Article

Relationships between Effects of Ag Nanoparticles and Ag Salt on Behavioral and Cognitive Functions of Mice and Ageing

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Abstract: Silver in different forms is used for medical purposes from ancient times. It is not yet well known, which form of silver is more biocompatible and less toxic. Here we considered silver nanoparticles and silver citrate. Also, the relationships of neurotoxicity of silver compaunds with ageing factor is not yet described. To assess the role of nanoform in neurotoxicity of silver and role of ageing a long-term experiment was conducted. We had four control groups of intact mice and four experimental groups which were exposed to silver nanoparticles and silver citrate for two months. Four groups of mice were introduced into the experiment since the age of five months to assess ageing factors. It was shown that the nanoform does play a certain role in neurotoxicity of silver. Silver citrate seems to be a more preferable silver compound. Ageing can be regarded as a positive factor that neutralizes toxic action of silver compounds. It may be due to the development of physiological/cognitive functions with the age as well as adaptation to unnatural content in the individual cages that is definitely stressful for mice.

Keywords: nanoform; nanoparticle; silver nanoparticles, silver citrate, ageing, behavioral functions, cognitive functions, stress, individual content, mice

1. Introduction

Silver is a well-known element that had been used as a medicine since Antiquity [1]. Metallic silver was used in the Ancient Egypt and Mesopotamia as well as in India. Silver salts were actively applied since XIX century for wound and burn treatments. Along with the development of nanotechnologies in the beginning of XXI century silver nanoparticles (AgNPs) found their application in light, food and cosmetic industries as well as in medicine and pharmaceutics [2]. AgNPs are usually regarded as a less toxic and more effective antiseptic in comparison with other forms of silver.

Today it is obvious that silver compounds may demonstrate toxic properties towards the healthy cells [3]. The mechanism of AgNPs action is not yet understood. It is supposed that they may penetrate into living cells, gradually release ions that interact with the cellular biomacromolecules [4]. Silver salts may release positively charged Ag+ ions as well as anions. Interaction of ions and Ag NPs with cellular biomacromolecules and organelles may lead to release of reactive oxygen species that may cause certain toxicity itself [5].

AgNPs and Ag salts have similar biokinetics profiles that differ quantitatively [6,7]. It should be noted that maximum threshold of Ag exposure is determines by the level of solubility of the compound in water media. Higher solubility of the compound determines higher biological activity. Silver salts dissociate into ions. Ag nanoparticles may form colloidal solutions. Thus, maximum threshold for metallic silver is 0,1 mg/m3 and the maximum threshold for all water-soluble forms of silver such as salts and hydrophilically coated nanoparticles is 0,01 mg/m3 [8].

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It is known that Ag NPs and Ag in ionic form may accumulate in different tissues [9-11], for instance in brain [12]. Certainly, accumulation of Ag in brain is not a sufficient condition for influence on cognitive functions due to the necessity of taking into account more complicated bounds between systems and tissue and neurovisceral integration itself [13]. Nonetheless, some works regard influence of Ag NPs on cognitive and behavioral functions. They demonstrate the presence of such an influence: negative [14-16] and positive [17]. However, there are no researches investigating influence of Ag salts on cognitive and behavioral functions despite the fact that silver in the salt form may accumulate in the brain [6,7].

Also, the role of ageing in nanotoxicology is studied even weaker. Roughly, it can be supposed that the growth of age and a harmful action of the xenobiotic are directly proportional. From the one side we consider how age affects the toxic outcome of chemicals, whereas from the other side we consider how chemicals accelerate aging (i.e. how chemicals act as gerontogens) [18]. It can be proved by the facts that aged organisms are at higher risks and more subject to negative actions of toxins [19]. However, one need to see difference between old as aged organisms and elder organisms. The compliance to the harmful action of the toxin may be conditioned by the biological clocks (the oscillations of the release of different substances). The amplitude of biorhythms rises till the age about 42-45 years old for a human (Fig. 1) [20]. The amplitude of oscillations is increasing till a certain time point and then start to fade away. Also, this increase/decrease of physiological functions are conjugated with the oscillations of cognitive functions. It is described that cognitive functions rise till a certain age during youth and adolescence [21]. The biological clocks for mice and other rodents are not fully described. However, the form of these dependances might be extrapolated to other mammalian's such as mice's physiological/cognitive functions. Thus, we believe that resistance and adaptation to xenobiotics grow with the age till a breaking point since that time resistance and adaptation start to decrease.

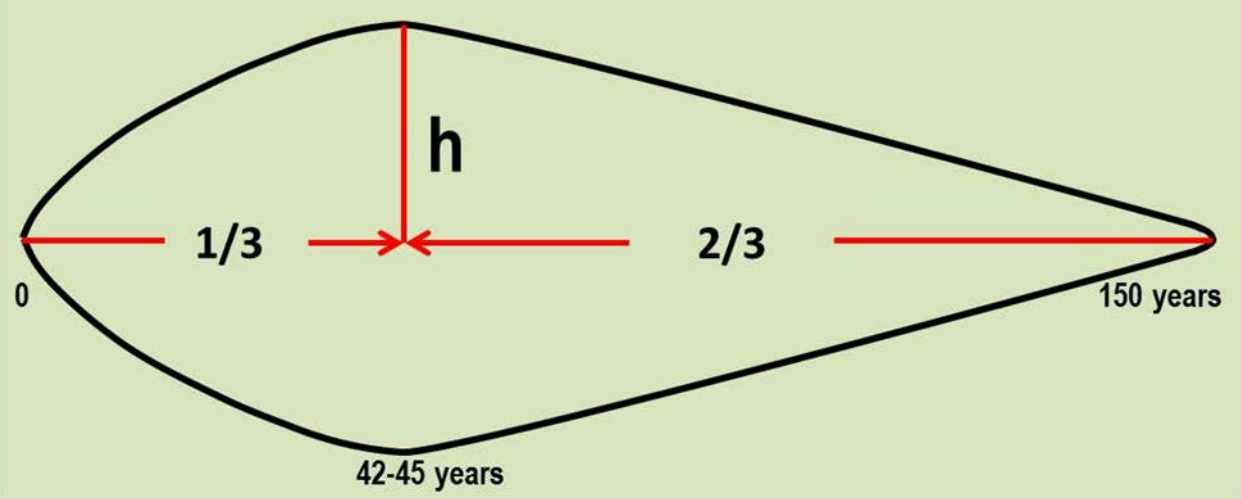

Figure 1. The normalized amplitude of oscillations of physiological functions during the whole human life.

Let us remind Paracelsus words "Solely the dose determines that a thing is not a poison". Each chemical may be regarded as a medicine and as a xenobiotic at the same time. The usage and the danger depend on the dose of that chemical compound.

Silver compounds may be considered as medicine and xenobiotics at the same time [1]. Here we regard silver compounds as xenobiotics. Namely, we choose Ag salt and Ag NPs as xenobiotics. No such works regarding relationships between Ag compounds and ageing are known at the present moment.

The other factor to get adapted for is keeping the animals in the individual cages. Normally the mice live in family groups [22]. Thus, keeping them separately is a non-natural way that can cause stress. This is an additional condition to get adapted to.

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Thus, our objective was to estimate the influence of two types of xenobiotics as AgNPs and Ag salt on cognitive and behavioral functions on mice along with their ageing and keeping separately in the individual cages. Thus, in the present study we have 3 important factors where 2 of them varied:

Introduction of a xenobiotics such as Ag NPs and Ag salt;

Stressful conditions caused by unnatural keeping in individual cages;

Influence of ageing.

It is important to explain why those conditions were included into the research. First of all, it is important to establish less harmful silver compound. The used AgNPs have been already fully described in other our works in part of their biokinetics as well [9, 23]. We choose Ag citrate (Ag+) as highly biocompatible and in the same time rather accessible Ag salt.

Mice were kept in the individual cages in order to estimate daily water consumption as well as for purity of the experiment. Mice were included into the study since the age of 2 months and 5 months. Both groups received listed Ag compounds for the same periods of administration. We conditionally call the group of mice which received Ag compounds since 2 month as "young" mice, and the second group as ''elder" mice. We cannot call them old mice because the age difference with the first group is not so large. Also, we could nott expect very drastic difference in behavior between those two groups. However, certain variabilities may occur.

Thus, in this study our goals were to assess neurotoxicity of two different xcenobiotics in mice in stressful conditions as individual content and to assume the presence of synergistic or antagonistic effects of ageing. More precisely to say not aging but growing up.

2. Materials and Methods

Colloidal solution of AgNPs namely Argovit-S (OOO Vector-Vita, Novosibirsk, Russia) was used as AgNPs. Silver citrate (Ag +) (SilverSalt, Saint-Petersburg) was used as silver biocompatible salt. We used AgNPs Argovit-S as a xenobiotic N 1 and Ag+ as a xenobiotic N 2. We have certain experience to work with Argovit-S [9,12,16,23]. The nanoparticles are stabilized with hydrophilic coating as polyvinylpyrrolidone. Ag+ was used as a biocompatible and accessible silver salt. Anions as well as cations may interact with cellular biomacromolecules and demonstrate toxic properties towards the living organisms. Our goal was to reduce negative influence of anions towards the living organisms. Therefore, we choose silver salt of a weak acid. Dynamical Light Scattering (DLS) was used to study size distribution of AgNPs.

Mice C57Bl/6 were used as a mammalian model. They were obtained from the "Stolbovaya" branch of the Federal Medical Biological Agency of Russia. Throughout the experiment, the animals were kept in the individual cages as it can be seen in Fig.2. They had unlimited access to food and water in rooms with automatically maintained temperature of 23 ± 2 ◦C and a 12/12-h day/night cycle. The room's humidity was also controlled at 45 ± 10%. A part of mice was introduced into the study since age of 2 months, the other part was introduced since age of 5 months in order to take into account the ageing factor. Each of two groups consisted of two subgroups: AgNP mice and Ag + mice. Those groups were divided into 2 other subgroups: experimental and control mice. Thus, we had 8 groups of model mammals in total (Table 1, Fig. 3). Both experiments (AgNP and Ag +) were conducted not simultaneously. AgNPs experiment was conducted 5 years before the second one. Both of them were carried out during the same seasons (November - December). Xenobiotic 1 + control 1, control 2 experiments were conducted simultaneously, xenobiotic 2 + control 3, control 4 were conducted simultaneously as well.

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Figure 2. An example of the animal content in the individual cages.

All the mice and their drinkers were weighted weekly during all the experiment to trace animal growth and to control the amount of the accepted solutions. All the experimental mice received orally daily 50 μg of silver of two kinds (Ag NPs and Ag +) from 2 months and 5 months excerpt in order to study influence of ageing factor. All the control mice received pure water ad libitum. Thus, regime of xenobiotic administration is presented in Table 1.

Table 1. The regime of xenobiotic administration.

N	Name	Regime of administration	Regime of administration	Working name
1	AgNP xenobiotic N 1	50 μg of Ag	Daily orally for 2 months since the age of 2 month	60 AgNPs
2	AgNP xenobiotic N 1	50 μg of Ag	Daily orally for 2 months since the age of 5 month	60 AgNPs elder
3	Ag+ xenobiotic N 2	50 μg of Ag	Daily orally for 2 months since the age of 2 month	60 Ag+
4	Ag+ xenobiotic N 2	50 μg of Ag	Daily orally for 2 months since the age of 5 month	60 Ag+ elder
5	Control 1	Pure water ad libitum	Daily orally for 4 months (comparison to xenobiotic N 1 group)	Control 1
6	Control 2	Pure water ad libitum	Daily orally for 7 months (comparison to xenobiotic N 1 group)	Control 2
7	Control 3	Pure water ad libitum	Daily orally for 4 months (comparison to xenobiotic N 2 group)	Control 3
8	Control 4	Pure water ad libitum	Daily orally for 7 months (comparison to xenobiotic N 2 group)	Control 4

Fig. 3 demonstrates the periods of xenobiotics/pure water administration. Xenobiotics were administered with the drinking water in the ad libitum mode for 2 months in each experiment. For 1st and 3rd groups it was administered after 2 months of maintaining in individual cages to level the extra stress out (so called, young mice).

2nd and 4th groups were administered with the xenobiotics 1 and 2 after 5 months of maintaining in the individual cages (60 elder groups, so called elder mice). Thus, it

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brought the possibility to assess the role of age in the adaptation processes to the xenobiotics. Xenobiotic administration was carried out till the end of the whole study.

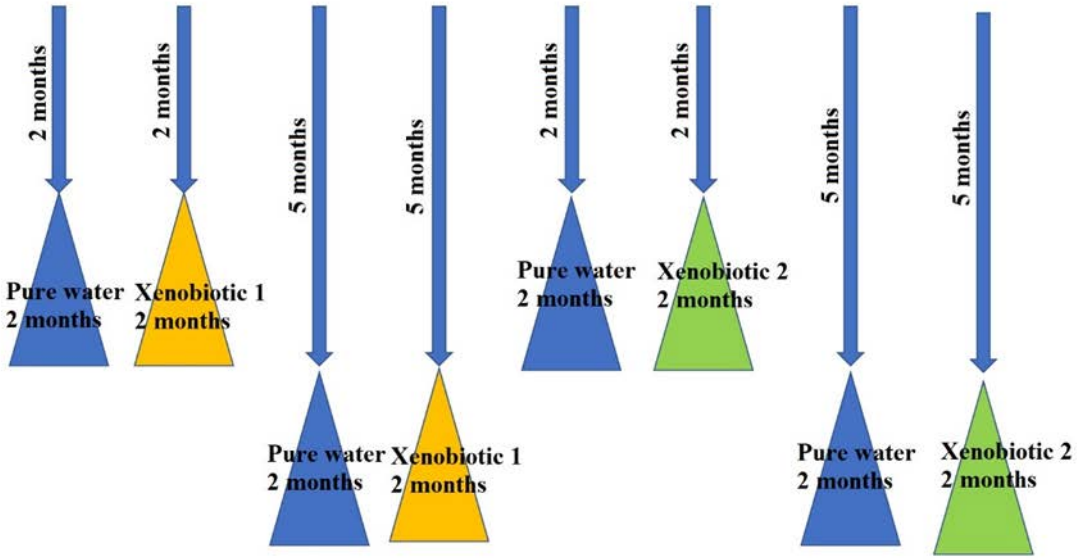

Figure 3. Scheme of water/xenobiotics administration during the experiment. Groups 5, 6, 7 and 8 did not receive any toxicants being referential to the experimental groups; groups 1 and 3 received xenobiotics for 2 months right away from maintaining for 2 months, groups 2 and 4 received xenobiotics after 5 months of maintaining. It gave the possibility to estimate influence of ageing. Names of the groups are listed in the Table 1.

At the end of each period of administration mice were tested in the Open Field Test (OF), Elevated Plus Maze (EPM), Light-Dark Box (LDB) to study their behavioral functions and in Freezing Conditioning Modelling (FC) to check their long-term memory under conditions of exposure to silver compounds [16]. Mice movement and tracks were video recorded and their parameters were automatically assessed.

Statistical analysis was performed with GraphPad Prizm 6 (La Jolla, CA, USA) by the nonparametric Mann-Whitney test. The differences were considered significant at $p < 0.05$. All data were expressed as means ± SEM.

3. Results

All the mice were gradually growing. No statistically significant difference in their body weight between experimental and control groups was detected (Fig. 1, Supplementary Materials (SM)).

Let us consider the behavior of intact mice regarding ageing. We compared control 1 vs control 2 and control 3 vs control 4 separately. Tables 2a and 2b (and Figures 2-9, SM) demonstrate differences in intact mice behavior that statically significant vary with the age. It is important to assess behavioral differences in intact mice with the age in order to understand the general trend of ageing. It should be noted that the stressful factor of the content of animals in the individual cages can not be excluded.

Table 2a. The quantitative comparison of the measured parameters in the behavioral tests of intact mice of different age: control 1 vs control 2. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Control 1	Control 2	Ref. to Figures (SM)
1	OF, total distance moved (cm)	3400 ± 440	2400 ± 200*	Fig. 2

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2	OF, distance moved in the intermediate area (cm)	1200 ± 140	790 ± 80*	Fig. 2
3	OF, Distance center/Distance total (%)	13 ± 1,9	9,3 ± 1,4*	Fig. 3
4	OF, Time spent in the Central area (s)	29 ± 5	60 ± 15*	Fig. 4
5	OF, Time spent in the Central area (%)	9,7 ± 1,8	20,0 ±5,0*	Fig. 5

*p < 0.05 in comparison with the Control 1.

Table 2b. The quantitative comparison of the measured parameters in the behavioral tests of intact mice of different age: Control 3 vs Control 4. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Control 3	Control 4	Ref. to Figures (SM)
1	OF, total distance moved (cm)	3050 ± 290	1800 ± 200**	Fig. 6
2	OF, distance moved in the intermediate area (cm)	790 ± 120	370 ± 89**	Fig. 6
3	OF, distance moved in the peripheral area (cm)	1900 ± 220	1250 ± 150*	Fig. 6
4	OF, entries into center (number)	7 ± 1	4 ± 1*	Fig. 7
5	OF, rearings (number)	26 ± 3	16 ± 3*	Fig. 8
6	EPM, distance traveled in closed arms (cm)	204 ± 56	410 ± 63*	Fig. 9

*p < 0.05, **p < 0.01 in comparison with the Control 3.

In general, as it can be seen from Tables 2a and 2b locomotor activity decreases with the age (decrease of total distance moved and distance moved in other areas), however research behavior even increases (increase of entries into center and rearing). Perhaps, thigmotaxis decreases as well that is indicated by increase of time spent in the central area of OF. Thigmotaxis is a natural behavior of mice implementing in the choosing of safest places. Such a trend in behavior may be interpreted as adaptation to the stressful factor as content in the individual cages which is unnatural for mice as a decrease of anxiety. Perhaps, initial high values of locomotor activity (Tables 2a, 2b) are due to extra excitation of mice housed in the individual cages that was neutralized with time. Increase of research behavior is also a positive factor that confirms adaptation of mice to the stressful conditions of the content.

Secondly, let us consider influence of AgNPs on young and old mice comparing experimental animals with control ones. Tables 3 and 4 as well as figures in the SM show quantitative characteristics of this comparison. Thus, Table 3 considers parameters from the tests for intact young mice (Control 1) and young mice that received AgNPs for 60 days.

Table 3. The quantitative comparison of the measured parameters in the behavioral tests between young mice: intact and 60 AgNPs mice. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Control 1	60 AgNPs	Ref. to Figures (SM)
1	OF, distance moved in the intermediate area (cm)	1200 ± 138	710 ± 135**	Fig. 10
2	OF, time spent in the intermediate area (s)	90 ± 10	52 ± 10*	Fig. 12
3	OF, time spent in the peripheral area (s)	180 ± 13	220 ± 12*	Fig. 12
4	OF, Entries into center (number)	7 ± 1	4 ± 1*	Fig. 15
5	LDB, time spent in the light chamber (s)	180 ± 32	100 ± 23*	Fig. 21

*p < 0.05, **p < 0.01 in comparison with the Control 1.

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According to the data from Fig. 10,12,15,21 SM and Table 3 it may be concluded that 60 days of AgNPs administration led to the development of anxiety in the young mice. Amplification of anxiety may be also due to the stressful content in the individual cages. Table 4 as well as Fig. 21 and 23 SM regard parameters from the same tests for intact elder mice and elder mice received AgNPs for 60 days.

Table 4. The quantitative comparison of the measured parameters in the behavioral tests between elder mice: intact and 60 AgNPs 60 elder mice. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Control 2	60 AgNPs elder	Ref. to Figures (SM)
1	LDB, time spent in the light chamber (s)	170 ± 22	92 ± 14*	Fig. 21
2	LDB, latency to entry into the dark chamber (s)	64 ± 18	19 ± 8*	Fig. 21
3	LDB, looking out (number)	8 ± 1	13 ± 1*	Fig. 23

*p < 0.05 in comparison with the Control 2.

The obtained data may evidence about the development of research behavior and some decrease of anxiety after treatment with AgNPs for 60 days. However, anxiety does not disappear completely.

In order to investigate the differences of influence of AgNPs taking place with the growing up of the animals, let us compare AgNPs and AgNPs elder mice. The data is present in the Table 5 and Figs. 10, 12, 14, 15, 17, 21 SM.

Table 5. The quantitative comparison of the measured parameters in the behavioral tests between AgNPs mice: 60 AgNPs and 60 AgNPs elder. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Ag NPs	Ag NPs elder	Ref. to Figures (SM)
1	OF, Distance moved in the central area, cm	240 ± 37	470 ± 55**	Fig. 10
2	OF, Time spent in the peripheral area, sec	220 ± 12	170 ± 18*	Fig. 12
3	OF, Time spent in the intermediate area, sec	52 ± 10	90 ± 11*	Fig. 12
4	OF, Time spent in the central area, sec	21 ± 3	43 ± 8*	Fig. 12
5	OF, Time spent in the Central area (%)	7,1 ± 1,0	14 ± 2,6*	Fig. 12
6	OF, Distance center/Distance total (%)	9,3 ± 1,4	19,0 ± 2,5*	Fig. 14
7	OF, Entries into center (number)	4 ± 1	7 ± 1**	Fig. 15
8	EPM, Total distance moved, cm	1400 ± 91	1050 ± 99*	Fig. 17
9	LDB, lookings out (number)	9 ± 1	13 ± 1*	Fig. 21

*p < 0.05, **p < 0.01 in comparison with AgNPs.

The data from the Table 5 shows increase of locomotor activity and research behavior in elder AgNPs exposed mice in comparison to young AgNPs exposed mice. The only parameter that does not fit the overall trend is total distance moved in the EPM. Younger mice moved longer distance than the older ones. It may be explained by the presence of the extra excitation in young mice that did not adapt to the individual contents.

Figure. 25a SM demonstrates long-term contextual memory of younger and elder mice exposed to Ag NPs. No statistically significant changes in the contextual memory between mice were observed .

Overall, we observed negative influence of exposure to AgNPs rather in younger mice than in elder. The negative effects of AgNPs exposure in elder mice were partially neutralized. Thus, it can evidence about higher liability of younger organisms to negative influence of xenobiotics which leaves up the hypothesis mentioned in the Introduction

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that resistance of an organism rises with the development of physiological and cognitive functions with the growing up till a certain age point. Also, the observed effects may be amplified by the effect of adaptation to the unnatural individual content with the time that we also found in the intact mice.

Let us consider Ag+ influence on mice in the same vein. The data shows the absence of the influence of Ag+ exposure to younger mice if we compare them with the intact ones (Control 3) (Figures 11, 13, 14, 16, 18, 20, 22, 24 SM). Table 6 demonstrates differences in behavior between intact mice and Ag + elder mice.

Table 6. The quantitative comparison of the measured parameters in the behavioral tests between elder mice: intact and 60 Ag+ elder. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Control 4	60 Ag+ elder	Ref. to Figures (SM)
1	OF, total distance moved (cm)	1850 ± 200	3000 ± 250**	Fig. 11
2	OF, distance moved in the central area (cm)	230 ± 50	400 ± 63*	Fig. 11
3	OF, distance moved in the intermediate area (cm)	370 ± 89	780 ± 110**	Fig. 11
4	OF, distance moved in the peripheral area (cm)	1250 ± 150	1800 ± 185*	Fig. 11
5	OF, Entries into center (number)	4 ± 1	8 ± 1*	Fig. 16

*$p < 0.05$, **$p < 0.01$ in comparison with the Control 4.

The data demonstrates increase of locomotor activity and research behavior at the same time. Distance moved in the peripheral area is also increased. It indicates that thigmotaxis is not decreased. It should be noted that we found earlier that mice get adapted to the individual content with the time of the content. This result may be regarded doubly. From the first side we can see improving of behavioral functions but from the other side it can be regarded as extra excitation and presence of pathology. In order to further determine which version from the listed above is more likely true let us consider the relationships between younger and older Ag + exposed mice. Table 7 and Fig. 13 SM demonstrates the comparison between Ag+ and Ag+ elder mice.

Table 7. The quantitative comparison of the measured parameters in the behavioral tests between Ag + mice: 60 Ag + and 60 Ag+ elder. Only parameters that are significantly different are listed in the table below, Mean ± SEM.

№	Parameter	Ag +	Ag + elder	Ref. to Figures (SM)
1	OF, Time spent in the peripheral area, sec	230 ± 10	180 ± 14*	Fig. 13
2	OF, Time spent in the central area, sec	27 ± 5	58 ± 14*	Fig. 13
3	OF, Time spent in the Central area (%)	9,1 ± 1,6	19,5 ± 4,6*	Fig. 13

*$p < 0.05$ in comparison with Ag+.

The data gives us the information, which could be related to anxiety or research behavior levels in mice of different age. It seems that exposure to Ag+ reduces anxiety with the age. This is a logical conclusion that fits to the previously obtained data about adaptation to the stressful individual content. Taking into account the data from Tables 6 and 7 as well as the fact of absence of statistically significant difference between Ag + mice vs Control 3 it should be concluded that that there are no sufficient grounds to speak about the presence of pathology during the exposure of Ag + to elder mice. Thus, we can see stimulation of behavioral functions.

No statistical difference was observed in long-term contextual memory of Ag+ exposed mice in comparison to Ag+ elder group. It evidences about the absence of influence of Ag + on cognitive functions (Fig. 25b SM).

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5. Conclusions

The aims of this experiment were to choose less toxic silver compound (AgNPs or Ag+), to assess the role of the nanoform and to estimate influence of ageing on vulnerability of mice to the toxicants. There had been 3 factors to be taken into account: content in the individual cages, ageing and administration of silver compound as xenobiotics. Elder age occurred to be a positive parameter as well as silver citrate. Negative influence on behavioral functions was traced for AgNPs injection. However, they partially improved with the age.

We suppose that the lesser negative influence of the xenobiotics on elder mice was due to the development of physiological and cognitive functions with the age. We also suppose that the elder mice did not cross the breaking point when age starts to play against. Also, individual content may be regarded as a negative factor and longer content of mice in the individual cages may neutralize toxic action of xenobiotic due to they get adapted for the second stressful factor. Thus, xenobiotic toxicity and individual content first show synergy but later adaptation of mice leads to antagonism of those negative factors. It should be noted that it is rather interesting and important to investigate development of physiological and cognitive functions with the age and their relationships with possible stressful factors. Thus, we can conclude that experienced organisms are less at risk than "blank sheet" ones.

The second important conclusion is that the nanoform is more toxic than ionic form of silver. Thus, probably it is safer for behavioral functions to use biocompatible silver salt than AgNPs. Perhaps, longer administration of a substance in a nanoform into an organism may cause more drastic neurotoxicity. In the same time it is complicated to find an explanation of this phenomena but definitely the root of it is in the different mechanisms of biological actions of nanoparticles and ionic silver. We suppose that in contrary to the popular belief mentioned above nanoform is more toxic than ionic form. Thus, acute attention in the problem of nanotoxicology, which began to be given to this area of science from the beginning of XXII, is quite justified. Also, it should be noted that salt action is regulated by anions as well. We choose more biocompatible silver salt. If we had different anion, for example, from nitric acid, the result might be different. Also, it should be noted that further research is required to clarify the issue of the Ag+ action. Can the improving of behavioral functions comparing to control mice be regarded as a positive factor or a pathology such as extra excitation?

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure 1: Changes in body weight of mice with the age; Figure 2: Distance moved in the OF: a –total, b – in the intermediate area (control 1 vs control 2); Figure 3: Distance moved in the central area/total distance in the OF (%) (control 1 vs control 2); Figure 4: Time spent in the central area of the OF (control 1 vs control 2); Figure 5: Time spent in the central area/total time in the OF (control 1 vs control 2); Figure 6: Distance moved in the OF: a –total, b – in the peripheral area, c – in the intermediate area (control 3 vs control 4); Figure 7: Number of entries into center area of the OF (control 3 vs control 4); Figure 8: Number of rearings in the OF (control 3 vs control 4); Figure 9: Distance moved in the closed arms of the EPM (control 3 vs control 4); Figure 10: Data received in the OF from Ag NPs, Control 1, Ag NPs elder, Control 2 mice: a - total distance moved, b – distance moved in the peripheral area, c - distance moved in the intermediate area, d distance moved in the central area; Figure 11: Data received in the OF from Ag +, Control 3, Ag + elder, Control 4 mice: a - total distance moved, b – distance moved in the peripheral area, c - distance moved in the intermediate area, d - distance moved in the central area; Figure 12: Data received in the OF from Ag NPs, Control 1, Ag NPs elder, Control 2 mice: a – time spent in the peripheral area (s), b – time spent in the intermediate area (s), c - time spent in the central area (s), d - time spent in the central area/total time in the OF (%); Figure 13: Data received in the OF from Ag +, Control 1, Ag + elder, Control 2 mice: a – time spent in the peripheral area (s), b – time spent in the intermediate area (s), c - time spent in the central area (s), d - time spent in the central area/total time in the OF (%); Figure 14: Central locomotion in the OF: a - Ag NPs, b - Ag +; Figure 15: Research behavior in the OF for Ag NPs mice: a number of entries into center, b – number of stands; Figure 16: Research behavior in the OF for Ag + mice: a - number of entries into center, b – number of rearings; Figure

17: Data received in the EPM from Ag NPs, Control 1, Ag NPs elder, Control 2 mice: a - total distance moved, b – distance moved in the closed arms, c - distance moved in the open arms; Figure 18: Data received in the EPM from Ag +, Control 3, Ag + elder, Control 4 mice: a - total distance moved, b – distance moved in the closed arms, c - distance moved in the open arms; Figure 19: Data received in the EPM from AgNPs mice: a - time spent in the open arms, b – overhangs; Figure 20: Data received in the EPM from Ag + mice: a - time spent in the open arms, b – overhangs; Figure 21: Data received in the LDB from Ag NPs, Control 1, Ag NPs elder, Control 2 mice: a – time spent in the light chamber, b – latency to entry into the dark chamber, c - distance moved in the light chamber; Figure 22: Data received in the LDB from Ag +, Control 3, Ag + elder, Control 4 mice: a – time spent in the light chamber, b – latency to entry into the dark chamber, c - distance moved in the light chamber; Figure 23: Data received in the LDB from AgNPs mice: a – transition (passages) between chambers, b – peeks from the dark chamber; Figure 24: Data received in the LDB from Ag + mice: a – transition (passages) between chambers, b – peeks from the dark chamber; Figure 25: Number of freezing actions as an indicator of long-term contextual memory: a – AgNPs exposed mice, b – Ag + exposed mice.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, A.A.A. and M.Yu.K.; methodology, M.Yu.K.; DLS, A.A.A.; biological experiment, M.Yu.K. formal analysis, M.Yu.K.; analysis and interpretation, A.A.; writing and editing, A.A.A; supervision, P.K.K; project administration, A.A.A.; funding acquisition P.K.K. All authors have read and agreed to the published version of the manuscript."

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