Asia Pacific Academy of Science Pte. Ltd.

Journal of Biological Regulators and Homeostatic Agents 2025, 39(4), 8244.
https://doi.org/10.54517/jbrha8244

Article

Stimulating effect on behavioral and cognitive functions of mice at silver citrate exposure

Anna A. Antsiferova[1,2,*], Pavel K. Kashkarov[1,2,3]

[1] National Research Center, Kurchatov Institute, 1, Akademika Kurchatova Sq., Moscow 123182, Russia
[2] Moscow Institute of Physics and Technology (National Research University), 9, Institutskii Lane, Moscow Region, Dolgoprudny 141700, Russia
[3] Department of Physics, Lomonosov Moscow State University, 1, GSP-1, Leninskiye Gory, 119991 Moscow, Russia
*** Corresponding author:** Anna A. Antsiferova, aiyoga@yandex.ru

CITATION

Antsiferova AA, Kashkarov PK. Stimulating effect on behavioral and cognitive functions of mice at silver citrate exposure. Journal of Biological Regulators and Homeostatic Agents. 2025; 39(4): 8244. https://doi.org/10.54517/jbrha8244

ARTICLE INFO

Received: 10 September 2025
Revised: 19 September 2025
Accepted: 22 September 2025
Available online: 10 December 2025

Abstract: Influence of long-term daily oral exposure of C57BL/6 male mice during up to 180 days to 50 μg/day/animal of silver citrate on their cognitive and behavioral functions was studied in the present work. The behavioral and cognitive functions' change can be expressed as the three-staged process, such as (1) anxiety increase, (2) susceptibility (sensitivity) increase, (3) tendency to improve long-term contextual memory at the background of decreased anxiety and locomotion increase. Thus, the influence of silver citrate on behavioral and cognitive functions of mice is complex, time-dependent alterations. The observed effect of stimulation of behavioral and cognitive functions at silver citrate exposure at the final stage can be applied in Medicine for treatment of neurodegenerative diseases as well symptoms of mental illness. Silver citrate may potentially replace silver nanoparticles, which are well-known from scientific literature to be toxic.

Keywords: behavioral functions; cognitive functions; mammals; silver citrate, silver chelate complex; stimulation

1. Introduction

Silver in different forms has been applied for medical purposes due to its antiseptic, anti-inflammatory, and regenerative properties [1,2]. Nowadays, silver is used in Medicine mostly as silver nanoparticles. There are the silver-based preparations such as Protargol, which is applied for infectious diseases' treatment [3]; Argosulfan ointment, which is used for wound and burn treatment [4]; dressings with silver nanoparticles' coating are applied in purulent surgery [5]; silver plating is used in pacemakers [6]; and implants [7] to improve their biocompatibility. Silver nanoparticles are applied in dentistry [8] as well, in packing materials to extend the shelf life of food [9], in cosmetology for acne treatment [10], in textiles such as protective masks and respirators [11], and actively used in agriculture for treatment of seeds and crops from pathogens [12].

At the same time, many relevant scientific papers and presentations at scientific conferences during 2010–2020s discuss the problem of silver nanoparticles' toxicity. Silver nanoparticles demonstrate their toxicity in *in vitro* [13–15] and *in vivo* [16–18] experiments, change behavioral and cognitive functions of laboratory animals [19,20], negatively impact fertility [21], show nephrotoxicity [22], and hepatotoxicity [23]. It was shown previously that silver nanoparticles are able to penetrate the blood-brain barrier and interact directly with the nervous tissue of the brain [24,25].

The problems of silver nanoparticles' safety and security, as well as approaches of their risk assessment, are actively discussed [26].

In general, silver nanoparticles are known as hazardous for human health and the environment. For that reason, the search for less toxic and more biocompatible silver compounds is relevant.

Colloidal silver (silver nanoparticles) and silver chelate complexes are permitted for use at production of food supplements for adults according to uniform sanitary and epidemiological and hygienic requirements for products subject to sanitary and epidemiological supervision (control) accepted by Eurasian Economic Union [27]. All chelate complex compounds are characterized by the fact that one of the terminal atoms of the polydentate ligand is bound to the central atom or complex-forming ion through unpaired electrons of a normal covalent bond, and the other is bound through a donor–acceptor bond. Metal chelate complexes are known to be highly bioavailable. Which form of silver is more biocompatible—whether it's silver chelate complexes or silver nanoparticles? Biocompatibility of silver chelate complexes is less studied than silver nanoparticles' toxicity. Therefore, the answer for the question is not clear today. Due to this, scientific studies on the problem of the toxicity of silver chelate complexes are rather relevant and demanded.

The purpose of the present work was to study influence of long-term oral exposure of laboratory mice to silver citrate, as an example of such a silver chelate complex, on their behavioral and cognitive functions.

2. Materials and methods

2.1. Materials

Silver citrate (SilverSalt, Saint Petersburg, Russia) was used as a silver chelate complex for exposure. Silver citrate was chosen due to its high commercial availability. It was a transparent water solution. The chemical formulae of silver citrate is $Ag_3C_6H_5O_7$. Silver citrate was performed as a transparent water solution with initial content of silver of 2400 ppm.

The exposure solutions were obtained by dissolution of silver citrate by purified tap water (OOO Pharmsystemy, Besedy village, Moscow region, Russia) to the required concentration.

Influence of silver citrate on behavioral and cognitive functions of laboratory animals is a novel topic and have been studied before just by our scientific group [28,29], unlike silver acetate's and silver nitrate's influence.

2.2. Animals

Male mice C57BL/6 ("Stolbovaya" branch of the Federal Medical Biological Agency of Russia, Moscow, Russia) were applied as a mammalian model. The animals were received from the supplier at the age of 2 months, with the average weight of 20–22 g. After the delivery to the National Research Center Kurchatov Institute, the mice were allowed to adapt to the new environment for 2 weeks. The animals were kept in individual cages in a room with an automatically maintained temperature of 23 ± 2°C and a 12/12-h day/night cycle during the whole experiment. The animals had unlimited access to food and water. All the manipulations with the animals were

implemented according to Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, as well as the requirements of the Local Ethical Committee on Biomedical Research of the National Research Center Kurchatov Institute approved this research (Protocol No. 2, 07 October 2021).

2.3. Methods

Behavioral tests such as Open Field (OF), Elevated Plus Maze (EPM), Light-Dark Box (LDB) were used to assess behavioral functions of mice. Namely, locomotion, anxiety, and exploration behavior were assessed in the tests. Susceptibility (sensitivity) was used as an additional behavior parameter. Contextual fear conditioning task (CFCT) was used for long-term contextual memory evaluation.

2.4. Experimental scheme

Body weight of mice was measured weekly using electronic scales Adventurer Pro (Ohaus Corporation, Pine Brook, NJ, USA).

Experimental mice received silver citrate in composition of drinking water with unlimited access to it. Thus, 3 mL of silver citrate solution were used each day to deliver 50 μg of silver/animal/day during 30, 60, 120, or 180 days. Control animals received purified water ad libitum. Thus, four groups of experimental mice (n = 12 in each group: "Ag+ 30", "Ag+ 60", "Ag+ 120"; n = 14 in "Ag+ 180") and 4 groups of control mice (n = 12 in each group: "Control+ 30", "Control+ 60", "Control+ 120"; n = 14 in "Control + 180") took part in the study. The dosages were chosen according to our previous experience on long-term exposure of mice to silver nanoparticles [19], where we used similar doses and similar periods of exposure. The dose is based on silver ion content according to the manufacturer's data.

Assessment of behavioral and cognitive functions of mice was carried out after the cessation of the periods of silver citrate exposure since 31st, 61st, 121st, and 181st days. Testing was implemented in the following order such as OF, EPM, and LDB, by one test per day. Silver citrate administration was maintained during testing in order to exclude influence of silver elimination processes. Administration of purified water to control mice was maintained during testing as well. Home cages were used for the transportation of animals from the animal holding area to the testing area and back. The animals were returned to their home cages after each testing. The parameters and conditions of the tests are described below.

2.4.1. Open field

A round arena with a diameter of 1200 mm, surrounded by 450 mm high walls, was used as OF. The arena floor and walls were made of gray polyvinylchloride. The illumination level of the arena was 115 lux. The walls and the floor were wiped with a 70% ethanol solution (ZAO Bryntsalov-A, Russian Federation) before placing of each subsequent animal in the arena.

Each animal was placed into the center of OF and was allowed to explore the OF freely during 300 s. Video recording of the animal's behavior during testing was performed using a WV-CP500G color analog video camera (Panasonic, Japan) installed above the center of the arena at a height of 2.5 m, and EthoVision XT 8.5 behavioral video recording system (Noldus Information Technology, the Netherlands).

The video recordings of mice behavior were analyzed using the EthoVision XT 8.5 program.

2.4.2. Elevated plus maze

A maze with 4 arms (*L* × *W*: 620 × 50 mm^2) intersecting at an angle of 90°, and a central platform at the intersection of the arms (L × W: 50 × 50 mm^2) was used as EPM. The base of the EPM is made of gray plastic and is raised 700 mm above the floor. Two closed arms of the maze are surrounded on three sides by transparent plexiglass 160 mm high walls. A 5 mm high transparent plexiglass side is installed along the edges of the open arms. The illumination of the closed arms was 60 lux, the illumination of the open arms was 77 lux, and the illumination of the central platform was 70 lux. The maze was wiped with a 70% ethanol solution (ZAO Bryntsalov-A, Russian Federation) before placing each subsequent animal in the EPM.

For testing, each animal was placed onto the central platform facing either open arm, and allowed to freely explore the maze for 300 s. Video recording of animal's behavior was carried out during testing with the use of analog video camera WV-CP500G (Panasonic, Japan) installed above the central platform of EPM at a height of 2.5 m, and the EthoVision XT 8.5 behavioral video recording system (Noldus Information Technology, the Netherlands).

2.4.3. Light-dark box

The test is used as a common model of anxiety behavior of rodents. The LDB consists of safe dark part and brightly illuminated open part, which forms a zone of disgust. The model creates a conflict situation for the mouse, which is inclined to explore the unfamiliar area, but initially wants to avoid the unknown circumstances, characterizing by neophobia. The interval of time spent in the dark compartment correlates with the level of anxiety, while the number of exits and the time spent exploring the illuminated compartment are indicators of risk-taking and exploratory activity.

The setup consists of two parts such as dark and light chamber (50 × 25 × 40 cm^3), which are connected by an opening (8 × 6 cm^2). The walls and floor of the chambers were wiped with a 70% ethanol solution (ZAO Bryntsalov-A, Russian Federation) before placing each subsequent animal in the LDB.

For testing, each animal was placed in the center of a light chamber and allowed to freely explore the setup for 600 s. Video recording of the animal's behavior in the light section of the setup during testing was performed using a WV-CP500G color analog video camera (Panasonic, Japan) installed above the center of the light chamber at a height of 2.5 m and an EthoVision XT 8.5 behavioral video recording system (Noldus Information Technology, the Netherlands). The resulting behavioral video recordings of animal's behavior were analyzed using the EthoVision XT 8.5 program.

2.4.4. Contextual fear conditioning task

The animals were trained and then tested in CFCT to assess silver citrate influence on forming and retention of long-term contextual memory using video fear conditioning system (MED Associates Inc., USA) and Video Freeze software (MED Associates Inc., USA).

Video recording of the mice's behavior was performed and the number and duration of freezing acts were automatically determined during learning and during testing. Freezing was defined as a specific form of mouse behavior characterized by a complete absence of any movements other than breathing.

Mice of each group were divided into two subgroups such as active control (AC) (n = 6 or 7) and fear conditioning (FC) (n = 6 or 7). Mice of the AC subgroups were placed in the experimental chamber for 6 min to explore the environment, without electrical stimulation. Mice of the FC subgroups were placed in the experimental chamber for 6 min: 3 min to explore the environment, 3 electric stimulations with current strength of 1 mA, duration of 2 s and an interval of 1 min to memorize the environment. The quality of learning of the mice was analyzed by assessing and comparing the percentage of freezing between the AC and FC subgroups of each group in 1 min after training. Intervals of interest were set in the Video Freeze program (0–178 and 300–360 s of the experiment) and the time (percentage) of freezing was automatically calculated for these intervals as the ratio of the total duration of freezing acts in the interval to the duration of the interval for this purpose.

The animals were tested for the determination of the level of long-term contextual memory, or, in other words, skill retention in 24 h after training. For this, each animal was placed into the same experimental chamber for 3 min. An interval of interest (0–180 s of the experiment) was set in the Video Freeze program, and the time (percentage) of freezing was automatically calculated for this interval as the ratio of the total duration of freezing acts in the interval to the total duration of the interval.

2.4.5. Parameters of assessment of mice behavioral and cognitive functions

The values of parameters characterizing behavioral, cognitive functions between experimental and control animals were compared within each period of exposure such as "Ag+ 30" and "Control+ 30", "Ag+ 60" and "Control+ 60", "Ag+ 120" and "Control+ 120", "Ag+ 180" and "Control+ 180".

These parameters are:

1) OF: total distance moved; distance moved in peripheral, intermediate and central areas; number of entrances into center; number of rearings;
2) EPM: total distance moved; distance moved in open arms, closed arms, center; time spent in open arms, closed arms; number of head dips;
3) LDB: number of lookings out from the dark chamber; number of transitions between chambers; distance moved in the light chamber; latency to exit from the light chamber.
4) We suggest to introduce additional parameter to assess behavioral functions of mice, such as freezing (percentage) time in 1 min after learning in CFCT. Apparently, it characterizes the sensitivity (susceptibility) of mice. Also, it shows the quality of learning. In our opinion, it is possible to define the sensitivity (susceptibility) of mice as the time (percentage) of freezing in 1 min after learning, due to increased freezing reflects enhanced responsiveness to the electric shock stressor.
5) Long-term contextual memory was assessed by the time of freezing (%) in 24 h after the learning in the CFCT.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prizm 6.01 software (La Jolla, San Diego, USA). Nonparametric Kruskal-Wallis analysis of variance with Dunn's post hoc test for multiple comparisons or Mann–Whitney *U* test were used. Differences were considered to be significant at $p < 0.05$.

3. Results

The **Figures 1–5** show statistically significant ($p < 0.05$) differences as well as a tendency ($0.05 < p < 0.07$) in the measured values. Reliable differences were observed only in EPM and CFCT as follows:

EPM: A decrease of time spent in the open arms by "Ag+ 30" mice compared to "Control+ 30" was observed after 30 days of the experiment (**Figure 1**). It shows the enhanced level of anxiety in "Ag+ 30" mice.

Increase of time spent in open arms of EPM was observed in "Ag+ 120" compared to "Control+ 120" (**Figure 1**). It shows anxiety decrease in "Ag+ 120" mice. Decrease of distance moved in closed arms of EPM was also observed in "Ag+ 120" compared to "Control+ 120" (**Figure 2**). At the background of unchanged total distance moved it shows anxiety decrease in "Ag+ 120" as well.

Increases of total distance moved and distance moved in the center of EPM were observed in "Ag+ 180" group compared to "Control+ 180" (**Figure 3**). It indicates locomotion increase in the "Ag+ 180" group.

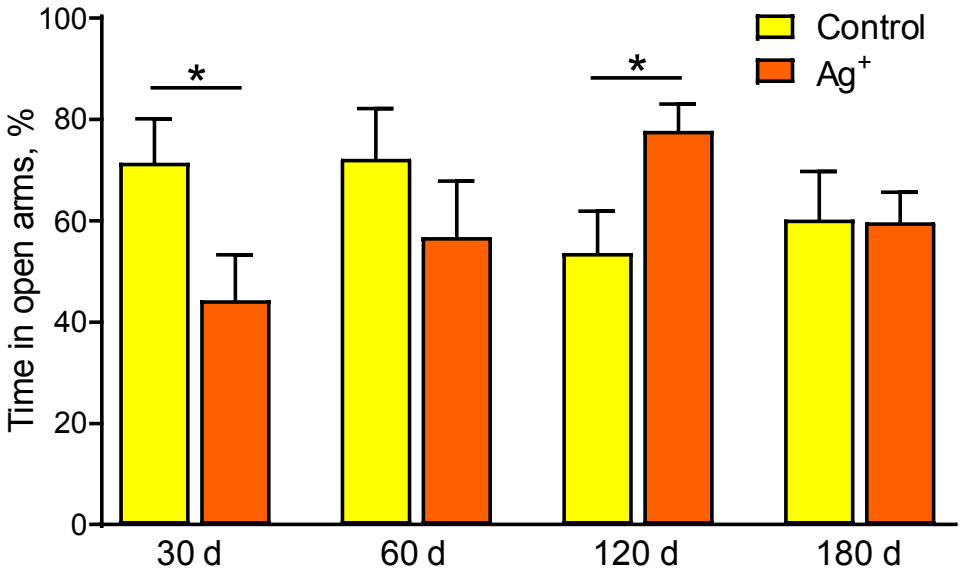

Figure 1. Time spent in the open arms of EPM. Values are presented as mean ± SEM. *$p < 0.05$. Decrease of time spent in open arms was observed in "Ag+ 30" compared to "Control+ 30" and increase of the time was observed in "Ag+ 120" compared to "Control+ 120." It shows reverse in the character of mice behavior at prolongation of silver citrate exposure periods. Increased anxiety is replaced by decreased anxiety.

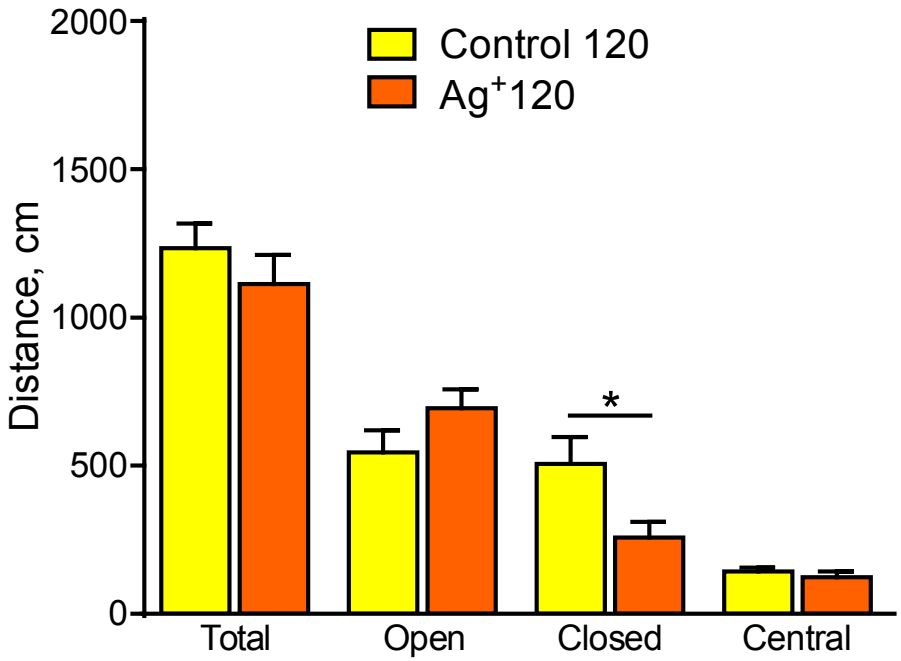

Figure 2. Distance moved in EPM by "Ag+ 120", "Control+ 120" such as total, in open arms, in closed arms, in center. Values are presented as mean ± SEM. *$p < 0.05$. Decrease of distance moved in closed arms was observed in "Ag+ 120" compared to "Control+ 120". It shows anxiety decrease in the mice exposed to silver citrate during 120 days.

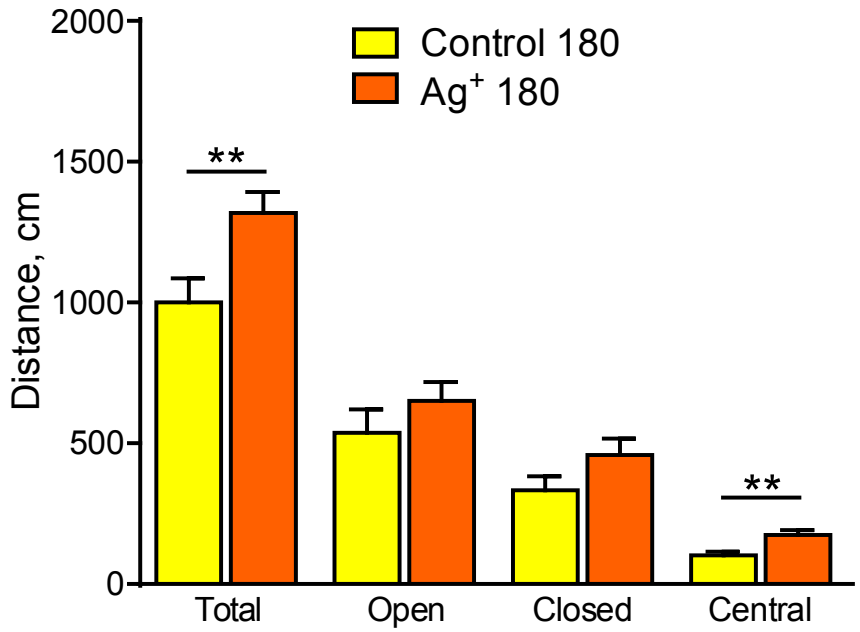

Figure 3. Distance moved in EPM by "Ag+ 180", "Control+ 180" such as total, in open arms, in closed arms, in center. Values are presented as mean ± SEM. *$p < 0.05$. Increase of total distance moved and distance moved in center of EPM was observed in "Ag+ 180". It shows locomotion increase in the experimental animals.

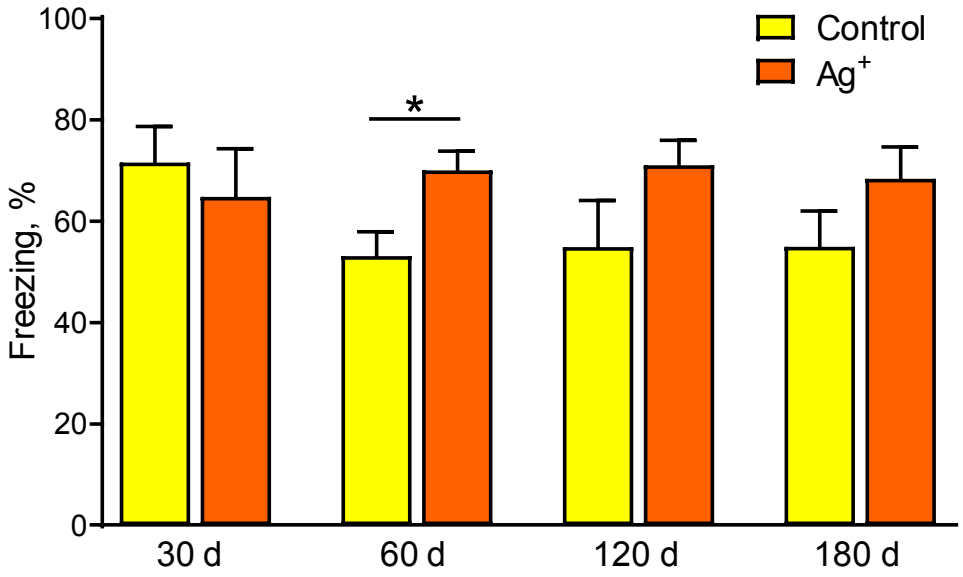

Figure 4. Freezing time in 1 min after learning in CFCT. Values are presented as mean ± SEM. *$p < 0.05$. An increase of freezing time was observed in "Ag+ 60" compared to "Control+ 60". The similar character of the ratios between experimental and control mice, however without significant differences, was observed for all the following by time groups as well. It may show increase of sensitivity (susceptibility) in the experimental mice.

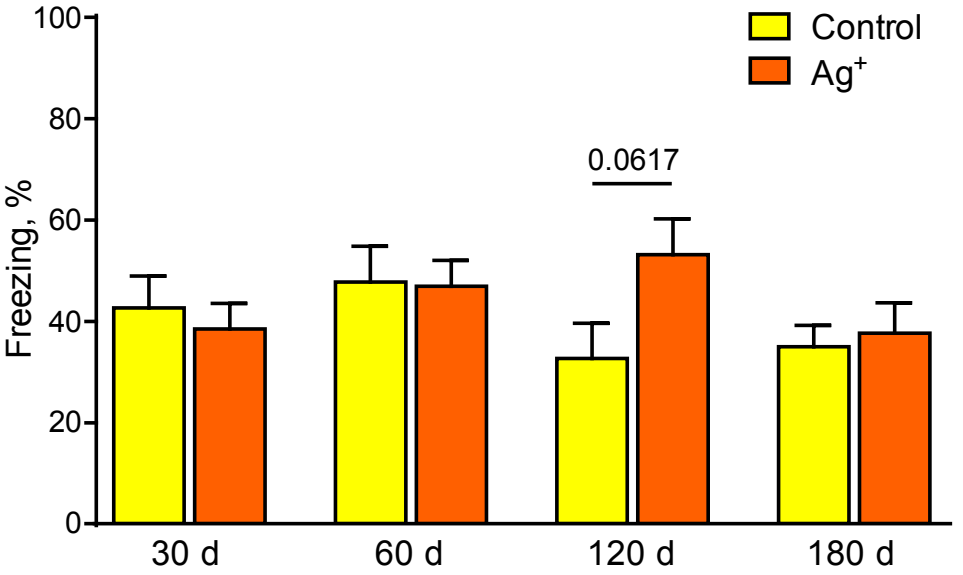

Figure 5. Freezing time in 24 h after learning in CFCT. Values are presented as mean ± SEM. *$p < 0.05$. Tendency to increase freezing time in 24 h after learning was observed in "Ag+ 120" compared to "Control+ 120". It shows tendency to improve long-term contextual memory in "Ag+ 120" mice.

CFCT: An increase of freezing time in "Ag+ 60" group compared to "Control+ 60" group was observed in 1 min after learning (**Figure 4**). The similar, however without significant differences, character of the ratios between experimental and control mice was observed in all the following by period of exposure groups as well. It may show increase of sensitivity (susceptibility) to the stressor in the form of negative experience of electric shocks in the mice exposed to silver citrate in comparison to the controls. Also, it shows that "Ag+ 60" mice learned better that "Control+ 60" mice. The trend remained for all the following by the period of exposure groups.

Tendency to increase of freezing time in "Ag+ 120" group compared to "Control+ 120" group ($p = 0.0617$) was observed in 24 h after learning in the CFCT (**Figure 5**). It demonstrates tendency to improve long-term contextual memory in "Ag+ 120" mice. Significant differences by the parameter were not observed for all the periods of exposure to silver citrate.

4. Discussion

Changes of behavioral and cognitive functions at silver citrate exposure can be expressed as a 3-staged process such as (1) anxiety increase, (2) susceptibility (sensitivity) increase, (3) tendency to improve long-term contextual memory at the background of decreased anxiety and locomotion increase. Anxiety increase at the early stage is rather expectable and associated with response of the organism to stress induced by the exogenous substance such as silver citrate exposure. The tendency to improve long-term memory and locomotion increase are likely compensation to the anxiety increase observed at the early stage.

Susceptibility (sensitivity) increase may be regarded as the marker of successful compensation of anxiety, stimulating effect appearance.

We observed the stimulating effect of behavioral and cognitive functions of mice at their long-term exposure to silver citrate in the present experiment. In our previous works on 60-days' exposure of mice to silver citrate we revealed absence of the influence in younger mice [28,29] as well as increase of exploration behavior and decrease of anxiety in elder mice [29]. The result in elder mice shows stimulating effect of silver citrate as well. Herewith, equivalent dose and exposure period of silver nanoparticles increased anxiety in younger and in elder mice, while the level of anxiety was higher in the younger mice compared to the older [30]. We have not found other works on silver citrate toxicity investigation in the scientific literature. Let's consider the known studies on the toxicity of other silver salts such as silver acetate, which is a chelate complex as well, and silver nitrate, which is not a chelate complex.

There is a work on silver acetate and silver nanoparticles' influence on behavioral and cognitive functions of laboratory rats at daily intranasal exposure to them for 20 days [31]. Anxiety increase, locomotion, and spatial memory disturbance were detected at exposure both to silver acetate and the nanoparticles. Herewith, silver acetate had greater toxic impact compared to the nanoparticles. A pronounced acidity of silver acetate and possible irritation of nose receptors that could strengthen the negative effect should be taken into account in this case. A relatively short period of exposure such as 20 days should be taken into account as well. We observed impairment of behavioral functions after 30 days of exposure to silver citrate as well such

as anxiety increase. Silver acetate influence on reproductive capacity, the offspring and the parents as well, which was introduced into male and female mice in different doses, was studied in Sprando et al. [32]. A decrease of the consumed liquid and food by parents as well as their increase of locomotion were observed. Decrease of fertility, number of pups, and number of alive pups as well as pup's body weight was observed as well. The most pronounced changes compared to controls by the major number of parameters were found at the exposure to maximal dose of silver acetate such as 40 mg/kg bw. The authors believe that the effects are due to the binding of selenium by silver. Selenium is essential and vital element for mammals, which takes a key part in the processes, which are important for reproductive health, while it's lack can cause different pathologies. Thus, granules of silver with selenium inclusions were observed in tissues after exposure of rats to silver acetate [33].

Toxicokinetics of silver powder, silver nanoparticles, silver acetate, and silver nitrate after 28-day exposure of rats to them were studied in Mertens et al. [34]. The highest bioavailability was found in the salts and the lowest related to the powder. The bioavailability of silver nanoparticles was in the middle.

Negative influence on long-term memory at 28-day exposure of rats to silver nitrate [35], as well as behavioral functions of fishes after 48- [36] and 72-h [37] exposure were noted. Silver nitrate led to the fish lethality [36]. Silver acetate and silver nitrate were more cytotoxic and genotoxic compared to silver nanoparticles in in vitro experiment [38]. IC_{50} for human hepatoblastoma inhibition by silver nitrate was 2-fold lower that IC_{50} for silver nanoparticles [39]. It demonstrates higher toxicity of silver nitrate compared to silver nanoparticles.

Toxicity of the salt is determined by the properties of both ions—cation and anion [40]. Toxicity of silver chelate complexes strongly depends on each anion's toxicity itself. Silver salts demonstrated higher bioavailability as well as higher toxicity in comparison to silver nanoparticles as reviewed above. It should be taken into attention that solely short-term effects at silver compounds' exposure for no longer than 28 days were assessed in the previous works reviewed above. In the present study, we esteemed effects of subchronic and chronic exposures to silver citrate from 30 to 180 days. Short-term effects are neutralized and long-term ones start to play significant role then. The long-term effects of silver nanoparticles onto living organisms are not sufficiently studied yet. However, we observed impairment of long-term contextual memory of mice after 180 days of daily exposure to silver nanoparticles [19].

The stimulating effect of silver citrate found in the present work can potentially be applied in Medicine, taking into account the existing permission for the use of silver chelate complexes in the production of food supplements for adults. Such a stimulating regenerative and wound healing effect of silver is known and successfully used in surgery today [41]. There is a patent that demonstrates the effective wound healing and regenerative properties of copper-silver citrate composite [42].

Anti-neurodegenerative properties of silver nanoparticles such as possibility to treat Alzheimer's and Parkinson's diseases as well as multiple sclerosis are actively studied today [43–45]. Along this, the stimulating effect of silver citrate on behavioral and cognitive functions found in this work can be potentially used to treat such neurodegenerative diseases. Also, it is reasoned to consider the prospects of its use in

the treatment of symptoms of mental illnesses such as depression, apathy, and negative symptoms in schizophrenia. It is especially relevant according to the known difficulties and sometimes inefficiency in treatment of neurodegenerative and mental diseases by standard therapy. Herewith, the possible side effects should be minimized.

Influence of silver citrate and silver itself is definitely manifested via stress at the general organism level [46]. Thus, the concept of the mechanism underlying the proposed silver citrate therapeutic action is in the words of well-known Canadian M.D. Hans Selye such as "Stress fallen on one system helps the other system to rest" [47]. The possible related mechanistic pathways of manifestation of stress in the observed stimulation such as binding of silver to selenium and the role of gut-brain axis should not be excluded. Identification and clarification of such mechanistic pathways may be the directions for future research. Despite the overall picture of silver citrate influence on behavioral functions of mice is rather complex, time-dependent alterations than a unequivocally beneficial stimulation, anxiety increase at the initial stage of the process is not surprising and is expectable within Selye's concept of General Adaptation Syndrome to any stressor and can be regarded as Nervous system's response to silver citrate.

The possible terminological confusion should be pointed separately. Silver citrate and citrate-coated silver nanoparticles are two completely different objects, such as salt and nanoparticles. We encountered such a confusion during the review of our previous manuscript in one European journal. The reviewers apparently using AI and having inattentively delved into our work stated the identity of these two objects. However, we should emphasize that these are principally different objects. Such a terminological confusion may bring to a wrong conclusion about the existing of sufficient knowledge on silver citrate toxic properties. In reality, however, there are enough data on citrate coated silver nanoparticles' toxicity and just a few works on toxicity of silver citrate itself [28,29].

5. Conclusion

We observed a stimulating effect at long-term exposure to silver citrate, such as tendency to improve long-term contextual memory and locomotion increase, which can be explained as compensation for increased anxiety at early stage and adaptation of the organisms of mice to the exogenous substance. Considering that silver chelate compounds are permitted for the production of food supplements for adults, such a stimulating effect may potentially be applied for a therapy of neurodegenerative diseases as well as symptoms of mental illness. Silver citrate may potentially replace the known to be toxic silver nanoparticles at the market.

Author contributions: Conceptualization, AA and PK; investigation, AA; writing—original draft preparation, AA; writing—review and editing, PK; supervision, PK; project administration, AA. All authors have read and agreed to the published version of the manuscript.

Funding: This work was carried out within the state assignment of the National Research Center 'Kurchatov Institute'.

Acknowledgments: The authors are grateful to Marina Kopaeva for fruitful discussion.

Ethical approval: The study was conducted according to GOST 33215-2014 "Guidelines for the Maintenance and Care of Laboratory Animals. Rules for Equipping Premises and Organizing Procedures" and approved by the Local Ethical Committee on Biomedical Research of the National Research Center 'Kurchatov Institute' (Protocol No. 2, 07 October 2021).

Conflict of interest: The authors declare no conflict of interest.

References

1. Alexander JW. History of the medical use of silver. Surgical Infections (Larchmt). 2009; 10(3): 289–292. doi: 10.1089/sur.2008.9941
2. Shcherbakov AB, Korchak GI, Surmasheva YeV, et al. Silver preparations: Yesterday, today, tomorrow (Russian). Pharmaceutical Journal. 2006; 5: 45–57.
3. Flexner S, Amoss HL. Chemical versus serum treatment of epidemic meningitis. Journal of Experimental Medicine. 1916; 23(5): 683–701. doi: 10.1084/jem.23.5.683
4. Samtsov AV, Telichko IN, Statsenko AV, Khairutdinov VR. Application of external drugs comprising silver compounds for the treatment of pyodermic patients. Vestnik Dermatologii i Venerologii. 2014; 90(1): 75–80. doi: 10.25208/0042-4609-2014-90-1-75-80
5. Rybka M, Mazurek Ł, Konop M. Beneficial effect of wound dressings containing silver and silver nanoparticles in wound healing-from experimental studies to clinical practice. Life (Basel). 2022; 13(1): 69. doi: 10.3390/life13010069
6. Shawcross J, Bakhai A, Ansaripour A, et al. In vivo biocompatibility and pacing function study of silver ion-based antimicrobial surface technology applied to cardiac pacemakers. Open-Heart Surgery. 2017; 4(1): e000357. doi: 10.1136/openhrt-2015-000357
7. Tagandurdyeva N, Yudin VE. Bioresorbable composites for bone reconstruction. Nanotechnologies in Russia. 2020; 15(7–8): 400–414. doi: 10.1134/S1995078020040151
8. Fernandez CC, Sokolonski AR, Fonseca MS, et al. Applications of silver nanoparticles in dentistry: Advances and technological innovation. International Journal of Molecular Sciences. 2021; 22(5): 2485. doi: 10.3390/ijms22052485
9. Rao MMV, Mohammad N, Banerjee Sh, et al. Synthesis and food packaging application of silver nano-particles: A review. Hybrid Advances. 2024; 6: 100230. doi: 10.1016/j.hybadv.2024.100230
10. Kapuścińska A, Nowak I. Silver nanoparticles as a challenge for modern cosmetology and pharmacology. In: Grumezescu AM (editor). Nanobiomaterials in Galenic Formulations and Cosmetics. William Andrew Publishing; 2016. pp. 395–417. doi: 10.1016/B978-0-323-42868-2.00015-2
11. Jadhav SA, Patil AH, Thoravat SS, et al. A brief overview of antimicrobial nanotextiles prepared by in situ synthesis and deposition of silver nanoparticles on cotton. Nanobiotechnology Reports. 2021; 16 (4): 543–550. doi: 10.1134/S2635167621040170
12. Khan S, Zahoor M, Khan RSh, et al. The impact of silver nanoparticles on the growth of plants: The agriculture applications. Heliyon. 2023; 9(6): e16928. doi: 10.1016/j.heliyon.2023.e16928
13. De Matteis V, Cascione M, Toma CC, et al. Silver nanoparticles: Synthetic routes, in vitro toxicity and theranostic applications for cancer disease. Nanomaterials. 2018; 8(5): 319. doi: 10.3390/nano8050319
14. Akter M, Sikder MT, Rahman MM, et al. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. Journal of Advanced Research. 2017; 9: 1–16. doi: 10.1016/j.jare.2017.10.008
15. Noga M, Milan J, Frydrych A, et al. Toxicological aspects, safety assessment, and green toxicology of silver nanoparticles (AGNPS)—Critical review: State of the art. International Journal of Molecular Sciences. 2023; 24(6): 5133. doi: 10.3390/ijms24065133
16. Kakakhel MA, Wu F, Sajjad W, et al. Long-term exposure to high-concentration silver nanoparticles induced toxicity, fatality, bioaccumulation, and histological alteration in fish (Cyprinus carpio). Environmental Sciences Europe. 2021; 33: 14. doi: 10.1186/s12302-021-00453-7
17. Ryan J, Jacob P, Lee A, et al. Biodistribution and toxicity of antimicrobial ionic silver (Ag^{+}) and silver nanoparticle ($AgNP^{+}$) species after oral exposure, in Sprague-Dawley rats, Food and Chemical Toxicology. 2022; 166: 113228. doi: 10.1016/j.fct.2022.113228

18. Zhang J, Liu S, Han J, et al. On the developmental toxicity of silver nanoparticles. Materials & Design. 2021; 203: 109611. doi: 10.1016/j.matdes.2021.109611
19. Antsiferova A, Kopaeva M, Kashkarov P. Effects of prolonged silver nanoparticle exposure on the contextual cognition and behavior of mammals. Materials. 2018; 11(4): 558. doi: 10.3390/ma11040558
20. Węsierska M, Dziendzikowska K, Gromadzka-Ostrowska J, et al. Silver ions are responsible for memory impairment induced by oral administration of silver nanoparticles. Toxicology Letters. 2018; 290(133). doi: 10.1016/j.toxlet.2018.03.019
21. Maciejewski R, Radzikowska-Büchner E, Flieger W, et al. An overview of essential microelements and common metallic nanoparticles and their effects on male fertility. International Journal of Environmental Research and Public Health. 2022; 19: 11066. doi: 10.3390/ijerph191711066
22. Nosrati H, Hamzepoor M, Sohrabi M, et al. The potential renal toxicity of silver nanoparticles after repeated oral exposure and its underlying mechanisms. BMC Nephrology. 2021; 22(1): 228. doi: 10.1186/s12882-021-02428-5
23. Belji Kangarlou M, Khavanin A, Nadri F, et al. Noise and silver nanoparticles induce hepatotoxicity via CYP450/ NF-Kappa B 2 and p53 signaling pathways in a rat model. Toxicology and Industrial Health. 2024; 40(4): 206–219. doi: 10.1177/07482337241233317
24. Buzulukov YuP, Arianova E, Demin VF, et al. Bioaccumulation of silver and gold nanoparticles in organs and tissues of rats studied by Neutron Activation Analysis. Biological Bulletin. 2014; 41(3): 255–263. doi: 10.1134/S1062359014030042
25. Antsiferova AA, Buzulukov YP, Demin VA, et al. Radiotracer methods and neutron activation analysis for the investigation of nanoparticlebiokinetics in living organisms. Nanotechnologies in Russia. 2015; 10(1–2): 100–108. doi: 10.1134/S1995078015010024
26. Shipelin VA, Gmoshinski IV, Khotimchenko SA. Risk assessment of silver nanoparticles. IOP Conference Series: Materials Science and Engineering. 2015; 98: 012010. doi: 10.1088/1757-899X/98/1/012010
27. Uniform sanitary and epidemiological and hygienic requirements for products subject to sanitary and epidemiological supervision (control) accepted by Eurasian Economic Union. Available online: https://eec.eaeunion.org/comission/department/depsanmer/regulation/P2_299.php (accessed on 2 August 2025).
28. Antsiferova AA, Kopaeva MY. Comparison of silver nanoparticle's and silver citrate neurotoxicity on the organisms of laboratory mammals (Russian). 2023; Medline.ru. 24: 819–828.
29. Antsiferova AA, Kopaeva MYu, Kashkarov PK. Relationships between effects of Ag nanoparticles and Ag salt on behavioral and cognitive functions of mice and ageing. Nanobiotechnology Reports. 2022; 17(6): 857–865. doi: 10.1134/ S2635167622340018
30. Antsiferova AA, Kopaeva MYu, Kochkin VN. Neurotoxicity of silver nanoparticles and non-linear development of adaptive homeostasis with age. Micromachines. 2023; 14: 984. doi: 10.3390/mi14050984
31. Farimaneh J, Tabandeh MR, Fatemi Tabatabaei SR. Nasal administration of silver nanoparticles and silver ions reduced cognitive performance in adult rats. Universal Journal of Pharmaceutical Research. 2023; 8(2): 71–76. doi: 10.22270/ujpr.v8i2.929
32. Sprando RL, Black T, Keltner Z, et al. Silver acetate exposure: Effects on reproduction and post natal development. Food and Chemical Toxicology. 2017; 106(Pt A): 547–557. doi: 10.1016/j.fct.2016.06.022
33. Loeschner K, Hadrup N, Qvortrup K, et al. Distribution of silver in rats following 28 days of repeated oral exposure to silvernanoparticles or silver acetate. Particle and Fibre Toxicology. 2011; 1(8): 18. doi: 10.1186/1743-8977-8-18
34. Mertens J, Alami A, Arijs K. Comparative in vivo toxicokinetics of silver powder, nanosilver and soluble silver compounds after oral administration to rats. Archives of Toxicology. 2023; 97(7): 1859–1872. doi: 10.1007/s00204-023-03511-6
35. Dziendzikowska K, Węsierska M, Joanna Gromadzka-Ostrowska J, et al. Silver nanoparticles impair cognitive functions and modify the hippocampal level of neurotransmitters in a coating-dependent manner. International Journal of Molecular Sciences. 2021; 22(23): 12706. doi: 10.3390/ijms222312706
36. Bilberg K, Hovgaard MB, Besenbacher F, et al. In vivo toxicity of silver nanoparticles and silver ions in zebrafish (Danio rerio). Journal of Toxicology. 2012; 2012: 293784. doi: 10.1155/2012/293784
37. Fu CW, Horng JL, Tong SK, et al. Exposure to silver impairs learning and social behaviors in adult zebrafish. Journal of Hazardous Materials. 2021; 403: 124031. doi: 10.1016/j.jhazmat.2020.124031
38. Guo X, Li Y, Yan J, et al. Size- and coating-dependent cytotoxicity and genotoxicity of silver nanoparticles evaluated using in vitro standard assays. Nanotoxicology. 2016; 10(9): 1373–1384. doi: 10.1080/17435390.2016.1214764

39. Vrček IV, Žuntar I, Petlevski R, et al. Comparison of in vitro toxicity of silver ions and silver nanoparticles on human hepatoma cells. Environmental Toxicology. 2016; 31(6): 679–92. doi: 10.1002/tox.22081
40. Petritskaya EN, Rogatkin DA, Rusanova EV. Comparative characteristics of antibacterial effect of silver and nanosilver in vitro (Russian). Almanac of Clinical Medicine. 2016; 44: 221.
41. Nandhini J, Karthikeyan E, Elizabeth Rani E, et al. Advancing engineered approaches for sustainable wound regeneration and repair: Harnessing the potential of green synthesized silver nanoparticles. Engineered Regeneration. 2024; 5(3): 306–325. doi: 10.1016/j.engreg.2024.06.004
42. Martin WJ, Benet Palmer S. Regenerative wound healing using copper-silver citrate composition. US Patent 2010/0099758A1, 22 April 2010.
43. Zhao S, Zhang J, Zhang J. Neuroprotective effects of biogenic silver nanoparticles in Parkinson's disease: In vitro and in vivo study. International Journal of Biological Macromolecules. 2024; 138236. doi: 10.1016/j.ijbiomac.2024.138236
44. Scarpa E, Cascione M, Griego A, et al. Gold and silver nanoparticles in alzheimer's and parkinson's diagnostics and treatments. Ibrain. 2023; 9(3): 298. doi: 10.1002/ibra.12126
45. Chountoulesi M, Demetzos C. Promising nanotechnology approaches in treatment of autoimmune diseases of central nervous system. Brain Science. 2020; 10(6): 338. doi: 10.3390/brainsci10060338
46. Antsiferova AA, Kashkarov PK. About therapeutic action of silver ions: A brief overview. Journal of Biological Regulators and Homeostatic Agents. 2025; 39(2): 3303. doi: 10.54517/jbrha3303
47. Selye H. Stress without distress (Russian). Progress Moscow. 1976; p. 126.