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RESEARCH ARTICLE

A study of sleep-wake cycle after transplantation of neural stem cells treated with gold nanoparticles in the suprachiasmatic nucleus lesion in the rat

Vahid Pirhajati Mahabadi[1,2*], Seyed Mohammad Amini[3*], Seyed Behnamedin Jameie[1], Mona Farhadi[4], Neda Eslahi[5]

[1] *Iran University of Medical Sciences, Neuroscience Research Center, Tehran, Iran.*

[2] *Iran University of Medical Sciences, Oncopathology Research Center,Tehran, Iran.*

[3] *Iran University of Medical Sciences, Radiation Biology Research Center, Tehran, Iran.*

[4] *Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.*

[5] *Iran University of Medical Sciences, Finetech in Medicine Research Center, Tehran, Iran*

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ABSTRACT

Objective(s): Numerous stem cells are distributed throughout various regions of the central nervous system. Currently, extensive research has been conducted on the potential use of neural stem cells (NSCs) for the repair of the central nervous system. These neural stem cells possess the ability to differentiate into several cell types, including neurons, astrocytes, and oligodendrocytes. The suprachiasmatic nucleus (SCN) is crucial in regulating the circadian rhythm as well as the sleep-wake cycle.

This research investigates the NSCs transplantation on the sleep-wake cycle in suprachiasmatic nucleus lesions of rats.

Methods: Apigenin-coated gold nanoparticles have been synthesized. NSCs were cultured in DMEM F12 enriched with apigenin-coated gold nanoparticles. Thirty adult male Wistar rats weighing between 220-250 g, were classified into three groups (Lesion, experimental, and control). The Subventricular zone (SVZ) of newborn rat brains was applied for NSCs separation. The animals received Suprachiasmatic nucleus lesions (2mA current, 8 seconds), (0.2 mm ML,-9.2 mm DV, -0.6 mm AP from bregma). To record the sleep cycle, 2 Electromyography (EMG) and 3 electroencephalogram (EEG) electrodes were situated in the neck, muscles, and skull, respectively. A transmission electron microscope (TEM) was used for ultrastructural analysis of cells.

Results: In this study, we observed the expression of Nestin and Sox2 in NSCs and neurospheres. The lesion group showed a significant increase of REM and NREM sleep compared to the control group. After NSCs transplantationin, a huge diminish in REM and NREM sleep was observed in the experimental group compared to the lesion group. In TEM analysis, NSCs labeled with gold nanoparticles were observed in the injury area. Nanoparticles were evident in the cytoplasm and mitochondria of these cells.

Conclusions: The current study demonstrates that gold nanoparticles can interact well with NSCs and can be used to investigate cell transplantation. Also, NSCs transplantation improves sleep-wake cycle disruption after Suprachiasmatic nucleus lesion.

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* Corresponding Author Email: *Mohammadamini86@gmail.com*
vpirhajati.1@gmail .com

INTRODUCTION

NSCs are found in the subventricular zone (SVZ) and hippocampus of the central nervous system [1]. The SVZ in the lateral wall of the ventricles is an important location of neurogenesis in the brain [2]. Under appropriate differentiation medium, NSCs are differentiated into neurons and glial cells [3]. The Suprachiasmatic nucleus is associated with sleep-wake cycle management. The main characteristic of sleep in mammals is that it occurs at a predictable time during the sleep-wake cycle. This specific sleep pattern shows that the circadian regulation procedure plays a crucial function in regulating sleep time [4]. Sleeping time is different for different species, but follows a main rule: creatures are active and awake during the hours they are looking for food. That is, the times when they need to be alert, sleep is minimized. A study on animals with lesions to the suprachiasmatic nucleus region of the hypothalamus shows that this nucleus is an important structure that regulates the timing of the 24-hour circadian rhythm [5]. This nucleus is located above the optic chiasm and it is connected with the areas involved in sleep and wakefulness [6]. In normal conditions, the suprachiasmatic nucleus is reset during the day with the light entering the retina and during the night with the melatonin secretion from the pineal gland [7, 8].

The results of various studies show that damage to the suprachiasmatic nucleus increases sleep and decreases wakefulness in rats, mice, and monkeys [9]. The study of Single-cell recordings has shown that the activity of the suprachiasmatic nucleus during wakefulness is higher than in slow-wave sleep, and this study also shows that the suprachiasmatic nucleus is effective in promoting awakening [10]. Today, metallic nanoparticles are used for various biomedical investigations [11, 12]. One of the most widely used nanoparticles in neurological studies is gold nanoparticles [13]. Due to their high electron density, gold particles can be used in investigations based on high-energy rays such as electron microscopes [14]. Therefore, the aim of this study was to evaluate of NSCs transplantation on the sleep-wake cycle in the suprachiasmatic nucleus lesion in the rat through neural stem cells decorated with gold nanoparticles.

MATERIALS AND METHODS

Gold nanoparticles synthesis and characterizations

Gold nanoparticles were synthesized through the green chemistry method using apigenin as a reducing and coating agent based on the previously reported method [15-17]. Briefly, apigenin with a concentration of 0.3 mM was dissolved in Deionized water (pH 9-10) and brought to boiling temperature under vigorous stirring. Then, an aqueous chloroauric acid solution with the same volume and a concentration of 1.2 mM was slowly added to the apigenin solution. After the synthesis of nanoparticles, they were washed by a series of centrifugation and decantation processes according to the previous method for washing gold and silver particles synthesized with other plant compounds [18-20]. UV-Vis spectroscopy (SPEKOL 2000, Analytik Jena, UK) was used to investigate the plasmonic peak specific to nanoparticles. A transmission electron microscope (Zeiss EM 900, Germany) was used to evaluate the size and shape of nanoparticles. The gold concentration of samples was investigated through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (model: VISTA-PRO, Varian Co, Australia)

Animals

Thirty adults male Wistar rats (weight = 220-250 g) were classified into three groups (Lesion, control, and experimental (NSCs Transplantation)). We harvested NSCs from the SVZ of the newborn rat brain. We housed each animal individually in one cage with a cycle of 12 hours of light and 12 hours of darkness (22 ± 2 °C). Free access to food and water was provided for all animals. All studies were conducted based on the ethical guidelines set by the "Ethical Committee of Iran University of Medical Sciences" (IR.IUMS.REC.1398.1402).

Cell studies

NSCs from the SVZs of the newborn rat brain were harvested in this study. SVZs were digested in 0.002% deoxyribonuclease I (Sigma, Germany) plus 0.02% trypsin (Invitrogen, UK) for 5 min at 37 °C. After dissociation of the tissues into single cells, the NSCs were cultured in DMEM/F12 (Gibco, USA) with 2% B-27 serum-free supplement (Gibco, USA), 1% penicillin (Invitrogen, UK), 2 mML-glutamine (Gibco, USA) and 1% streptomycin (Invitrogen, UK) for 24 h. Then the cells were grown in 20 ng/ml hFGF (Royan, Iran), and 20 ng/ml hEGF (Calbiochem, USA) for two weeks. In the above media, isolated single cells formed neurospheres by day 14. The neurospheres grew as a globular structure during culture. The cells were

passaged every 14 days [21].

Immunocytochemistry

For identification of NSCs, we used 4% paraformaldehyde (Sigma, Germany) as fixation of cells for 20 min and washed trice in PBS. Then the cells were incubated in HCl, washed with buffer borate, and incubated with a blocking solution containing 0.3% Triton X-100 (Sigma, Germany) and 10% goat serum (Invitrogen, UK) in PBS for 30 min. For the immunocytochemistry technique, polyclonal anti-human Sox-2 antibody (Abcam, UK) and polyclonal anti-Nestin antibody (Abcam, UK) were used as primary antibodies. These primary antibodies were used overnight at 4°C. Then after washing the cells with PBS, we used polyclonal secondary antibody FITC-conjugated Goat anti-Rabbit IgG (Abcam, UK) [21]. Next, a fluorescent microscope (Olympus, Japan) was used for examination.

Surgery

Xylazine 2% (10 mg/kg) and ketamine 10% (100 mg/kg) were applied for animal anesthetization. Then rats received Suprachiasmatic nucleus lesions (2 mA current, 8 seconds), (-0.6 mm AP, 0.2 mm L, 9 mm V from bregma) in the stereotaxic apparatus [22]. Three EEG electrodes and Two EMG electrodes were implanted in the skull and into the dorsal neck muscle, respectively. EEG electrodes were implanted in the left frontal, right parietal, and left occipital cortex [23]. In the lesion group, we implanted a stainless-steel guide cannula (23 gauge) 1mm above SCN. For cell transplantation, seven days after the lesion, we injected 3,000,000 cells that had been treated with gold nanoparticles for 16 h, through the tail vein.

Sleep recordings

For sleep recording, we transferred the rats to a Faraday cage as a recording box (40 × 40 ×40 cm). We attached a flexible, shielded cable to the skull of the rats. A polygraph amplifier (SienceBeam, Tehran, Iran), amplified (×100) the signals and low-passed filtered at 100 Hz for EEG and 1 kHz for EMG recordings. 30-seconds epochs were used to analyze EEG/EMG recordings. The wake episode when it is more than 300 sec is called long duration wake (LDW). Also when it is less than 300 sec it is called a brief wake (BW). Then LDW episodes were used to recognize one sleep episode from another vigilance cycling [24].

For sleep recordings, 4-hour polygraphic recordings (EMG, EEG) [from 10.00 to 14.00 h] were performed. At least 24 hours of adaptation to the cage was applied before each recording. The Recordings were taken before the lesion (7 days after electrode implantation), and then 7, 14, 28, and 49 days following the lesions.

Sleep analysis

REM (Rapid Eye Movement) waves were marked by low-amplitude, high-frequency activity similar to wakefulness waves in the EEG, but EMG activity was absent. NREM (Non-Rapid Eye Movement) waves were marked by low-frequency, high-amplitude activity [25]. To secure the correct designation of vigilance states and remove any periods with artificial noise, all scores were inspected visually.

Transmission electron microscopy

For the TEM technique, brain tissues including SCN were washed with PBS, and then for primary fixation, 2.5% glutaraldehyde for 2 hours. Tissues were washed, 2-3 times with PBS to remove free glutaraldehyde. Then, for secondary fixation, we used 1% osmium tetroxide for 1.5 hours. Tissues were dehydrated in acetone (50, 70, 90, 100%), infiltrated by resin/acetone (1/1, 2/1, 3/1, and pure resin), and finally, for embedding we used pure resin (TAAB, Epon 812, UK). For light and electron microscopy we performed Semi-thin (500 nm) and thin (50 nm) sections respectively. Before imaging with TEM (LEO 906; Zeiss), we transferred thin sections on the 200-mesh grids and stained them with uranyl acetate and lead citrate[26].

Statistical Analysis

One-way analysis of variance (ANOVA) was employed to assess the differences among groups in the obtained data. The results are expressed as the mean ± standard error (SE) with a significance level of $P \leq 0.05$.

RESULTS

Characterization of gold nanoparticles

The size, shape, and size distribution of synthesized gold nanoparticles were characterized through TEM. Based on the TEM micrographs the particles are almost spherical with an average diameter of 22.1 ± 9.3 nm (Fig.1). UV-Vis absorption spectroscopy evaluation showed the synthesis of gold nanoparticles with a plasmonic

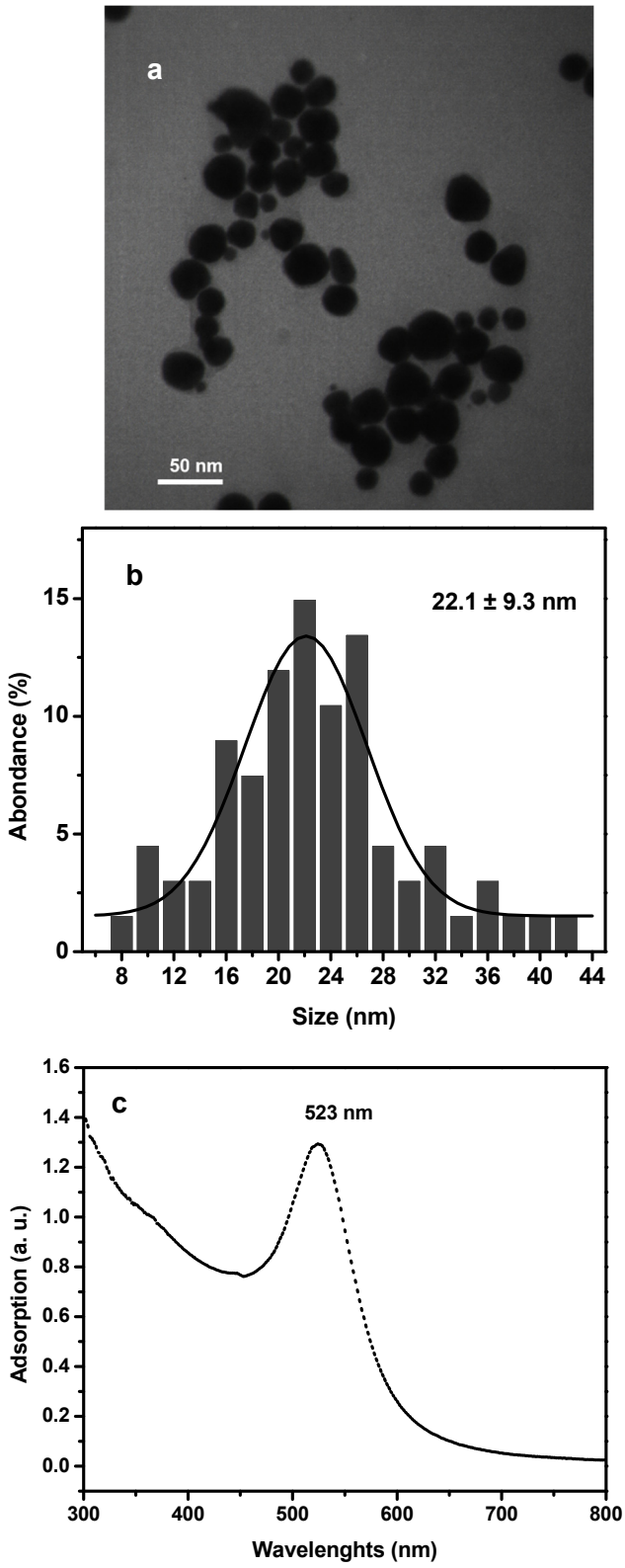

Fig. 1. Characterization of gold nanoparticles. Electron micrograph sample of gold nanoparticles (a), size distribution curve obtained from particles (b), A UV-Vis spectra of the gold nanoparticles (c).

peak specific to these nanoparticles at the maximum absorption of 523 nm.

Characterization of NSCs

Here, the cells were cultured in the form of neurospheres or clusters (Fig. 2). Immunocytochemistry with the individual antibodies against specific markers for NSCs including Nestin and Sox-2 was performed. As shown in Fig. 3, Nestin and Sox-2 markers were positive in neurospheres.

Effects of suprachiasmatic nuclei lesion and NSCs transplantation on the sleep-wake cycle

Following SCN lesions, the sleep-wake cycle was fragmented. Our results indicate lesions of SCN enhance NREM sleep, The EEG activity was composed of high-voltage, low-frequency waves in NREM episodes. After the lesion of SCN, the number of REM episodes increased. After the lesion, the amount of NREM sleep in the lesion group was significantly increased compared to the control group. After transplantation of NSCs, NREM sleep in the experimental group was significantly decreased in comparison with the lesion group. There were significant differences in REM sleep between the experimental and Lesion groups. Our results show that NSCs transplantation has the ability to improve the sleep-wake cycle after a SCN lesion (Fig. 4).

Ultrastructure in the NSCs and SCN after treatment with GNPs

We used TEM for ultrastructural analysis of NSCs and SCN after treatment with GNPs. In the electron microscope examination, the NSCs were healthy after treatment with gold nanoparticles (Fig. 5). Thin sections were prepared and examined with an electron microscope after staining. In ultrastructural analysis of SCN, normal cell

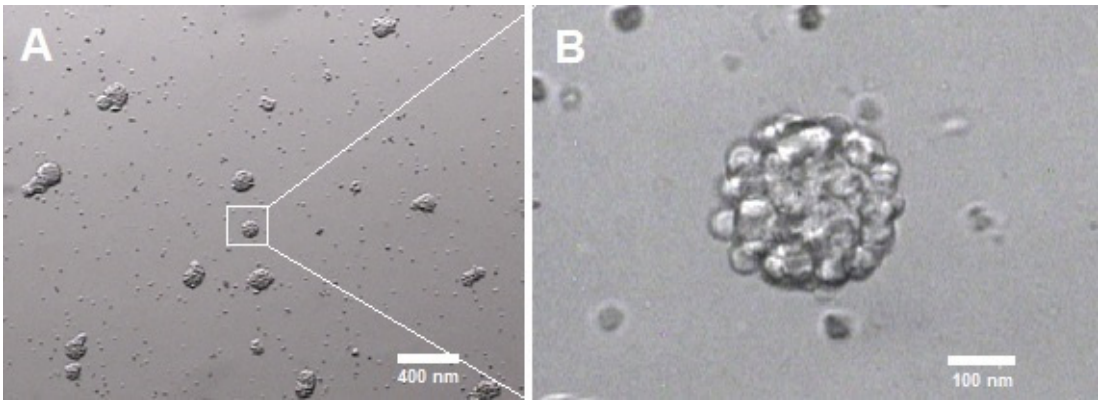

Fig. 2. Phase contrast images of NSCs. Neural stem cells have been harvested from the SVZ. NSCs proliferate to form 'neurospheres'.

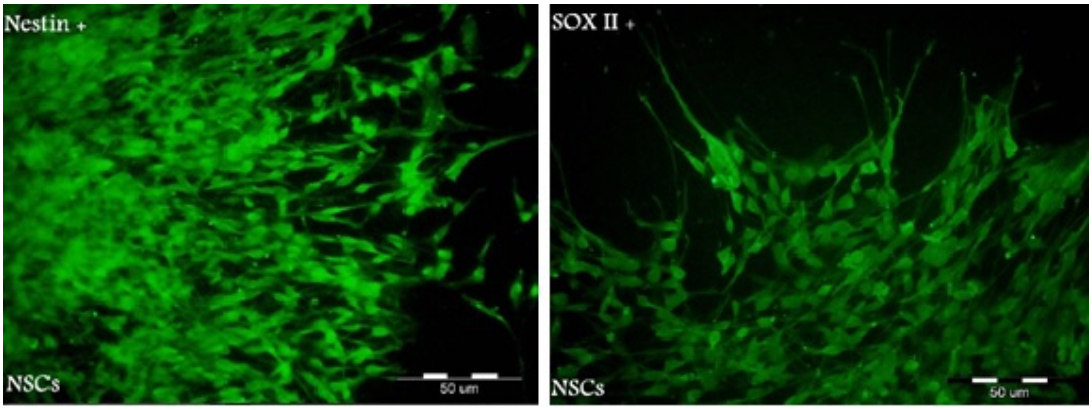

Fig. 3. Immunocytochemical analysis of neurosphere with the antibodies against Nestin, and Sox- 2 markers for neural stem cells.

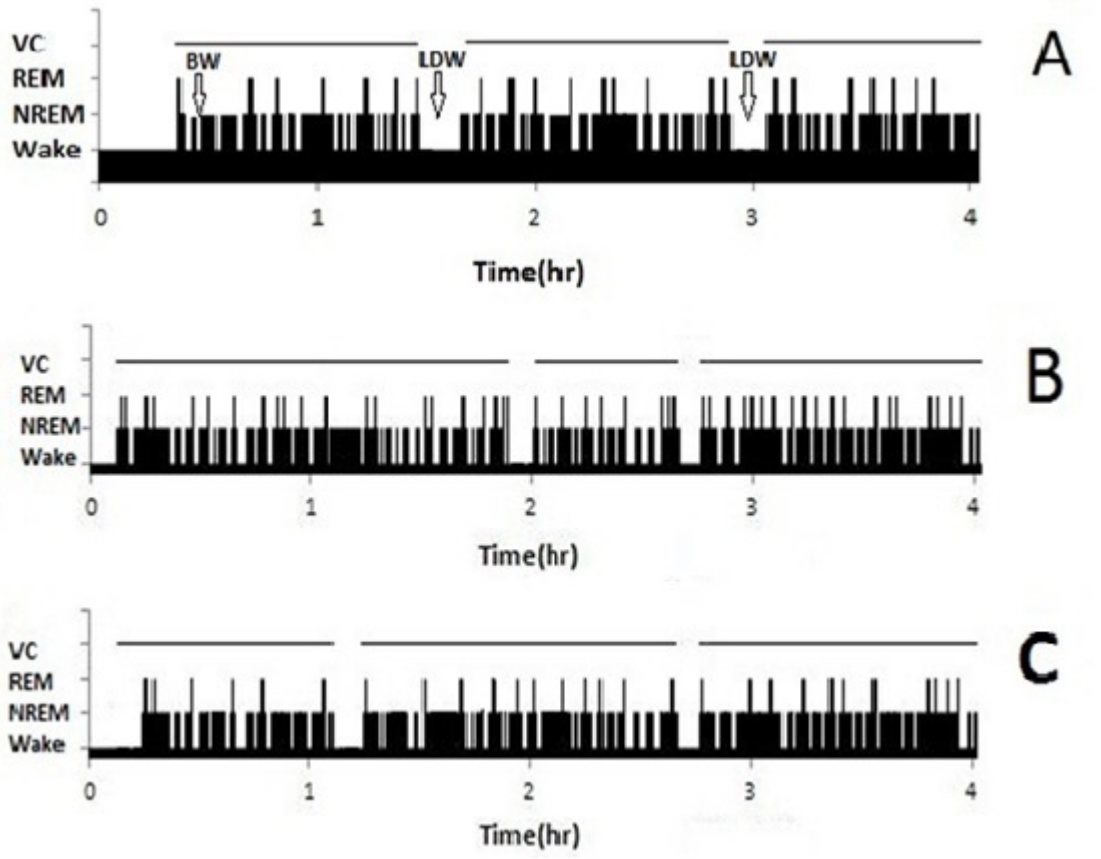

Fig. 4. Hypnograms from three representatives sleep recording. Control (A), lesion (B), experimental groups (C). Long Duration Wake (LDW), Brief Wake (BW).

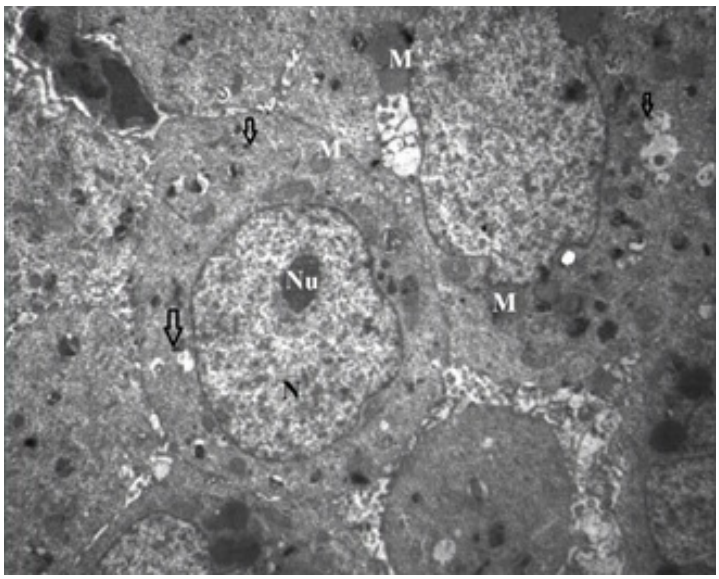

Fig. 5. Representative transmission electron micrographs from NSCs With gold nanoparticles. Nucleus (N), Nucleolus (Nu), Mitochondria (M). Gold nanoparticles were observed in the mitochondria and cytoplasm (black arrow).

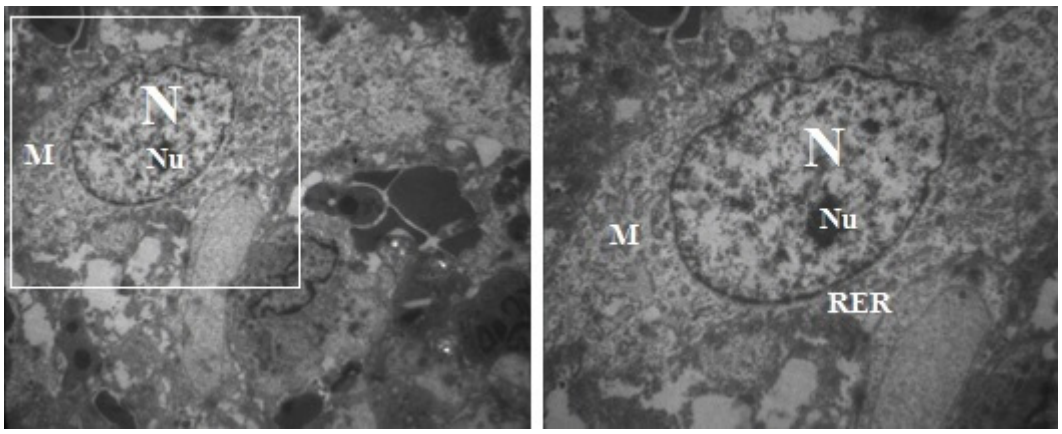

Fig. 6. Representative transmission electron micrographs from suprachiasmatic nucleus in the control group. The heterochromatin nucleus (N), nucleolus (Nu), Rough endoplasmic reticulum (RER) and Mitochondria (M) were observed in cells.

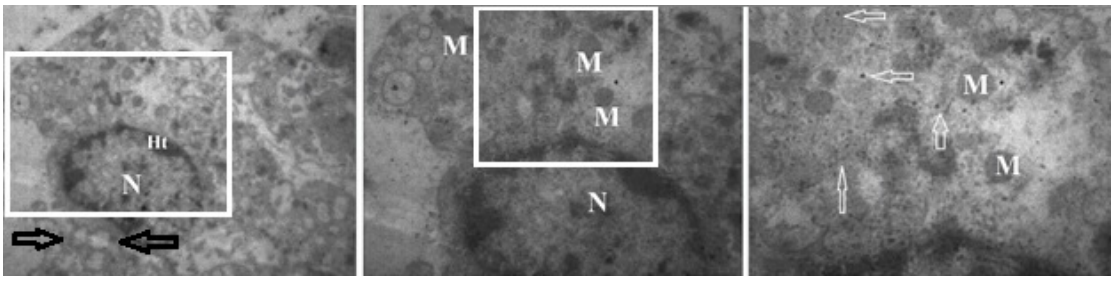

Fig. 7. Representative transmission electron micrographs from suprachiasmatic nucleus in the experimental group. The heterochromatin nucleus (N), Heterochromatin (Ht), and Mitochondria (M) were observed in cells. Gold nanoparticles were observed in the mitochondria and cytoplasm white arrow). Early neuronal connection (black arrow).

membrane, heterochromatin nucleus with intact membrane and three layers, rough endoplasmic reticulum, and mitochondria were observed (Fig. 6).

In the transplanted group, after preparing semithin sections and confirming the damaged area, thin sections were prepared and after staining, they were examined with an electron microscope. Cells labeled with gold nanoparticles were observed in the injury area. Nanoparticles were evident in the cytoplasm and mitochondria of these cells. In these cells, the nucleus was heterochromatin and had a normal membrane. In the examination of the tissue ultrastructure in the transplanted group, early neuronal connections were observed. Gold nanoparticles were detected in the cytoplasm, mitochondria, and autophagic vacuoles (Fig. 7).

DISCUSSION

Our study shows that NSCs can proliferate and form neurospheres. These cells require a cell culture medium supplemented with hEGF and hFGF without serum. Neurospheres expressed Nestin and Sox-2 markers. There are some studies on the lesion of SCN, however, such a procedure was not applied to the model for the study sleep-wake cycle [27, 28].

Previous research indicates that lesions in the suprachiasmatic nucleus (SCN) lead to alterations in the quantity of non-rapid eye movement (NREM) sleep. The average percentage of NREM sleep in the group with lesions was significantly higher when compared to the control group [29]. Consistent with earlier findings, our results demonstrate that an increase in REM sleep occurs following lesions to the suprachiasmatic nucleus.

Conversely, following cell transplantation, the average percentages of NREM sleep were notably reduced when compared to the lesion group. Prior research involving SCN lesions in rats

has demonstrated that such lesions promote an increase in the occurrence of REM sleep. Wei-Min et al showed that the SCN lesion affects the amount of REM and NREM but does not change the daily amount of sleep over 24h [27]. Certain studies indicate that the suprachiasmatic nucleus (SCN) enhances the frequency of attempts to achieve rapid eye movement (REM) sleep. While the SCN plays a crucial role in regulating the circadian rhythm of sleep, the findings suggest that lesions in the SCN do not affect the overall quantity of REM sleep or the transitions from non-rapid eye movement (NREM) to REM sleep [27, 30].

CONCLUSION

Our research collectively demonstrates that the size of the SCN lesion decreased following cell transplantation. Additionally, we observed that the experimental group exhibited a significant reduction in the amount of NREM sleep when compared to the lesion groups. These findings may prove valuable for investigations in the field of neurological diseases associated with frontal and other brain region impairments.

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REFERENCES

1. Fontán-Lozano, Á., et al., To become or not to become tumorigenic: subventricular zone versus hippocampal neural stem cells. Frontiers in Oncology, 2020. 10: p. 602217. https://doi.org/10.3389/fonc.2020.602217
2. Jurkowski, M.P., et al., Beyond the hippocampus and the SVZ: adult neurogenesis throughout the brain. Frontiers in cellular neuroscience, 2020. 14: p. 576444. https://doi.org/10.3389/fncel.2020.576444
3. Yoon, S.-H., et al., Efficient generation of neural stem cells from embryonic stem cells using a three-dimensional differentiation system. International Journal of Molecular Sciences, 2021. 22(15): p. 8322. https://doi.org/10.3390/ijms22158322
4. Deboer, T., Circadian regulation of sleep in mammals. Current Opinion in Physiology, 2020. 15: p. 89-95. https://doi.org/10.1016/j.cophys.2019.12.015
5. Mendelson, W.B., B.M. Bergmann, and A. Tung, Baseline and post-deprivation recovery sleep in SCN-lesioned rats. Brain research, 2003. 980(2): p. 185-190. https://doi.org/10.1016/S0006-8993(03)02896-8
6. Ma, M.A. and E.H. Morrison, Neuroanatomy, nucleus suprachiasmatic. 2019.
7. Blume, C., C. Garbazza, and M. Spitschan, Effects of light on human circadian rhythms, sleep and mood. Somnologie, 2019. 23(3): p. 147. https://doi.org/10.1007/s11818-019-00215-x
8. Arendt, J. and A. Aulinas, Physiology of the pineal gland and melatonin. Endotext [Internet], 2022.
9. Easton, A., et al., The suprachiasmatic nucleus regulates sleep timing and amount in mice. Sleep, 2004. 27(7): p. 1307-1318. https://doi.org/10.1093/sleep/27.7.1307
10. Sakai, K., Single unit activity of the suprachiasmatic nucleus and surrounding neurons during the wake-sleep cycle in mice. Neuroscience, 2014. 260: p. 249-264. https://doi.org/10.1016/j.neuroscience.2013.12.020
11. Shirkhanloo, H., et al., Novel Semisolid Design Based on Bismuth Oxide (Bi2O3) nanoparticles for radiation protection. Nanomedicine Research Journal, 2017. 2(4): p. 230-238.
12. Oroskhani N.,et al., Anti-Proliferative Activity of Poloxamer Cobalt Ferrite Nanoparticles against Human Prostate Cancer (DU-145) Cells: In-Vitro Study. IET Nanobiotechnol. 2024 Mar 20;2024:8929168. doi: 10.1049/2024/8929168. PMID: 39144409. https://doi.org/10.1049/2024/8929168
13. Amini, S.M., et al., Curcumin-gold nanoformulation: Synthesis, characterizations and biomedical application. Food Bioscience, 2024. 57. https://doi.org/10.1016/j.fbio.2023.103446
14. Amini, S.M., et al., Gold cluster encapsulated liposomes: theranostic agent with stimulus triggered release capability. Medical Oncology, 2023. 40(5): p. 126. https://doi.org/10.1007/s12032-023-01991-1
15. Mohammadi, E. and S.M. Amini, Green synthesis of stable and biocompatible silver nanoparticles with natural flavonoid apigenin. Nano-Structures and Nano-Objects, 2024. 38. https://doi.org/10.1016/j.nanoso.2024.101175
16. Neshastehriz A., et al., Overcoming breast cancer cell treatment resistance by optimizing sonodynamic therapy and radiation sensitizers on lncRNA PVT1 and miR-1204 expression. Photodiagnosis Photodyn Ther. 2024 Dec 5;51:104433. doi: 10.1016/j.pdpdt.2024.104433. https://doi.org/10.1016/j.pdpdt.2024.104433
17. Neshastehriz, A., et al., Combined sonodynamic therapy and X-ray radiation with methylene blue and gold nanoparticles coated with apigenin: Impact on MCF7 cell viability. International Journal of Radiation Research, 2024. 22(2): p. 509-513. https://doi.org/10.61186/ijrr.22.2.515
18. Amini, S.M. and S. Shahroodian, Antibacterial activity of silver and gold nanoparticles that have been synthesized by curcumin. Inorganic and Nano-Metal Chemistry: p. 1-7. https://doi.org/10.1080/24701556.2024.2352352
19. Akbari, A., et al., In-vitro investigation of curcumin coated gold nanoparticles effect on human colorectal adenocarcinoma cell line. Nanomedicine Research Journal, 2022. 7(1): p. 66-72.
20. Salavati, M.S., et al., Enhanced Colloidal Stability of Silver Nanoparticles by Green Synthesis Approach: Characterization and Anti-Leishmaniasis Activity. Nano, 2022. 17(07): p. 2250052. https://doi.org/10.1142/S1793292022500527
21. Wang, L., et al., Neural progenitor cell transplantation and imaging in a large animal model. Neuroscience research, 2007. 59(3): p. 327-340. https://doi.org/10.1016/j.neures.2007.08.011
22. Paxinos, G. and C. Watson, The rat brain in stereotaxic coordinates: hard cover edition. 2006: Elsevier.

23. Pirhajati Mahabadi, V., et al., Effects of bilateral lesion of the locus coeruleus on the sleep-wake cycle in the rat. Physiology and Pharmacology, 2015. 19(1): p. 22-30.
24. Simasko, S.M. and S. Mukherjee, Novel analysis of sleep patterns in rats separates periods of vigilance cycling from long-duration wake events. Behavioural brain research, 2009. 196(2): p. 228-236. https://doi.org/10.1016/j.bbr.2008.09.003
25. Takahashi, K., et al., Locus coeruleus neuronal activity during the sleep-waking cycle in mice. Neuroscience, 2010. 169(3): p. 1115-1126. https://doi.org/10.1016/j.neuroscience.2010.06.009
26. Eslahi, N., et al., In vitro cytotoxicity of folate-silica-gold nanorods on mouse acute lymphoblastic leukemia and spermatogonial cells. Cell Journal (Yakhteh), 2019. 21(1): p. 14.
27. Liu, X.-G., et al., Lesions of suprachiasmatic nucleus modify sleep structure but do not alter the total amount of daily sleep in rats. Sleep and Biological Rhythms, 2012. 10: p. 293-301. https://doi.org/10.1111/j.1479-8425.2012.00572.x
28. Purnell, B.S. and G.F. Buchanan, Free-running circadian breathing rhythms are eliminated by suprachiasmatic nucleus lesion. Journal of Applied Physiology, 2020. 129(1): p. 49-57. https://doi.org/10.1152/japplphysiol.00211.2020
29. Lee, M.L., B.E. Swanson, and H.O. de la Iglesia, Circadian timing of REM sleep is coupled to an oscillator within the dorsomedial suprachiasmatic nucleus. Current Biology, 2009. 19(10): p. 848-852. https://doi.org/10.1016/j.cub.2009.03.051
30. Wurts, S.W. and D.M. Edgar, Circadian and homeostatic control of rapid eye movement (REM) sleep: promotion of REM tendency by the suprachiasmatic nucleus. Journal of Neuroscience, 2000. 20(11): p. 4300-4310. https://doi.org/10.1523/JNEUROSCI.20-11-04300.2000