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COMPARATIVE STUDY OF THE EFFECTS OF DETONATION NANODIAMONDS WITH VARIED PROPERTIES ON FUNCTIONAL STATE OF BRAIN NERVE TERMINALS

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The aim of the study was to compare the effects of detonation nanodiamond preparations from different batches cleaned from impurities by diverse methods of chemical treatment on the membrane potential and glutamate transport characteristics of rat brain nerve terminals.

The size of nanodiamond particles vary from 10-20~nm to $10~\mu\text{m}$. There are carbonyl, hydroxyl and carboxyl functional groups on the surface of the particles. Physical-chemical properties such as a magnetic susceptibility and the amount of incombustible residue in samples of detonation nanodiamond vary depending on the synthesis regime and the method of chemical cleaning of the product and therefore, the neuroactive properties of nanodiamonds from different batches can be different.

It was shown by dynamic light scattering analysis that nanodiamond preparations from different batches treated by diverse technologies of chemical treatment had varied average size of particles and distribution of particles by size. Nanodiamond preparations from different batches changed the plasma membrane potential and caused membrane depolarization of nerve terminals. Analysis of the effects of nanodiamonds on transporter-mediated L-[14C]glutamate uptake by nerve terminals also revealed that all studied nanodiamond preparations decreased abovementioned parameter. Therefore, detonation nanodiamonds from different batches have similar principal effects on functional state of nerve terminals, however variability in their physical and chemical properties is associated with diverse strength of these effects.

Key words: nanodiamond, glutamate, Na+-dependent uptake, brain nerve terminals.

In nanobiotechnology, a lot of attention was attracted by carbon materials due to their new unusual properties that can be implemented in practice. Among carbons, nanodiamonds are one of the most perspective and promising nanoparticles due to their unique excellent mechanical and optical properties, huge surface areas and tunable surface structures that allow them to bond with other materials. Nanodiamonds have a wide range of potential applications in drug delivery, bioimaging and as a filler material for nanocomposites [1–3]. Nanodiamonds can be synthesized using high temperature/high pressure or

detonation, and also laser ablation and plasma-enhanced chemical vapor deposition can be employed [1-7].

Nowadays the method of detonation of explosives in special explosive chambers (method of explosive decomposition of mixed explosives with negative oxygen balance, e.g. trotyl/hexogen, grade TG-40/60) is most widely used for the production of nanodiamonds. It is known that the properties of the synthesized nanodiamonds depend on many factors such as the size and configuration of the chamber, cooling methods, composition of the explosive, detonation regime [8, 9].

Method of extraction of nanodiamonds from product of detonation is also very important [7].

The size of nanodiamond particles vary from 10-20 nm to 10 µm. Nanodiamond particle is a kind of specific carbon complex, the core of which is sp^3 hybridization carbon (diamond), and a surface layer composed of sp^2 hybridization carbon (non-diamond carbon) and firmly connected with the core [8]. The non-diamond carbon content is substantially reduced due to chemical treatment (Fig. 1*). There are carbonyl, hydroxyl and carboxyl functional groups on the surface of the particles (Fig. 2) [7, 8].

Physical-chemical properties such as a magnetic susceptibility and the amount of incombustible residue in samples of detonation nanodiamonds vary depending on the synthesis regime and the method of chemical cleaning of the product and therefore, the neuroactive properties of nanodiamonds of different samples from different batches can be different [7, 10, 11].

Nanodiamonds are considered to be non-toxic that makes them appropriate to a wide range of biomedical applications [4]. The interaction of nanodiamonds with animal organs and tissues, their circulation in the organism, and clearance in the animal body has not yet been studied in details [6]. Despite great potential of nanodiamonds in drug delivery, the fundamental mechanisms of their interaction with the cells are still poorly understood.

Recently, we revealed significant neuromodulatory features of carbon nanodots, nanoparticles with other than *nanodiamonds type of hybridization. Carbon nanodots are characterized by carbon core with sp^2 hybridized graphene islands and diamond-like sp^3 hybridized elements. Fluorescent carbon dots obtained from β -alanine by microwave heating significantly influenced presynaptic transport of glutamate and γ -aminobutyric acid (GABA), which are key excitatory and inhibitory neurotransmitters in the mammalian central nervous system [12].

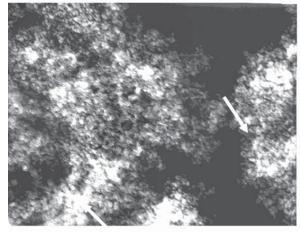
Na $^+$ -coupled glutamate transporters play a key role in the termination of neurotransmission and mediate uptake of glutamate into the cytosol of presynaptic nerve terminals. The transporters are plasma membrane proteins with several transmembrane domains, and they use Na $^+$ /K $^+$ electrochemical gradients as a driving force for transfer of the neurotransmitters across the plasma membrane [13–18]. In turn, depolarization of the plasma membrane decreases the rate of glutamate uptake.

The present study focused on the effects of nanodiamond preparations from different batches, which possessed variable properties, on the membrane potential and glutamate transport characteristics of brain nerve terminals, thereby uncovering their possible different neuroactive properties.

Materials and Methods

Synthesis of nanodiamonds

Nanodiamonds for experiments were obtained according to Orel et al. [6] by the method of detonating synthesis using a



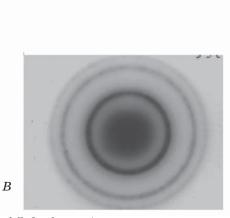


Fig. 1. Electron microscope image of flake formations: A — the fields of solid particle aggregates (indicate by arrows); B — single particle of nanodiamond of detonation synthesis

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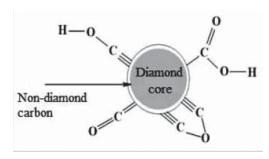


Fig. 2. Structure of nanodiamond particle with a cover of carbonyl, hydroxyl, and carboxyl functional groups on the surface

detonation wave at the explosion of powerful explosive material with negative oxygen balance (trotyl/hexogen, grade TG-40/60), using the different methods of chemical cleaning according the ISM technologies (to remove impurities): preparation # 1 is up to quality of technical standard of Ukraine TU U 26.8-05417377-177:2007 "Ultradispersed diamond powders" [7, 10] (DND, below preparation #1) and preparation # 2 is up to quality of technical standard of Ukraine TU U 23.9-05417377-219:2010 "Synthetic diamond nanopowders for stable suspension preparation" [7, 11] (ASUD-99, below preparation #2). Specific magnetic susceptibility of the preparations consisted of $154.7 \cdot 10^{-8} \text{ m}^3/\text{kg}$ and $(-0.5-20) \cdot 10^{-8} \text{ m}^3/\text{kg}$ respectively. Non-combustible residue in the preparations, e.g. metals and ceramics, was equal to 4.7% in preparation #1 and 0.5-1.8%in preparation #2[6].

Physical-chemical characteristics of nanodiamond samples: preparation #1: the total amount of elements (Fe, Ti, Mn, Cu, Cr) — 0.8% (atom.); specific surface area is $120\text{--}300 \text{ m}^2/\text{g}$; density is $2.7\text{--}3.4 \text{ g/cm}^3$; moisture content is 1.0--2.0%; pH value is 3.5--7.0; preparation #2: Zeta potential, Z — no more than 10 mV; the degree of chemical heterogeneity, K — no more than 10%; specific resistance, $\rho = 5.0 \cdot 10^9 \text{ Ohm·m}$; mass fraction of impurities (non-combustible residue) — no more than 0.5%; total amount of elements (Fe, Ni, Mn, Cu, Cr, Ti) — no more than 0.2% [10, 11].

Analysis of the size of nanodiamonds by dynamic light scattering method

The size of particles of nanodiamonds in different preparations was measured by dynamic light scattering method using laser correlation spectrometer ZetaSizer-3 (Malvern Instruments, UK), equipped with He-

Ne laser LGN-111 (P = 25 mW, l = 633 nm). The range of the instrument is from 1 nm to 20 microns. 1 ml of suspension of nanodiamonds in water was placed in a cylindrical quartz cuvette, 10 mm in diameter, which is injected into the laser correlation spectrometer with maintaining a constant temperature. Registration and statistical processing of laser scattered from water (n = 1.33) suspensions of particles, performed repeatedly for 120 s at +22 °C with scattering angle 90°. The results of measurements processed using computer software service PCS-Size mode v1.61. Laser correlation spectrometer equipped with multi computing correlator type 7032 ce.

Ethical Approval

Wistar male rats, 100-120 g body weight, were obtained from the vivarium of Strazhesko Institute of Cardiology of the National Academy of Medical Sciences of Ukraine. Animals were kept in animal facilities of the Palladin Institute of Biochemistry, housed in a quiet, temperature-controlled room (22–23 °C) and were provided with water and dry food pellets ad libitum. All procedures were conducted according to the Declaration of Helsinki ("Scientific Requirements and Research Protocols" and "Research Ethics Committees"). Experimental protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol from 19/09-2011). Before removing the brain, rats were sacrificed by rapid decapitation.

Isolation of rat brain nerve terminals (synaptosomes)

Cerebral hemispheres of decapitated animals were rapidly removed and homogenized in ice-cold 0.32 M sucrose, 5 mM HEPES-NaOH, pH 7.4, and 0.2 mM EDTA. One animal was used to obtain one synaptosomal preparation, and each measurement was performed in triplicate. The synaptosomes were prepared by differential and Ficoll-400 density gradient centrifugation of rat brain homogenate according to the method of Cotman [19] with slight modifications [20]. All manipulations were performed at 4 °C. The synaptosomal suspensions were used in experiments during 2-4 h after isolation. The standard saline solution was oxygenated and contained (in mM): NaCl 126; KCl 5; MgCl₂ 2.0; NaH₂PO₄ 1.0; CaCl₂ 2; HEPES 20, pH 7.4; and D-glucose 10. Protein concentration was measured as described by Larson et al. [21].

Measurement of synaptosomal plasma membrane potential (E_m)

Membrane potential was measured using a potentiometric fluorescent dye rhodamine 6G (0.5 μ M) based on its potential-modulated binding to the plasma membrane [22]. The suspension of synaptosomes (0.2 mg/ml of final protein concentration) after preincubation at 37 °C for 10 min was added to stirred thermostated cuvette. To estimate changes in the plasma membrane potential the ratio (F) as an index of membrane potential was calculated according to Eq 1:

$$F = F_t / F_0 , \qquad (1)$$

where F_0 and F_t are fluorescence intensities of a fluorescent dye in the absence and presence of the synaptosomes, respectively. F_0 was calculated by extrapolation of exponential decay function to t=0.

Fluorescence measurements with Rhodamine 6G were carried using a Hitachi MPF-4 spectrofluorimeter at 528 nm (excitation) and 551 nm (emission) wavelengths (slit bands 5 nm each).

 $L\text{-}[^{14}C]$ glutamate uptake by nerve terminals

of L-[14C]glutamate synaptosomes was measured as follows. Synaptosomal suspension (125 µl; of the suspension, 0.2 mg of protein/ml) was preincubated in standard saline solution at 37 °C for 10 min, then nanodiamonds (0.6 mg/ml) were added to the synaptosomal suspension and incubated for 5 min. Uptake was initiated by the addition of 10 μM L-glutamate supplemented with 420 nM L-[14 C]glutamate (0.1 μ Ci/ml), incubated at 37 °C during different time intervals (1 and 10 min) and then rapidly sedimented using a microcentrifuge (20 s at 10,000 g). L-[14 C]glutamate uptake was determined as a decrease in radioactivity in aliquots of the supernatant (100 µl) and an increase in radioactivity of the pellet (SDStreated) measured by liquid scintillation counting with ACS scintillation cocktail (1.5 ml) [23, 24]. Data collected in triplicate in four independent experiments performed with different synaptosomal preparations each are presented as mean \pm SEM.

Statistical analysis

Results were expressed as mean \pm S.E.M. of n independent experiments. The difference between two groups was compared by two-tailed Student's t-test. Differences were considered significant when $P \le 0.05$.

Materials

EDTA, HEPES, D-glucose, sucrose, analytical grade salts were purchased from Sigma (St. Louis, MO, USA); Ficoll 400, L-[¹⁴C] glutamate, aqueous counting scintillant (ACS), were from Amersham (Little Chalfont, UK).

Results and Discussion

Assessment of the size of the particles in different preparations of nanodiamonds by dynamic light scattering method.

Analysis of the size of the particles in the suspension of nanodiamonds was performed by dynamic light scattering method using ZetaSizer-3 (Malvern Instruments) with helium-neon laser. Before the measurements nanodiamonds were subjected to treatment with ultrasound at 22 kHz for 1 min. In the graphs of dynamic light scattering, nanodiamonds (concentration was equal to 0.6 mg/ml) were presented as several heterogenic fractions. The average size of the particles in the suspension of nanodiamonds in the standard salt solution was calculated based on 5 measurements, each for 1 min. It was revealed that different preparations of nanodiamonds had different average size of particles and different distribution of particles by the size. In the first preparation, the average size of nanodiamond particles was equaled to 1500 ± 346 nm (Fig. 3, A); in the second one, it consisted of 1 100 \pm 240 nm (Fig. 3, B). Large size of nanodiamonds even after sonication can be explained by their possible aggregation [6].

We used isolated rat brain nerve terminals (synaptosomes) in the experiments. Synaptosomes retain all characteristics of intact nerve terminals, they are able to maintain membrane potential, accomplish glutamate uptake, exocytosis, etc. [25–31]. The analysis of the size of particles in synaptosomeal suspension was performed with the same instruments. The size of synaptosomes was equaled to $3.24 \pm 0.10~\mu m$ [24]. Therefore, the size of nanodiamonds was comparable to or lower than the size of nerve terminals, so they presumably could interact with the synaptosomal plasma membrane and alter their molecular machinery.

Effects of nanodiamonds on membrane potential of nerve terminals.

One of the parameters that can considerably alter the functioning of Na⁺-dependent glutamate transporters is the potential of the plasma membrane of nerve terminals,

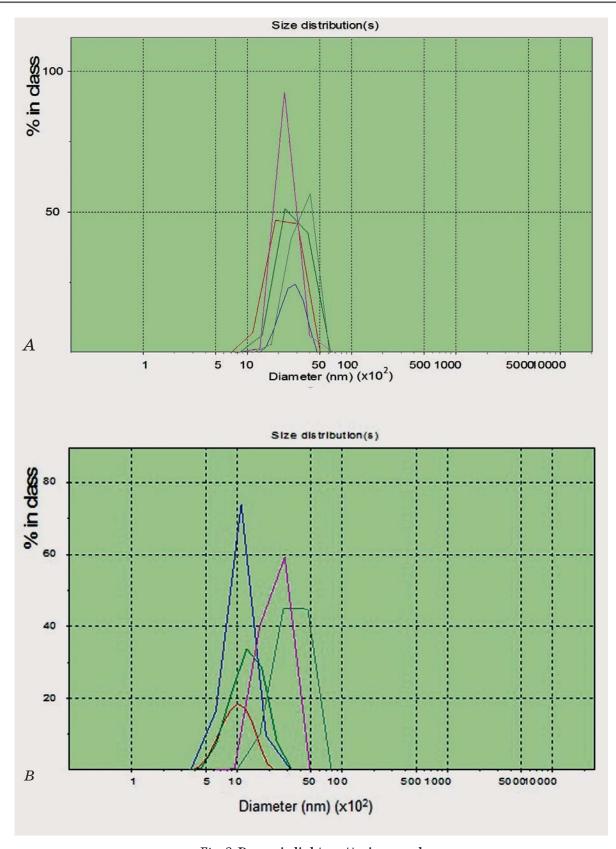


Fig. 3. Dynamic light scattering graphs: the analysis of the size of particles of the nanodiamond preparation #1 (A) and #2 (B) (0.6 mg/ml) in a standard salt solution. 5 measurements each during 1 min, sequentially 1—red, 2—blue, 3—green, 4—gray, 5—purple, are present in each graphs. The measurements were performed with Zetasizer-3 (Malvern Instruments) using helium-neon laser

because Na⁺/K⁺ electrochemical gradient across the plasma membrane serves as a driving force for transporter functioning. The potential was measured using the cationic potentiometric dye rhodamine 6G, which binds to the negative charges of the membranes. In the next series of the experiments, it was analyzed whether or not different preparation of nanodiamonds differently influenced the fluorescence of rhodamine 6G. No significant changes were found in the emission spectrum of rhodamine 6G in response to the addition of different preparation of nanodiamonds at concentration 0.6 mg/ml (Fig. 4). It should be noted that without synaptosomes the fluorescence signal of rhodamine 6G was quenched in response to addition of nanodiamonds to the cuvette (Fig. 5, preparation #1). Similar results were obtained with preparation #2.

As shown in Fig. 6, the addition of synaptosomes to the medium containing the dye rhodamine 6G was accompanied by a partial decrease in fluorescence due to its binding to the plasma membrane. $F_{\rm st}$, the membrane potential index at the steady state level, was achieved after 3 min. It was demonstrated that nanodiamond preparation #1 at a concentrations of $0.2{\text -}0.6$ mg/ml changed in the membrane potential, and so caused membrane

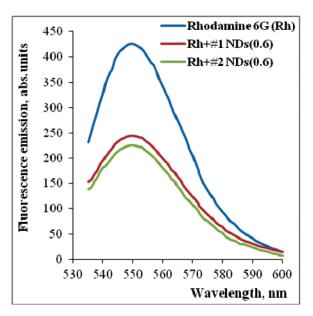


Fig. 4. Fluorescence emission spectra of rhodamine 6G (0.5 μ M) in the standard salt solution before and after application of different preparations of nanodiamonds (0.6 mg/ml): the preparation #1 — red line; the preparation #2 — green line

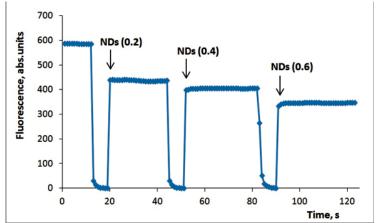


Fig. 5. Quenching of fluorescence signal of rhodamine 6G (0.5 μ M): in the presence preparation #1 of nanodiamonds (0.2–0.6 mg/ml) without synaptosomes

depolarization. Nanodiamond preparation #2 at a same concentrations exhibited stronger effect on membrane potential. Nanodiamonds did not affect completely the ability of synaptosomes to be depolarized in respond to the addition of high-KCl (Fig. 6, 7).

Effect of nanodiamonds on transportermediated L-[14 C]glutamate uptake by nerve terminals

In the experiments, the nanodiamonds were added to synaptosomal suspension 5 min before starting high-affinity transporter-mediated uptake process by L-[14C]glutamate,

so the acute effects of nanodiamonds were analyzed. Before the experiments with synaptosomes, water suspension of nanodiamonds at a concentration of 6 mg/ml was subjected to ultrasound treatment at 22 kHz for 1 min.

Influence of different preparation of nanodiamonds on the initial velocity of L-[$^{14}\mathrm{C}$]glutamate uptake by synaptosomes was analyzed. The addition of preparation of nanodiamonds #1 to synaptosomes caused significant changes in their initial velocity of L-[$^{14}\mathrm{C}$]glutamate uptake that was equal to $2.97\pm0.15\,\mathrm{nmol\,min^{-1}\,mg^{-1}}$ protein in the control experiments, and $2.42\pm0.14\,\mathrm{nmol\,min^{-1}\,mg^{-1}}$

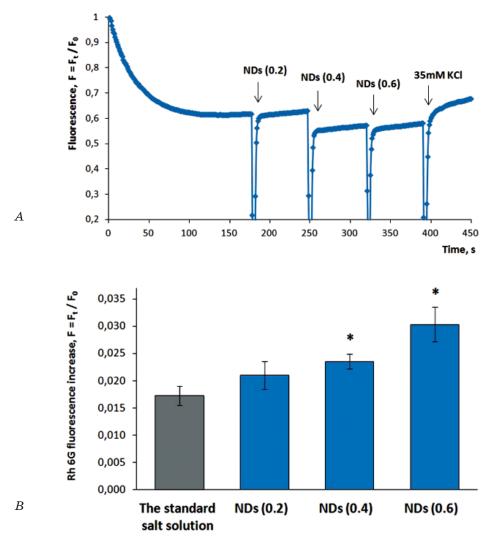


Fig. 6. A — the effects of preparation #1 of nanodiamonds on the membrane potential of synaptosomes: the suspension of synaptosomes was equilibrated with potential-sensitive dye rhodamine 6G (0.5 μ M); when the steady level of the dye fluorescence had been reached, the nanodiamonds at concentrations 0.2–0.6 mg/ml were added (marked by arrows) to synaptosomes. Trace represents four experiments performed with different preparations;

B — an increase in the fluorescence signal of rhodamine 6G in response to application: of preparation #1 of nanodiamonds (0.2–0.6 mg/ml). Thereafter: data is mean \pm SEM. *, P < 0.05 as compared to the level of the dye fluorescence when standart salt solution was added

protein in the presence of nanodiamonds at a concentration of 0.6 mg/ml (P < 0.05, Student's t-test, n = 4). The addition of preparation #2 to synaptosomes also decreased the initial rate of L-[14 C]glutamate uptake that was equal to 2.97 ± 0.15 nmol min $^{-1}$ mg $^{-1}$ protein in the control experiments, a nd 2.08 ± 0.18 nmol min $^{-1}$ mg $^{-1}$ protein in the presence of nanodiamonds at a concentration of 0.6 mg/ml (P < 0.05, Student's t-test, n = 4).

Accumulation of L-[14 C]glutamate by synaptosomes for 10 min consisted of 9.65 ± 0.45 nmol mg $^{-1}$ protein in the control experiments, and 7.23 ± 0.43 nmol mg $^{-1}$

protein in the presence of preparation of nanodiamonds #1 at a concentration of 0.6 mg/ml; and 5.95 \pm 0.38 nmol mg⁻¹ protein — preparation #2 at a concentration of 0.6 mg/ml (P < 0.05, Student's t-test, n = 4). Thus, we observed that both preparations of nanodiamonds #1 and #2 inhibited L-[14 C]glutamate uptake and its accumulation by synaptosomes.

Biocompatibility and toxicity of nanodiamonds has been intensively investigated and a progress concerning their biological and medical application for imaging and therapy has been achieved [1, 6, 32]. A low toxicity of nanodiamonds

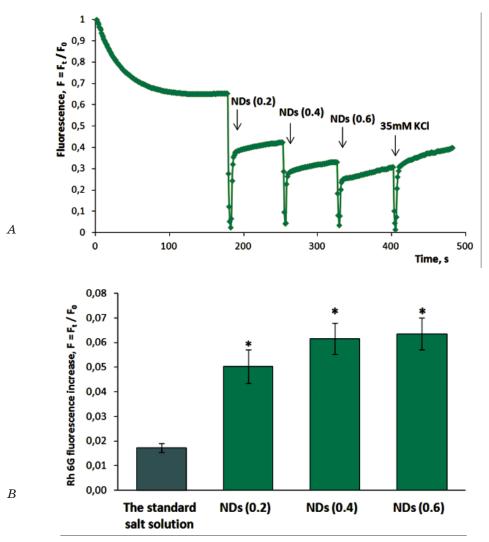


Fig. 7. A — the effects of preparation #2 of nanodiamonds on the membrane potential of synaptosomes: the suspension of synaptosomes was equilibrated with potential-sensitive dye rhodamine 6G (0.5 μ M); when the steady level of the dye fluorescence had been reached, the nanodiamonds at concentrations 0.2–0.6 mg/ml were added (marked by arrows) to synaptosomes. Trace represents four experiments performed with different preparations;

B — an increase in the fluorescence signal of rhodamine 6G in response to application: of preparation #2 of nanodiamonds (0.2–0.6 mg/ml). Data is mean \pm SEM. *, P < 0.05 as compared to the level of the dye fluorescence when standart salt solution was added

with the size of approximately 100 nm was shown suggesting biocompatibility of nanodiamonds in the cellular level [1]. However, the fundamental mechanisms of action of nanodiamonds on the cells are still poorly understood.

In this study, we revealed that nanodiamonds of different synthesis possessed different size and distribution of particles in the suspension. Large size of nanodiamonds even after sonication can be explained by their possible aggregation [6]. Also, we confirmed neuromodulatory properties of uncoated detonation nanodiamonds using nerve terminals [12, 14, 33]. The nanodiamonds of both preparations at the concentration of 0.6 mg/ml caused membrane depolarization and attenuated the initial velocity of transporter-mediated uptake of $L-[^{14}C]$ glutamate in the preparation of nerve terminals (Fig. 4-7). However, the strength of above effects was different. Both parameters namely the rate of glutamate uptake and membrane potential of nerve terminals are in tight relation with each other because the latest is a driving force for glutamate transporter functioning.

Comparing obtained results with our recent data concerning the properties of carbon nanodots synthesized from β -alanine [12], it can be concluded that despite of different type of hybridization

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in these nanoparticles, their principal neuromodulatory effects were almost similar. Whereas, the strength of their effects was diverse.

The size of nanodiamonds was less than 10 nm, and so they can enter nerve terminals during synaptic vesicle recycling, and in turn can affect transporter-mediated uptake of neurotransmitters [26]. Also, based on our data it is suggested that nanodiamonds are able to bind to the plasma membrane and change directly its membrane potential. We suggest that combination of neuroactive and recently discovered radioactive [34] properties of nanodiamonds is promising. Nanodiamonds irradiated in the core of a commercial-scale reactor demonstrated the resulting radioactivity. It was associated with the presence of metal-containing impurities in the initial nanodiamonds. The dose rate of γ-radiation achieved for nanodiamonds was about 180 µSv/h, and the dose rate of combined γ + β -radiation was ~ 720 μ Sv/h [34].

The authors declare no financial and non-financial competing interests exist.

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ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ВПЛИВУ НАНОАЛМАЗІВ ДЕТОНАЦІЙНОГО СИНТЕЗУ З РІЗНИМИ ВЛАСТИВОСТЯМИ НА ФУНКЦІОНАЛЬНИЙ СТАН НЕРВОВИХ ЗАКІНЧЕНЬ ГОЛОВНОГО МОЗКУ

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Метою роботи було порівняльне дослідження впливу зразків наноалмазів детонаційного синтезу різних партій, очищених від домішок різними технологіями хімічної обробки, на мембранний потенціал і транспорт глутамату в нервових закінченнях головного мозку щурів.

Розмір частинок наноалмазу варіює від 10 нм до 10–20 µм. На поверхні частинки знаходяться карбонільні, гідроксильні і карбоксильні функціональні групи. Фізико-хімічні властивості, наприклад, питома магнітна сприйнятливість та кількість неспалюваного залишку в зразках детонаційного наноалмазу, змінюються в залежності від особливостей синтезу, способу хімічної очистки продукту, та, отже, нейроактивні властивості зразків наноалмазу із різних партій можуть відрізнятися.

Методом динамічного розсіювання світла показано, що зразки наноалмазів різних партій, що були очищені різними технологіями хімічної обробки, відрізняються як за розміром частинок, так і за розподілом їх за розміром. Встановлено, що зразки наноалмазів різних партій впливають на мембранний потенціал плазматичної мембрани нервових закінчень та транспортерзалежне накопичення ними L-[14C]глутамату. Таким чином, наноалмази детонаційного синтезу різних партій здійснюють схожий вплив на функціональний стан нервових закінчень, однак його інтенсивність може несуттєво змінюватися.

Ключові слова: наноалмази, глутамат, Na⁺-залежне поглинання, нервові закінчення мозку.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ВЛИЯНИЯ НАНОАЛМАЗОВ ДЕТОНАЦИОННОГО СИНТЕЗА С РАЗНЫМИ СВОЙСТВАМИ НА ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ НЕРВНЫХ ОКОНЧАНИЙ ГОЛОВНОГО МОЗГА

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Целью работы было сравнительное исследование влияния образцов наноалмазов детонационного синтеза разных партий, очищенных от примесей различными способами химической обработки, на мембранный потенциал и транспорт глутамата в нервных окончаниях головного мозга крыс.

Размер частиц наноалмаза варьирует от 10 нм до 10–20 µм. На поверхности частицы находятся карбонильные, гидроксильные и карбоксильные функциональные группы. Физико-химические свойства, например, удельная магнитная воспримичивость и количество несгораемого остатка в образцах детонационного наноалмаза, меняются в зависимости от особенностей синтеза, способа химической очистки продукта, и, следовательно, нейроактивные свойства образцов наноалмазов из разных партий могут отличаться.

Методом динамического рассеяния света показано, что образцы наноалмазов из разных партий, очищенные различными технологиями химической обработки, отличаются как по размеру частиц, так и по распределению их по размерам. Установлено, что образцы наноалмазов из разных партий влияют на мембранный потенциал плазматической мембраны нервных окончаний и транспортерзависимое накопление ими L-[¹⁴C] глутамата. Таким образом, наноалмазы детонационного синтеза разных партий оказывают подобное воздействие на функциональное состояние нервных окончаний, однако его интенсивность может незначительно изменяться.

Ключевые слова: наноалмазы, глутамат, Na⁺-зависимое поглощение, нервные окончания мозга.