; Zhang, Q. Silver nanoparticles affect the neural development of zebrafish embryos. *J. Appl. Toxicol.* **2015**, *35*, 1481–1492. [CrossRef]
31. Pourali, P.; Nouri, M.; Ameri, F.; Heidari, T.; Kheirkhahan, N.; Arabzadeh, S.; Yahyaei, B. Histopathological study of the maternal exposure to the biologically produced silver nanoparticles on different organs of the offspring. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **2020**, *393*, 867–878. [CrossRef] [PubMed]
32. Lyu, Z.; Ghoshdastidar, S.; Rekha, K.R.; Suresh, D.; Mao, J.; Bivens, N.; Kannan, R.; Joshi, T.; Rosenfeld, C.S.; Upendran, A. Developmental exposure to silver nanoparticles leads to long term gut dysbiosis and neurobehavioral alterations. *Sci. Rep.* **2021**, *11*, 6558. [CrossRef] [PubMed]
33. Powers, C.M.; Slotkin, T.A.; Seidler, F.J.; Badireddy, A.R.; Padilla, S. Silver nanoparticles alter zebrafish development and larval behavior: Distinct roles for particle size, coating and composition. *Neurotoxicol. Teratol.* **2011**, *33*, 708–714. [CrossRef] [PubMed]
34. González, E.A.; Carty, D.R.; Tran, F.D.; Cole, A.M.; Lein, P.J. Developmental exposure to silver nanoparticles at environmentally relevant concentrations alters swimming behavior in zebrafish (*Danio rerio*). *Environ. Toxicol. Chem.* **2018**, *37*, 3018–3024. [CrossRef]
35. Ema, M.; Okuda, H.; Gamo, M.; Honda, K. A review of reproductive and developmental toxicity of silver nanoparticles in laboratory animals. *Reprod. Toxicol.* **2017**, *67*, 149–164. [CrossRef]
36. Tang, J.; Xiong, L.; Wang, S.; Wang, J.; Liu, L.; Li, J.; Wan, Z.; Xi, T. Influence of silver nanoparticles on neurons and blood-brain barrier via subcutaneous injection in rats. *Appl. Surf. Sci.* **2008**, *255*, 502–504. [CrossRef]
37. Krawczyńska, A.; Dziendzikowska, K.; Gromadzka-Ostrowska, J.; Lankoff, A.; Herman, A.P.; Oczkowski, M.; Królikowski, T.; Wilczak, J.; Wojewódzka, M.; Kruszewski, M. Silver and titanium dioxide nanoparticles alter oxidative/inflammatory response and renin-angiotensin system in brain. *Food Chem. Toxicol.* **2015**, *85*, 96–105. [CrossRef]
38. Hritcu, L.; Stefan, M.; Ursu, L.; Neagu, A.; Mihasan, M.; Tartau, L.; Melnig, V. Exposure to silver nanoparticles induces oxidative stress and memory deficits in laboratory rats. *Cent. Eur. J. Biol.* **2011**, *6*, 497–509. [CrossRef]
39. Pulgar, V.M. Transcytosis to cross the blood brain barrier, new advancements and challenges. *Front. Neurosci.* **2019**, *13*, 1019. [CrossRef]
40. Saraiva, C.; Praça, C.; Ferreira, R.; Santos, T.; Ferreira, L.; Bernardino, L. Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier to treat neurodegenerative diseases. *J. Control. Release* **2016**, *235*, 34–47. [CrossRef]
41. Pinheiro, R.G.R.; Coutinho, A.J.; Pinheiro, M.; Neves, A.R. Nanoparticles for targeted brain drug delivery: What do we know? *Int. J. Mol. Sci.* **2021**, *22*, 11654. [CrossRef] [PubMed]
42. Greish, K.; Alqahtani, A.A.; Alotaibi, A.F.; Abdulla, A.M.; Bukelly, A.T.; Alsobyani, F.M.; Alharbi, G.H.; Alkiyumi, I.S.; Aldawish, M.M.; Alshahrani, T.F.; et al. The Effect of Silver Nanoparticles on Learning, Memory and Social Interaction in BALB/C Mice. *Int. J. Environ. Res. Public Health* **2019**, *16*, 148. [CrossRef] [PubMed]
43. Antsiferova, A.; Kopaeva, M.; Kashkarov, P. Effects of prolonged silver nanoparticle exposure on the contextual cognition and behavior of mammals. *Materials* **2018**, *11*, 558. [CrossRef] [PubMed]
44. Ghaderi, S.; Tabatabaei, S.R.F.; Varzi, H.N.; Rashno, M. Induced adverse effects of prenatal exposure to silver nanoparticles on neurobehavioral development of offspring of mice. *J. Toxicol. Sci.* **2015**, *40*, 263–275. [CrossRef] [PubMed]
45. Tabatabaei, S.R.F.; Moshrefi, M.; Askaripour, M. Prenatal exposure to silver nanoparticles causes depression like responses in mice. *Indian J. Pharm. Sci.* **2015**, *77*, 681–686. [CrossRef]

46. Zhao, G.; Wang, Z.Y.; Xu, L.; Xia, C.X.; Liu, J.X. Silver nanoparticles induce abnormal touch responses by damaging neural circuits in zebrafish embryos. *Chemosphere* **2019**, *229*, 169–180. [CrossRef]
47. Dziendzikowska, K.; Węsierska, M.; Gromadzka-Ostrowska, J.; Wilczak, J.; Oczkowski, M.; Męczyńska-Wielgosz, S.; Kruszewski, M. Silver nanoparticles impair cognitive functions and modify the hippocampal level of neurotransmitters in a coating-dependent manner. *Int. J. Mol. Sci.* **2021**, *22*, 12706. [CrossRef]
48. Wu, J.; Yu, C.; Tan, Y.; Hou, Z.; Li, M.; Shao, F.; Lu, X. Effects of prenatal exposure to silver nanoparticles on spatial cognition and hippocampal neurodevelopment in rats. *Environ. Res.* **2015**, *138*, 67–73. [CrossRef]
49. Liu, P.; Huang, Z.; Gu, N. Exposure to silver nanoparticles does not affect cognitive outcome or hippocampal neurogenesis in adult mice. *Ecotoxicol. Environ. Saf.* **2013**, *87*, 124–130. [CrossRef]
50. Davenport, L.L.; Hsieh, H.; Eppert, B.L.; Carreira, V.S.; Krishan, M.; Ingle, T.; Howard, P.C.; Williams, M.T.; Vorhees, C.V.; Genter, M.B. Systemic and behavioral effects of intranasal administration of silver nanoparticles. *Neurotoxicol. Teratol.* **2015**, *51*, 68–76. [CrossRef]
51. Morishita, Y.; Yoshioka, Y.; Takimura, Y.; Shimizu, Y.; Namba, Y.; Nojiri, N.; Ishizaka, T.; Takao, K.; Yamashita, F.; Takuma, K.; et al. Distribution of Silver Nanoparticles to Breast Milk and Their Biological Effects on Breast-Fed Offspring Mice. *ACS Nano* **2016**, *10*, 8180–8191. [CrossRef] [PubMed]
52. Petritskaya, E.N.; Abaeva, L.F.; Rogatkin, D.A.; Litvinova, K.S.; Bobrov, M.A. To the question of the toxicity of silver nanoparticles after oral administration of a colloidal solution. *Almanac Clin. Med.* **2011**, *25*, 9–12.
53. Petritskaya, E.N.; Abaeva, L.F.; Rogatkin, D.A.; Litvinova, K.S.; Bobrov, M.A. Some aspects of the toxicity of silver nanoparticles in an experiment with adult mice with oral intake of colloidal solutions. *Nanotechnics* **2013**, *1*, 108–112.
54. Ivlieva, A.L.; Petritskaya, E.N.; Lopatina, M.V. Preliminary data on the effect of nanoparticles on the cognitive abilities of young animals. In *Proceedings of the Sixth International Forum on Cognitive Modeling, Pittsburgh, PA, USA, 30 July–1 August 2004*; Springer: Berlin, Germany, 2018.
55. Ivlieva, A.L.; Petritskaya, E.N.; Rogatkin, D.A.; Demin, V.A.; Glazkov, A.A.; Zinicovscaia, I.; Pavlov, S.S.; Frontasyeva, M.V. Impact of Chronic Oral Administration of Silver Nanoparticles on Cognitive Abilities of Mice. *Phys. Part. Nucl. Lett.* **2021**, *18*, 250–265. [CrossRef]
56. Zinicovscaia, I.; Pavlov, S.S.; Frontasyeva, M.V.; Ivlieva, A.L.; Petritskaya, E.N.; Rogatkin, D.A.; Demin, V.A. Accumulation of silver nanoparticles in mice tissues studied by neutron activation analysis. *J. Radioanal. Nucl. Chem.* **2018**, *318*, 985–989. [CrossRef]
57. Ivlieva, A.L.; Petritskaya, E.N.; Lopatina, M.V.; Rogatkin, D.A.; Zinkovskaya, I. Evaluation of the cognitive abilities of mice exposed to silver nanoparticles during prenatal development and lactation. In Proceedings of the Seventh International Forum on Cognitive Modeling, Montreal, QC, Canada, 19–22 July 2019.
58. Zinicovscaia, I.; Grozdov, D.; Yushin, N.; Ivlieva, A.; Petritskaya, E.; Rogatkin, D. Neutron activation analysis as a tool for tracing the accumulation of silver nanoparticles in tissues of female mice and their offspring. *J. Radioanal. Nucl. Chem.* **2019**, *322*, 1079–1083. [CrossRef]
59. Rudi, L.; Zinicovscaia, I.; Cepoi, L.; Chiriac, T.; Peshkova, A.; Cepoi, A.; Grozdov, D. Accumulation and effect of silver nanoparticles functionalized with spirulina platensis on rats. *Nanomaterials* **2021**, *11*, 2992. [CrossRef]
60. Morris, R. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* **1984**, *11*, 47–60. [CrossRef]
61. Ivlieva, A.L.; Petritskaya, E.N.; Rogatkin, D.A.; Demin, V.A. Methodological Characteristics of the Use of the Morris Water Maze for Assessment of Cognitive Functions in Animals. *Neurosci. Behav. Physiol.* **2017**, *47*, 484–493. [CrossRef]
62. Petritskaya, E.N.; Rogatkin, D.A.; Rusanova, E.V. Comparative characteristics of antibacterial effect of silver and nanosilver in vitro. *Alm. Clin. Med.* **2016**, *44*, 221–226. [CrossRef]
63. Cepoi, L.; Zinicovscaia, I.; Rudi, L.; Chiriac, T.; Rotari, I.; Turchenko, V.; Djur, S. Effects of PEG-coated silver and gold nanoparticles on spirulina platensis biomass during its growth in a closed system. *Coatings* **2020**, *10*, 717. [CrossRef]
64. Hadrup, N.; Sharma, A.K.; Loeschner, K. Toxicity of silver ions, metallic silver, and silver nanoparticle materials after in vivo dermal and mucosal surface exposure: A review. *Regul. Toxicol. Pharmacol.* **2018**, *98*, 257–267. [CrossRef] [PubMed]
65. Behra, R.; Sigg, L.; Clift, M.J.D.; Herzog, F.; Minghetti, M.; Johnston, B.; Petri-Fink, A.; Rothen-Rutishauser, B. Bioavailability of silver nanoparticles and ions: From a chemical and biochemical perspective. *J. R. Soc. Interface* **2013**, *10*, 20130396. [CrossRef] [PubMed]
66. Rumyantsev, K.A.; Shemetov, A.A.; Nabiev, I.R.; Sukhanova, A.V. Structural and functional aspects of the interaction of proteins and peptides with nanoparticles. *Nano Technol. Russ.* **2013**, *8*, 700–720. [CrossRef]
67. Leonenko, N.; Leonenko, O. Factors Influencing the Manifestation of Toxicity and Danger of Nanomaterials. *Innov. Biosyst. Bioeng.* **2020**, *4*, 75–88. [CrossRef]
68. Dănilă, O.O.; Berghian, A.S.; Dionisie, V.; Gheban, D.; Olteanu, D.; Tabaran, F.; Baldea, I.; Katona, G.; Moldovan, B.; Clichici, S.; et al. The effects of silver nanoparticles on behavior, apoptosis and nitro-oxidative stress in offspring Wistar rats. *Nanomedicine* **2017**, *12*, 1455–1473. [CrossRef]