



Research article

Electrophysiological effects of polyethylene glycol modified gold nanoparticles on mouse hippocampal neurons

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ABSTRACT

Gold nanoparticles (AuNPs) can cross the blood brain barrier, thus can be used as nanocarriers in brain drug delivery. However, the effect of bare and polyethylene glycol-modified (PEGylated) AuNPs on normal neural function has not been extensively investigated. In this study, bioelectrical properties of neuronal functions of male BALB/c mice were explored *ex vivo* and *in vivo* by using 5 nm bare AuNPs and PEGylated AuNPs. Electrophysiological properties of neurons from hippocampal CA1 region sections were recorded by patch clamp method. *Ex vivo*, firing rate of action and membrane potentials in response to negative current stimuli significantly altered only after bare AuNP exposure compared to control ($p < 0.05$). After *in vivo* injections, anxiety levels of animals were similar. Amplitude of action potentials reduced only in bare AuNP group ($p < 0.05$). In conclusion, excitability of hippocampal neurons is increasing with bare AuNP exposure, and PEGylation might be more biocompatible for medical applications.

1. Introduction

Gold nanoparticles (AuNPs) have a potential to be used in many different medical applications including cancer treatment, gene or drug delivery and imaging techniques such as positron emission tomography (Feng et al., 2014; Chen et al., 2009, 2010; Lasagna-Reeves et al., 2010). Their small size, ability to penetrate into cells and less cytotoxicity compared to many other nanomaterials makes them a promising candidate to be used in human applications. In addition, their synthesis is easy to control, and the synthesized particles are stable in colloidal form for longer periods (Huang et al., 2010; Raftis and Miller, 2019; Shukla et al., 2005; Boisselier and Astruc, 2009).

AuNP surfaces can also be modified and decorated with various chemicals depending on the desired application. One such molecule is polyethylene glycol (PEG). PEG modified AuNPs (PEGylated AuNPs) have recently attracted attention recently to several diagnostic imaging and therapeutic applications specifically due their longer circulation time (6–12 h) in rodents' blood (Liu et al., 2014; Huang et al., 2010). On the other hand, PEGylated AuNPs with various diameters (20–30 nm and 50 nm) were reported to be accumulated in the brain when it was compared

to bare AuNPs (Takeuchi et al., 2018; Lee et al., 2016) whereas other studies showed that PEGylated AuNPs were less cytotoxic compared to bare AuNPs (Harris et al., 2001; Niidome et al., 2006).

Phase 1 clinical studies of a drug conjugated with PEGylated AuNPs are currently ongoing for the treatment of cancer patients (Libutti et al., 2010). The promising results of these studies encourage their use in other fields such as neurological diseases, brain tumor diagnostics and therapy, manipulation of neurogenesis and neural activity (Polak and Shefi, 2015). However, one of the obstacles against drug delivery to the brain is blood brain barrier (BBB) which protects brain homeostasis by its selective permeability feature. In a study by Oh et al. it was reported that AuNPs up to 16 nm diameter size could enter COS-1 cells whereas 2.4 nm AuNPs were seen in cell nuclei, and 5.5–8.2 nm AuNPs in the cytoplasm (Oh et al., 2011). Either bare AuNPs or PEGylated AuNPs were reported to penetrate through the BBB in animal studies (Kang et al., 2019; Takeuchi et al., 2018; De Jong et al., 2008). PEGylated AuNPs can penetrate largely through brain endothelial cells specifically with a diameter less than 4 nm (Etame et al., 2011). Therefore, using sub-10 nm AuNPs is a wise approach to penetrate through BBB.

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In the literature, functional neurotoxicity, which is defined as modulations in neural electrical activity or membrane potential, of PEGylated AuNPs attracted less attention and the interaction with normal neuron function has not been reported yet. Most of the studies report changes in body weight, and the fluctuations in biochemical and hematological parameters (Adewale et al., 2019). In a previous work, we have demonstrated the bioelectrical effect of glucose, oligonucleotide, or peptide modified AuNPs on hippocampal mouse neurons after *ex vivo* administration where we observed differences in neuronal firing rates of action potentials upon administrating AuNPs with various surface modifications (Tuna et al., 2019). In this context, two reports by other groups demonstrated increased excitability of neurons exposed to bare spherical or star-shaped AuNPs in mice brain slices by using patch clamp recordings (Salinas et al., 2014; Jung et al., 2014). The first study by Jung et al. showed that *ex vivo* intracellular application of 5 nm and 40 nm bare AuNPs on mice brain hippocampal slices increased number of spikes and spontaneous firing rate of action potentials (Jung et al., 2014). In addition, intracellular applications of AuNPs on mice brain during seizure-like activity have even worsen the epileptiform activity (Jung et al., 2014). The second study which was reported by Salinas et al. demonstrated increased firing rate of mice hippocampal neurons due to extracellular application of 180 nm gold nanostars and suggested that the effect occurs due to potassium channel blockage (Salinas et al., 2014). Moreover, negatively charged quantum dots and metallic nanoparticles such as silver and zinc oxide stimulated spontaneous electrical activity in neurons (Dante et al., 2017; Liu et al., 2012; Zhao et al., 2009).

Therefore, if AuNPs are going to be used in bioapplications, it is important to elucidate their possible influence and functional effects specifically in neurons. Moreover, although toxic and genotoxic effects of AuNPs on brain and neurons were established, more studies are necessary to better understand whether surface modifications such as PEGylation can prevent electrical activity alterations of neurons both *ex vivo* and *in vivo*.

This study aimed to investigate the effect of PEGylated AuNPs on neuronal electrical activities. For this purpose, bare or PEGylated AuNPs were administered either *ex vivo* on brain hippocampal slices or *in vivo* via intraperitoneal injection to mice. Then, electrophysiological properties of neurons were measured by using patch-clamp technique from hippocampal brain slices. In addition, to observe behavioral changes, anxiety levels of mice were determined after AuNP injection. To the best of our knowledge on existing literature, this study is the first report on the topic (Adewale et al., 2019; Boyes and van Thriel, 2020).

2. Methods

2.1. AuNP synthesis, characterization, and surface modification with PEG

In this study, 5 nm-diameter sized spherical AuNPs were synthesized. For the synthesis of this sub-10 nm diameter AuNPs capped with sodium citrate, the method developed by Piella et al. was used (Piella et al., 2016). According to their seed-mediated growth method, briefly, 1 ml of 150 mM potassium carbonate and 0.1 ml 2.5 mM tannic acid containing 2.2 mM 150 ml sodium citrate was heated to 70 °C in a 250 ml three-necked round bottom flask that was kept in a heating mantle to prevent heat loss throughout the reaction. Once the mixture reached to 70 °C, 1 ml of 25 mM HAuCl₄ was injected to the flask and the reaction continued for 5 more minutes to allow enough time for reaction to occur. The obtained 3.5 nm diameter AuNP seeds were further grown by taking out 55 ml of the mixture and replacing it with fresh 55 ml 2.2 mM sodium citrate. Once the reaction reached back to 70 °C, 0.5 ml 25 mM HAuCl₄ was injected twice with 10 min intervals. This sodium citrate addition and HAuCl₄ injection step was repeated once again to obtain 5 nm citrate capped AuNPs, ready to be modified with thiolated PEG.

PEGylation was made through direct interaction with thiolated PEG (mw: 800, Sigma Aldrich catalog no: 729108-1G) under vigorous shaking overnight at room temperature. The next day PEG-coated AuNPs were dialyzed to get rid of unbound PEG from the colloid by using a Spectra/

Por® 7 dialysis cassette (cut off value 2000 Dalton). Both bare and PEGylated AuNPs (PEG-AuNPs) were stored at room temperature (25 °C) until use. AuNPs are well known for their long-term stability (up to several months at room temperature) and has been used extensively over several years by our group (Elbert et al., 2018; Tuna et al., 2019). Therefore, in addition to visual observation, UV-Vis spectrophotometry proved that the particles did not precipitate nor lost stability during the period of experiments at room temperature (25 °C) (data not shown).

Bare AuNPs and PEG-AuNPs were characterized by using PerkinElmer Lambda 25 UV-Vis spectrophotometer. For dynamic light scattering (DLS) and zeta potential measurements Malvern ZetaSizer Nano ZS was used. TEM images of bare AuNP and PEG-AuNP were obtained in METU central laboratories.

2.2. Ethics statement and animals

The local committee on the Ethics of Animal Experiments of the Yeditepe University, Istanbul Yeditepe University Animal Care and Use Committee (YUDETAM, Permit Number: DMF102880) approved all animal care, maintenance and experimental procedures. All efforts were made to minimize suffering. In total, 41 male BALB/c-mice at 4–6 weeks of age (20 mice for *ex vivo* and 21 mice *in vivo*) were housed at 22–24 °C on a 12 h light (06:00) and dark (18:00) cycle with ad libitum access to water and standard mouse chow unless otherwise noted.

2.3. Brain slice preparation

Mice were decapitated and 250 µm-thick coronal brain slices containing the hippocampal CA1 were sectioned by using vibratome (Campden instrument 5100 MHz). Slices were prepared in chilled cutting solution containing: 234 mM sucrose, 28 mM NaHCO₃, 7 mM dextrose, 2.5 mM KCl, 7 mM MgCl₂, 0.5 mM CaCl₂, 1 mM sodium ascorbate, 3 mM sodium pyruvate and 1.25 mM NaH₂PO₄, aerated with 95% O₂ and 5% CO₂. Then, they were transferred to artificial cerebrospinal fluid (aCSF) containing: 119 mM NaCl, 25 mM NaHCO₃, 11 mM D-glucose, 2.5 mM KCl, 1.25 mM MgCl₂, 2.0 mM CaCl₂ and 1.25 mM NaH₂PO₄, aerated with 95% O₂ and 5% CO₂. Slices were incubated 1 h at room temperature (20–24 °C) and then maintained and recorded at same conditions.

2.4. Patch-clamp recordings for electrophysiological studies

For *ex vivo* electrophysiological recordings, 0.01 mM bare AuNPs, PEG (mw: 800) or PEG-AuNPs were added into the perfusion solution and recordings were obtained from hippocampal CA1 neurons during 5–60 min of application. Spontaneous action potentials were measured by whole-cell patch configuration at -65mV. Glass pipettes (Harvard Apparatus) having 3–5 MΩ tip resistance were filled with a potassium gluconate based internal solution during whole-cell recordings. Pipette solution contained: 145 mM K-gluconate, 1 mM MgCl₂, 10 mM HEPES, 1.1 mM EGTA, 2 mM Mg-ATP, 0.5 mM Na₂-GTP, and 5 mM Na₂-phosphocreatine (pH 7.3 with KOH; 290–295 mOsm). Recordings were corrected for liquid junction potential.

Membrane potentials and stimulated action potentials were generated by current injection protocol, which was set for 500 ms pulses of 50 pA steps starting from -150 pA to +150 pA by whole cell patch configuration. Average of 500 ms were used to plot current-voltage (I-V) relationship.

2.5. In vivo experiments

To investigate the effects of systemic administration of bare AuNPs and PEG-AuNPs, mice were injected with 200 µL PBS (control/sham), AuNP (2.2 µg/g) or PEG-AuNP (2.2 µg/g) every day for 3 days with intraperitoneal injection (n = 7 mice for each group).

Locomotor activity of all mice was measured before the injection and at the end of 3 days by open field test to determine the anxiety-like

behavior of the animals. Each mouse was placed in the center of the open field apparatus ($60 \times 60 \times 42$ cm) and their activity was tracked for 10 min ($n = 3$ mice). The center of the floor was illuminated at 60 lux. The total distance travelled, the number of crossings into the middle portion of the arena (center quadrant, 20×20 cm), and percentage of time spent in the center quadrant were determined by offline analysis (Ethovision Nodus).

Then, mice were decapitated, and 250 μm -thick coronal brain slices were sectioned by using vibratome. After 1 h recovery, brain slices were incubated in aCSF and cell-attached recordings were obtained from hippocampal CA1 neurons at 31°C using glass pipette electrodes with 5–7 M Ω tip resistances and firing rate of neurons was measured. aCSF was used for the intracellular solution during cell-attached recordings.

All patch clamp recordings were performed using a MultiClamp 700 B amplifier and a Digidata1550 (Axon instruments, Inc, USA), and the acquired data were analyzed using the pCLAMP version 10.7 (Axon Instruments). Signals were filtered at 5 kHz and digitized at a sampling rate of 10 kHz.

2.6. Determining the amount of AuNP accumulated in mice brains

To determine the brain uptake of bare AuNPs and PEG-AuNPs in mice, inductively coupled plasma mass spectrometry (ICP-MS), Perkin-Elmer Nexion 300XX, was used with nickel sampler and skimmer cones 3 d after injection ($n = 3$ mice for each group). A microwave digestion system, Titan MPS Microwave Sample Preparation System (PerkinElmer, USA), with a rotor for sixteen Teflon digestion vessels was used for sample digestion process. Whole mice brains were homogenized and 200 mg of each homogenate was used for measurements.

2.7. Data analysis and statistics

Electrophysiological recordings were analyzed by using Clamp fit (version 9.2). All values shown in the results section are the mean \pm

standard deviation (STD). Data were analyzed by one-way analysis of variance (ANOVA) followed Bonferroni multiple comparison using SPSS Statistics version 18.0. In cases where the data distributed asymptotically (Welch), data were analyzed by Dunnett T3 test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Characterization of AuNPs

The particle size distribution of both bare AuNPs and PEG-AuNPs were detected as 5–7 nm with TEM and images indicated that the particles had uniform size and spherical shape (Figure 1).

The spectrum obtained from UV-Vis spectroscopy showed a peak maximum at 516 nm, which is typical for a colloidal suspension containing 5 nm diameter AuNPs as consistent with reports in the literature (Haiss et al., 2007). After surface modification with PEG, the peak blue-shifted from 516 to 512 nm for PEG-AuNPs (Figure 1).

Hydrodynamic mean diameter obtained from DLS for bare AuNPs was 7.53 ± 0.26 nm, and for PEG-AuNP, it was 12.31 ± 0.87 nm (Figure 1). The zeta potential of particles were negative with values of -24 ± 11.2 mV and -18.3 ± 8.79 mV for AuNP and PEG-AuNP, respectively.

Mouse hypothalamic GnRH neuronal cell line, GT1-7, was treated with AuNPs (5, 10, 25, 50, and 100 nM) to test the in vitro cytotoxicity. The AuNPs doses were not cytotoxic and did not affect the viability of GT1-7 cells (Supplementary file).

3.2. Ex vivo effects of bare and PEGylated AuNPs on mice hippocampal neural activity

The acute effect of AuNPs or PEG-AuNPs on spontaneous action potentials was investigated by current injection protocol by whole cell patch configuration. Example of recordings was provided in Figure 2. Spontaneous action potentials were observed in bare AuNP-treated

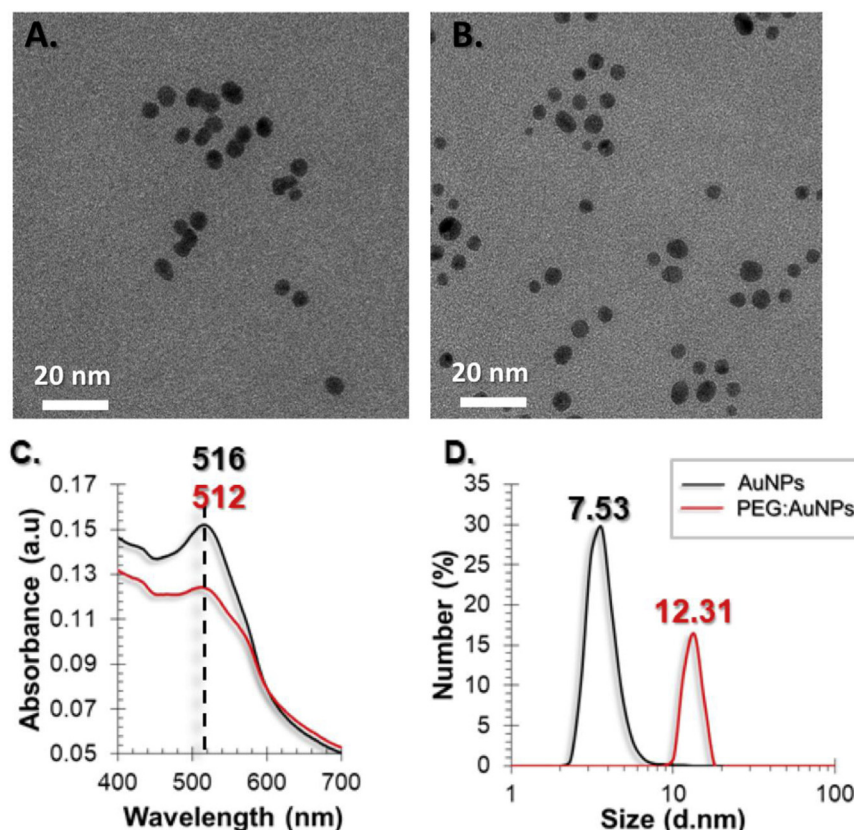


Figure 1. TEM images of A) bare AuNPs and B) PEGylated AuNPs C) UV-Vis D) DLS characterization of bare and PEGylated AuNPs.

neurons even after the current injection protocol was over (Figures 2a and 2b). However, PEG-AuNP treatment did not cause additional action potentials.

Firing rate of action potentials was higher in AuNP-treated group and not different in PEG-AuNP-treated group compared to control (Figure 2c, $p < 0.05$). Current-voltage relationship was significantly shifted upward at the presence of AuNPs (Figure 2d).

In addition, peak amplitude, half width, rise slope, area and firing frequency of spontaneous action potentials was determined at -65mV by current clamp whole cell patch configuration (Table 1). Amplitude of spontaneous action potentials was higher in the AuNP-treated group compared to control (Table 1, $p < 0.05$). There was no difference between control and the PEG-AuNP-treated neurons in any characteristic parameters of action potentials.

3.3. In vivo effects

3.3.1. Anxiety-like behavior of the animals determined by open field test

There was no statistical difference between the groups of the parameters determined by open field test. The total distance travelled, the number of crossings into the middle portion of the arena and percentage of time spent in the center quadrant were similar (Table 2). However, although it is not statistically significant, all these parameters representing anxiety-like behavior was higher in PEG-AuNP-injected group compared to control and AuNP-injected groups.

3.3.2. In vivo effects of bare and PEGylated AuNPs on mice hippocampal neural activity

The effect of AuNPs or PEG-AuNPs on spontaneous current spikes was investigated by voltage clamp cell-attached patch configuration after 3 days of bare AuNP or PEG-AuNP injection. Example of recordings was provided in Figure 3 a and b. The firing frequency of spontaneous current spikes of hippocampal neurons after 3 days of bare AuNP (7.04 ± 1.52 Hz) injection was similar compared to control (5.66 ± 1.02 Hz) and PEG-AuNP injected groups (8.35 ± 1.98 Hz) (Figure 3c).

The amplitude of spontaneous current spikes of hippocampal neurons was significantly lower in AuNP-injected group (-106.99 ± 9.42 mV/events, $n = 37$) compared to the control (-237.01 ± 25.77 mV/events, n

$= 34$) and PEG-AuNP groups (-185.69 ± 28.72 mV/events, $n = 33$) ($p < 0.05$) (Figure 3d).

3.3.3. The amount of AuNP and PEG-AuNP in mice brain

The elemental amount of AuNPs after 3 days of injection was determined by ICP-MS in mice brain. The control ($0.24\mu\text{g} \pm 0.01$) and AuNP group ($0.21\mu\text{g} \pm 0.02$) of mice had similar amount of AuNPs in their brains. The amount of PEG-AuNPs, was higher ($0.74\mu\text{g} \pm 0.03$) compared to control and bare AuNP-injected mice groups ($n = 3$). However, there was no significant difference between groups. Thus, PEG modification was observed to increase the AuNP accumulation amount in the brain of the animals, up to 3 times compared to control as well as bare AuNP-injected group.

4. Discussion

The present study demonstrated the effect of PEGylated AuNPs on electrical activity of neurons both *ex vivo* and *in vivo*. Although elemental amount of gold in mice brain increased as a result of AuNP surface modification with PEG, PEGylated AuNP-treatment did not alter action potential characteristics of hippocampal CA1 neurons both *ex vivo* and *in vivo*. In addition, PEG-AuNPs did not significantly affect anxiety-like behavior of mice represented by locomotor activity. As a result, PEGylation of AuNP suggested to be safer for neural function and might be useful for biomedical applications to target brain or brain related diseases. In a study by Chen et al., bare AuNPs of 17 and 37 nm diameters were tested for their cognition impairment (Chen et al., 2010). 17 nm AuNPs were seen to penetrate more to brain hippocampal region and compared to 37 nm AuNPs, 17 nm AuNPs impaired cognition more. In our study, more penetrance of PEG-AuNPs did not result in increased anxiety-like behavior, which indicated safer use of PEGylated AuNPs compared to bare ones.

Bare AuNPs were synthesized at 5 nm diameters and characterization was made using by UV-Vis spectroscopy, DLS and TEM. The measurements showed that after modification with PEG, the particles were intact and had around 10 nm hydrodynamic diameter size. The TEM images supported the diameter size results. The negative values of zeta potential approached to more positive values upon surface modification with PEG,

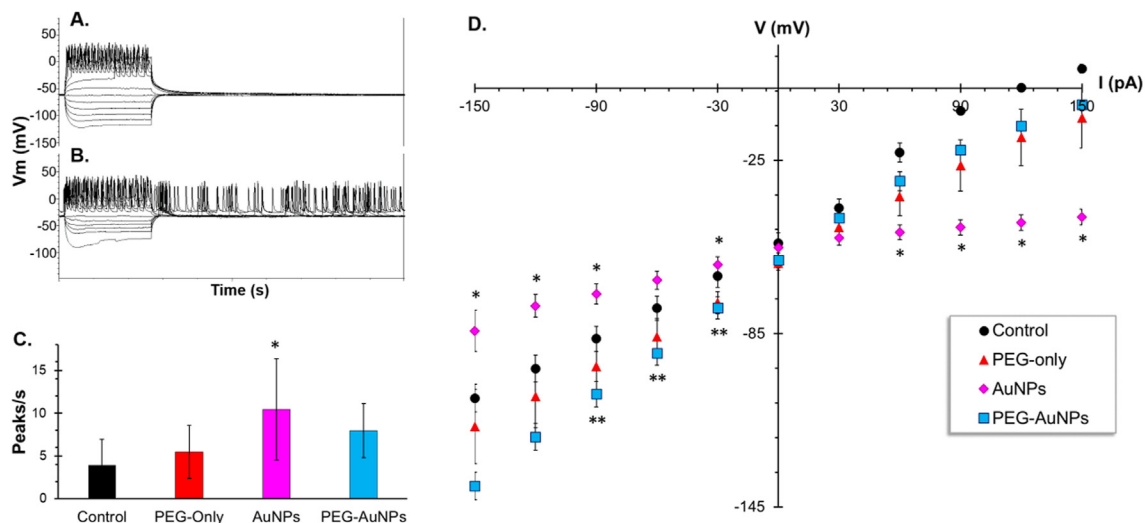


Figure 2. The effects of AuNPs and PEG-AuNPs on action potential characteristics of mice hippocampal neurons. Exemplary images of *ex vivo* responses obtained from A) control and B) bare AuNPs by using whole-cell current clamp configuration. Recordings of membrane potentials and action potentials were stimulated by current injections during 500 ms in the range of -150 pA and $+150$ pA with 50 pA steps. C) The changes in the number of spontaneous action potentials (peaks/s) at -65 mV current clamp. The number of neurons investigated was; control ($n = 10$), PEG-only ($n = 14$), AuNPs ($n = 28$), PEG-AuNPs ($n = 21$). At least 4 animals were used for each group. D) Current-voltage (I/V) plot to show the fluctuations in membrane potential of hippocampal neurons upon injected current. (*) represents the significant difference between control versus AuNPs. (**) represents the significant difference between control versus PEG-AuNPs ($p < 0.05$, Dunnett's T3 test). At least 4 animals were used for each group. 'n' numbers are representing the number of neurons.

Table 1. Properties of spontaneous action potentials at -65 mV.

	Control	PEG	AuNPs	PEG-AuNPs
Half width (ms)	5.1 ± 0.9	3.9 ± 0.6	4.9 ± 0.5	2.9 ± 0.9
Rise slope (mV/ms)	13.6 ± 3.6	7.3 ± 2.2	15.9 ± 3.6	5.9 ± 3.1
Area (mV.ms)	318.1 ± 41.4	156.7 ± 34.6	153.8 ± 19.4	188.7 ± 45.7
Firing frequency (peaks/s)	4.1 ± 0.7	5.5 ± 0.8	10.4 ± 1.3 (*)	7.9 ± 0.9

(*) represents the significant difference compared to control. The highest mean value of each row was shown in bold.

Table 2. Open field test. The anxiety-like behavior of animals determined by open field test was similar for all groups (n = 5).

	Total distance travelled (cm)	Time spent center/periphery	Number of crossings into the middle/periphery	Average speed (cm/s)
Control	3516 ± 318	3.0 ± 0.5	1.7 ± 0.2	5.9 ± 0.5
AuNPs	3376 ± 402	2.9 ± 0.8	2.2 ± 0.3	5.6 ± 0.7
PEG-AuNPs	3880 ± 124	5.2 ± 1.1	2.7 ± 0.5	6.5 ± 0.2

'n' numbers are representing the number of mice. The highest mean value of each column was shown in bold.

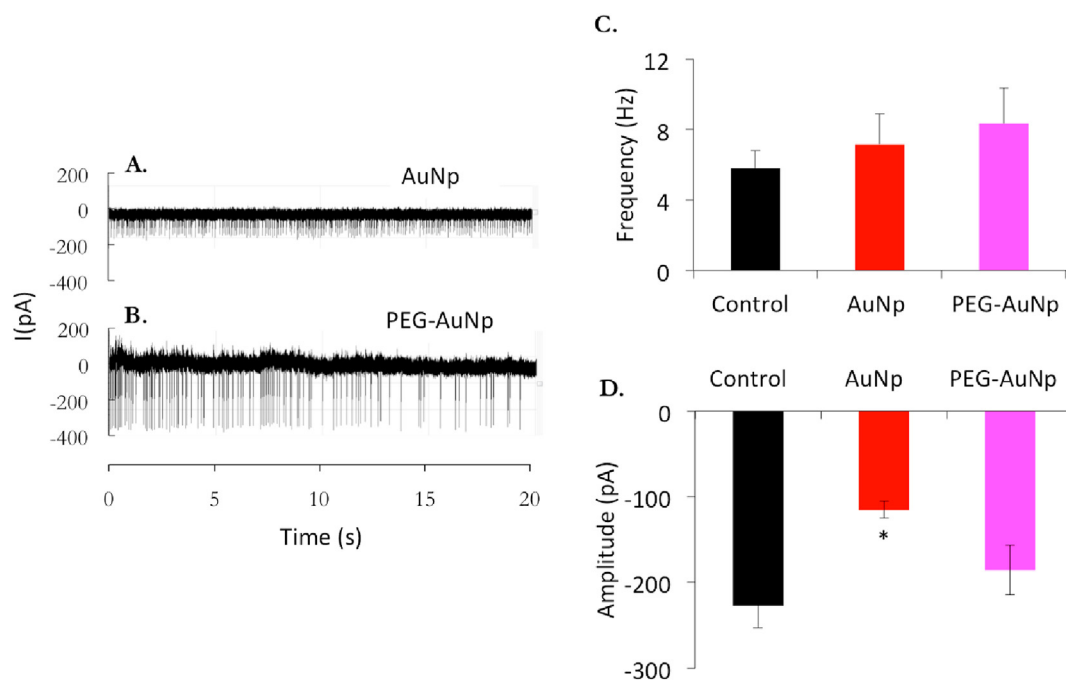


Figure 3. The effects of AuNPs and PEG-AuNPs on action potential characteristics of mice hippocampal neurons after daily 3 injections of bare AuNP or PEG-AuNP. Exemplary images of *ex vivo* responses obtained from A) control and B) bare AuNPs by using cell attached voltage clamp configuration. C) The firing frequency of spontaneous current spikes of hippocampal neurons after 3 days of bare AuNP injection was similar compared to control and PEG-AuNP injected groups. D) The amplitude of spontaneous current spikes of hippocampal neurons was significantly lower in AuNP-injected group (n = 37) compared to the control (n = 34) and PEG-AuNP groups (n = 33) (p < 0.05) (Figure 3d). (*) represents the significant difference between control versus AuNPs. (**) represents the significant difference between control versus PEG-AuNPs (p < 0.05, Dunnett's T3 test). 'n' numbers are representing the number of neurons.

which is a sign of successful surface coverage with PEG. Recently, alterations of neuronal electrical activity have been reported for quantum rods (Dante et al., 2017). The surface charge of nanoparticles, represented by zeta potential values, was found out to determine neuron-nanoparticle interactions (Dante et al., 2017); the particles with more negative zeta potential values accumulated more in neurons. More studies are necessary to understand how AuNPs are affecting membrane potentials or sodium/potassium currents.

Biodistribution of AuNPs and PegAuNPs in different organs via intraperitoneal injection were investigated in literature. For example, Zhang et al. reported that 5nm particles are distributed widely to liver, heart and kidney of mice received an intraperitoneal injection (Zhang et al., 2011). In the literature, penetration of PEGylated AuNPs through

brain endothelial cells was reported (Etame et al., 2011). In addition, Takeuchi et al. demonstrated the biodistribution of bare and PEGylated AuNPs and showed that the amount of PEG-AuNP in brain of mice were significantly more than bare AuNPs 2 d after intravenous injection (Takeuchi et al., 2018). In line with these studies, we have also showed more elemental gold accumulation in PEGylated AuNP injected mice brains although it was not statistically significant.

AuNPs (bare or PEGylated) were applied *ex vivo* to mice hippocampal brain slices and spontaneous and stimulated action potentials of neurons from CA1 region were recorded by whole-cell patch clamp method. Functional characteristics of neurons were compared by analyzing properties of action potentials such as spontaneous action potential rate and voltage-current (V-I) responses. The increase in firing rate might

lead to epileptic form of activity in brain (Jung et al., 2014). The increased firing rate of action potentials due to 5 nm AuNP and 180 nm nanostar AuNP treatment was previously reported (Jung et al., 2014; Salinas et al., 2014). In our study, as an addition to the literature, the effect of PEG modification on firing rate of spontaneous action potentials was shown and PEG-AuNP can be considered as more “biofriendly” for neuronal function than bare AuNPs since the firing rate of neurons in PEG-AuNP group did not increase compared to the control group. Moreover, membrane potential significantly shifted upwards in response to negative current stimuli (-150 to 0 pA) in bare AuNP-treated group compared to both control and PEG-AuNP-treated groups. These alterations through positive potential might also lead to increased excitability in neurons and epileptic form of activity in brain. However, PEG-AuNP did not alter neuronal activity characteristics as much as bare AuNPs did.

Bare AuNPs or PEG-AuNPs were injected into mice every day for 3 days and there was no difference between groups in terms of anxiety levels of animals *in vivo*. Even though there was no difference in the firing rate of spontaneous currents recorded by cell-attached configuration between the groups, the magnitude of the amplitude was observed to reduce in the bare AuNPs group. To the best of our knowledge, this is the first study investigating the effect of AuNPs on electrical activity of neurons after repeated injection. In consistent with *ex vivo* results, electrical activity of neurons treated with PEG-AuNPs *in vivo* was similar to the control.

In conclusion, the electrophysiological properties of hippocampal neurons treated with AuNPs were altered. Higher amount of PEGylated AuNPs were observed to penetrate to the even though causing less elicitation in neurons compared to bare AuNPs. To better understand the effect of nanoparticles on neural function, further studies might focus on how AuNPs are affecting membrane potentials or sodium/potassium currents. Another focus area should be in behavioral changes in the offspring of mice exposed to bare and PEGylated AuNPs in order to open up the way for smarter and safer designs for neuronal nanomedical applications (Alimohammadi et al., 2019).

Declarations

Author contribution statement

Bilge Guvenc Tuna: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gamze Yesilay & Yavuz Yavuz: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Bayram Yilmaz & Akif Maharramov: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Mustafa Culha: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Soner Dogan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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