Original Article

Neuronal Spines Can be Affected by Static Magnetic Fields: The Impact on Microtubule Dynamic Nature

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Abstract

Recently, the hypothesis in which memory and information would be stored as magnetic forms in astrocytes is expanding and neuromagnetic interactions between neurons and neighboring astrocytes in neocortex have potential to be the basis of memory formation. It has been proposed that all sorts of information may be maintained in form of neuronal activity-associated magnetic fields (NAAMFs) and thereby alterations of magnetic fields in the brain may potentially affect the memory function. On the other hand, microtubules (MTs), the most essential elements of cytoskeleton, are crucial in regulation of spine development and morphology, brain cognitive behavior, consciousness and information storage. Because of MT dynamic nature, it can produce local magnetic field in neurons through vibration. According to size, number, structure and function of microtubule proteins, they are the most eligible components of neurons to be affected by endogenous and exogenous magnetic fields. In this study we tried to investigate the possible effects of exogenous static magnetic fields (SMFs) on memory through examining the structural and functional changes in MT dynamic activity and neural cell morphology. MT activity results revealed that MT polymerization process was not attained to steady state at the right time in the presence of SMF at 300 mT and the ascending slope at the steady state phase was found as abnormal. In addition, MT structure was relatively changed. On the influence of SMF, PC12 neuron-liked cells' spines decreased significantly and their morphology altered to pyramidal form.

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Introduction

There are several components in the human brain which are capable of producing magnetic fields (MFs) including brain cells (i.e. neurons and glial cells), biogenic magnetites, microtubule proteins, ferritin (an iron storage protein), iron in the blood of the brain, etc. Most human brain cells are neurons and glial cells which produce electric pulses due to ion currents through their membrane, which result in generation of their local surrounding MFs. The neuronal networks are electrically excitable and communicate with the neighboring cells through action-potential, a phenomenon by which waves of openings and closures of voltage-gated channels are spreading, that lead to trigger exocytosis of neurotransmitters (1). Glial cells supply essential energy for neuronal function, regulate efficacy of synapses and have also supportive roles for neurons (2). Astrocytes, a

sub-type of glial cells in the central nervous system (CNS), are crucial for controlling cerebral blood flow, synapse architecture and release of neurotransmitters for regulation of neuronal excitability (3). Neurons communicate with astrocytes through cell-cell adhesion junctions and intracellular Ca2+ signaling (2). Astrocytes tend to produce steady-state magnetic fields (dc) while neurons generate time-varying magnetic fields (ac) (4, 5). The strength of MFs are proportional to intracellular action currents but are inversely related to the distance from the cells (5). Circulating electric currents from nervous and other excitable tissues are able to produce MFs in an extremely low-frequency range (6). Since a single astrocyte connects to thousands of adjacent neurons via the relative synapses, it seems that the axonal MFs would alter resting astrocyte MFs (7). In recent studies, the hypothesis in which memory and information would be stored as magnetic forms in astrocytes is expanding. It is mentioned that neuromagnetic interactions between neurons and neighboring astrocytes in neocortex would be the basis of memory formation (2, 6-8). Astrocytes may serve as a dynamic information sink for neurons and the memory would be stored by organizing the activity of ion channels (9). However, there is a principal unanswered question about the genus of information and memory in the brain; how could we recall the events in a variety of times or forget them? It has been proposed that nearly all sorts of information could be maintained as the form of neuronal activity-associated magnetic fields (NAAMFs) (6). Thus, alterations of MFs in the brain is expected to affect memory function. The collaboration of the bundles of NAAMFs in close proximity to other neuronal circuits with neighboring NAAMFs would describe the ability of brain to recall or fade recent and remote events. This ability could be changed through the alterations of the principal characteristics (frequency, intensity and spatial orientation) of NAAMFs in a specific neuronal circuit (6). NAAMFs may polarize the static astrocyte MFs in the vicinity of neuronal networks affecting the memory. Alterations of static astroglial MFs around the neuronal networks would probably modify some passive membrane properties of neurons. It seems that levels of consciousness and wakefulness are generally linked to the neuronal and astroglial activities across the cerebral cortex (7).

Biogenic ferromagnetic magnetites (Fe3O4) were discovered in various regions of human brain associated with memory (10, 11). Magnetites are also distributed in cell membrane of neurons and glial cells and could play some roles in biochemical or biophysical processes of membrane ion channels (12, 13). Biogenic magnetites constitute permanent magnetic crystals as single-domain forms which are mentioned as biological bar magnets (12). It is suggested that magnetites could have an impact on magnetic signals inside the neocortex. NAAMFs tend to affect the orientation and distribution of biomagnetites in neuronal and astroglial membrane. It seems that the brain magnetites which are distributed through neuronal and astroglial networks, are conserved during the evolution and play roles in information transduction and storage in the neocortex (13, 14).

On the other hand, several studies have suggested that microtubule (MT) proteins are involved in brain cognitive behaviors, consciousness, information storage, etc. (15-17) and are able to generate electromagnetic fields (EMFs) around themselves with strong electric components through vibration (18, 19). MTs are one of the most important proteins in the brain cells especially neuronal axons, which play key roles in signal transduction and vesicle transport (20-22). Furthermore, MT dysfunction is observed in neurodegenerative diseases (23-25). MTs are composed of polar subunits called tubulin which has dipole moments about 1714 Debye (26, 27) demonstrating magnetic anisotropic properties (28). MTs which are negatively charged are mostly located on C-terminal regions (27, 29). Vibrations of tubulin heterodimers could result in EMF generation (19, 30, 31) which participate in conduction pathways for electron mobility and consciousness (32). The essential energies for excitation of MT vibration are possibly supplied by energy released from GTP hydrolysis during dynamic instability of MTs, energy transferred from moving of motor proteins on MTs and energy released from mitochondria as "wasted energy". The frequencies of MT oscillations are estimated to range between kHz to GHz (19, 33). MT proteins are able to constitute parallel orientation under the exposure of exogenous MFs (34). Since MTs represent ferromagnetic behaviors (35, 36), changes of environmental MFs can affect the MT stabilization and function(37).

According to above evidences, MT networks could be involved in magnetic communication in neocortex. Therefore, the interactions between MTs and MFs are crucial and must be taken into account. Hence, PC12 cells were utilized as a model of sympathetic neurons to survey the effects of static magnetic fields (SMFs) on morphological properties of neural cells. In this study, we investigated the effects of external SMF on MT structure and function as an important molecule in

neocortical magnetic communication and magnet bars were exploited as a source of SMF producer. MT polymerization and structure were studied by turbidity method, electron microscopy and fluorescence spectroscopy.

Materials and Methods

- *Tubulin preparation and purification*

Tubulin was prepared freshly from sheep brain after homogenization in PEM buffer (100 mM PIPES pH 6.9, 2 mM MgSO4 and 1 mM EGTA) followed by two cycles of temperature-dependent polymerization-depolymerization. Polymerization of MT protein was accomplished by adding 1 mM GTP and 3.4 M glycerol at 37^{0}C. Depolymerization process took place at 4^{0}C (38, 39). Purified tubulin (without MT-associated proteins) was obtained after application of phosphocellulose P11 column chromatography (39). Tubulin fractions were immediately frozen in the liquid nitrogen and stored at -70^{0}C for further experiments. Acquired tubulin was highly pure as analyzed by SDS-PAGE (data was not shown) and two distinct bonds of α and β subunits were clearly observed. Tubulin concentration was measured 2 mg/ml using Bradford method (40).

- *Analysis of tubulin assembly*

Tubulin assembly was recorded by turbidity measurement (41) using a Cary-100 Bio UV/Visible spectrophotometer equipped with a temperature controlled accessory (Varian, Australia). Two magnet bars were located at two opposite sides of cuvette holder to study the effect of SMFs during tubulin polymerization process. Tubulin assembly was initiated by addition of 1 mM GTP at 37^{0}C and monitored at 350 nm for 30 min.

- *Measurement of fluorescence emission*

All fluorescence experiments were carried out using a Cary eclipse fluorescence spectrophotometer (Varian, Australia). A magnet bar was located on the top of cuvette holder to produce SMFs during the detection of tubulin fluorescence emission.

8-anilino-1-naphthalenesulfonic acid (ANS) was used for further investigation on tubulin conformational changes under the SMF exposure. Tubulin was incubated with 50 μM ANS at 4^{0}C for 7 min. Fluorescence emission of ANS bounded to tubulin was recorded between 450-650 nm every 10 min followed by excitation at 380 nm (42, 43).

- *Transmission electron microscopy (TEM)*

Tubulin proteins were assembled to the MTs during the exposure of SMFs generated by a magnet bar. MT proteins were diluted to 0.5 mg/ml in PEM buffer with 3.4 M glycerol at 37^{0}C. The samples containing assembled MTs (10 μl) were loaded on formvar-coated TEM grids and incubated at room temperature for 1 min. The liquid was subsequently wicked-off with a filter paper. Uranyl acetate 1% (10μl) was loaded on the grids for negative staining and wicked-off with a filter paper after 1 min. Sample grids were visualized using a HU-12A transmission electron microscope (Hitachi, Japan).

- *Cell culture*

PC12 cells were utilized to assess the effect of SMFs on neural cells. PC12 cells were derived from a rat pheochromocytoma, a tumor of the adrenal medulla (44). A frozen stock of PC12 cells was grown for 7 days in RPMI-1640 (Gibco) medium containing 5% horse serum (Gibco), 15% fetal bovine serum (Gibco), 100 mg/ml streptomycin and 100 mg/ml penicillin in a 96 well cell culture plate in a 5% CO2 incubator at 37°C. Afterwards, a magnet bar was located at one side of the plate to produce the SMF gradient along the plate. PC12 cells were growth under the described conditions for 48 – 72 hours and were visualized using Axiovert 25 inverted microscope (Ziess, Germany). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay were performed in a 96 well cell culture plate (45) to assess the cells viability in the presence of the SMF gradient using Powerwave XS2 microplate spectrophotometer (BioTek, USA).

- *Statistical analysis*

All experiments were conducted at least three times and the final data were presented as the mean±S.E.M. In this study, the difference between means was determined by one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test for multiple comparisons and the changes were considered significant at $P< 0.001$. All statistics were performed with Sigma Plot Version 12 (Systat Software Inc., CA).

Results

- *Enhancement of tubulin assembly*

Tubulin was polymerized in the presence of the SMF generated by the magnet bar. The absorbance of tubulin solutions at 350 nm was increased 10% and 30% in the presence of 150 mT and 300 mT SMF respectively (Figure 1A) meaning the enhancement of MT levels. The linear slope of MT

elongation phase was also increased 3% and 17% respectively as indicated in Fig. 1B. MT polymerization process was not attained to steady state at the right time in the presence of SMF at 300 mT and the ascending slope at the steady state phase was abnormally observed in Figure 1A. As a result, it seems that the SMF could also disturb the steady state phase of MT polymerization. To evaluate the effects of SMFs on MT proteins, polymerization was performed normally until the end of elongation phase of tubulin assembly and then the magnet bar was employed to produce SMF for formed MTs. No differences in absorbance at 350 nm were observed (data was not shown), meaning that MT polymers were not affected by the SMF. It seems that SMF affects the tubulin dimers during polymerization.

TEM was employed to support the existence of MT polymers in the presence of SMF. MTs were visualized clearly as hollow cylindrical rods. The elevated levels of MTs were observed in the presence of SMF (Figure 2).It seems that the straight orientation of MT rods was affected and reclined under SMF exposure (Figure 2). MT density was measured as high as 14±5 MTs/μm^2 in the presence of SMF while the control was 5±1 MTs/μm^2 at the same conditions. Consequently, acquired results of UV/Visible spectrophotometer was supported by TEM outcomes.

- *SMF alters the hydrophobic pockets of tubulin structure*

Fluorescence emission of ANS binding to tubulin was recorded between 450–650 nm followed by excitation at 380 nm for further investigations. The fluorescence intensity was considerably increased 35% after 60 min exposure of 50 mT SMF (Figure 3). Bovine serum albumin (BSA) was used instead of tubulin as a control and the same experiment with the same conditions was repeated. No significant changes were found in the fluorescence emission of ANS binding to BSA in the presence of SMF (data was not shown). It indicates that the SMF affects the tubulin structure specifically and makes tubulin hydrophobic regions to be exposed.

PC12 cells were incubated in the presence of SMF gradient for 72 hours using a magnet bar. We found PC12 cells to appear abnormally near the magnet bar (Figure 4B). Whatever the cells shifted far from the magnet bar where the strength of SMF became weaker, spindle and spherical forms of PC12 cells were normally found (Figure 4A). This indicates that SMF affects the shape of PC12 cells. MTT colorimetric assays did not indicate any remarkable changes in PC12 cells viability neither near nor far from the magnet bar.

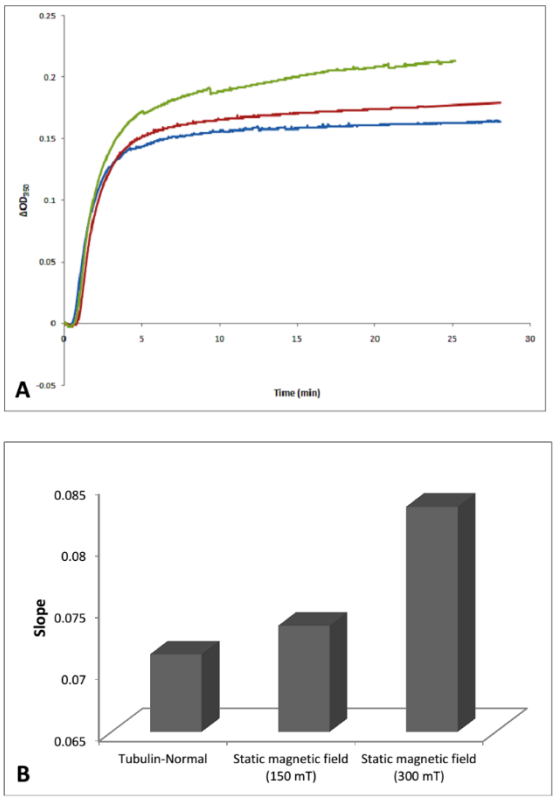

Figure 1. A) Tubulin assembly in the presence of 150 mT (red line) and 300 mT (green line) SMF, respectively. B) The linear slope of MT elongation phase; The absorbance at 350 nm was recorded at 37°C in the presence of Mg2+GTP 1 mM.

Discussion

Static magnetic fields (SMFs) affect macromolecules and living cells through three mechanisms; 1) SMFs exert effective forces on molecules to make ion flows leading to electric currents, 2) SMFs induce magnetic torques on ferromagnetic materials and certain molecules which possess dipole moments, 3) SMFs modify energy levels and spin orientation of electrons in macromolecules (46, 47).

Dynamic property of MT proteins is crucial for neural cells function. Hence, changes of such specificity affect the axonal functions. Analysis of UV-Visible spectroscopy results revealed that SMFs increased MT polymerization. Tubulin has anisotropic properties and MT displays ferromagnetic behavior which has been shown to be affected by magnetic particles (48). Since MFs interact directly with ferromagnetic and anisotropic

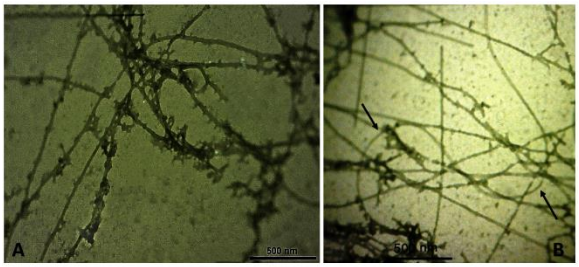

Figure 2. TEM images of polymerized MTs in the absence (A) and presence of 300 mT SMF (B). The arrows indicate the recline of MT rods under SMF exposure; Tubulin assembly was initiated by addition of 1 mM GTP at 37^{0}C.

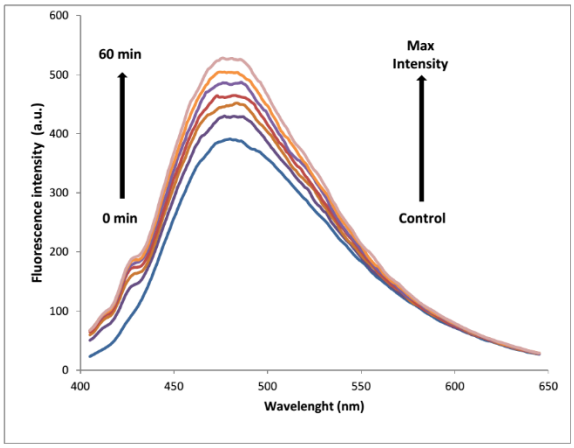

Figure 3. Tubulin was incubated with 50 µM ANS at 4^{0}C for 7 min. Fluorescence emission of ANS bounded to tubulin was recorded between 450-650 nm every 10 min under the SMF exposure followed by excitation at 380 nm.

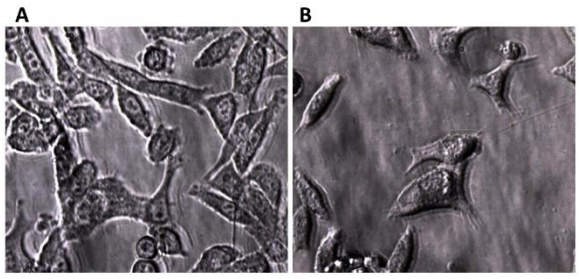

Figure 4. Light microscopy images of PC12 cells in the absence (A) and presence of 300 mT SMF (B)

materials resulting in charge currents (46), SMF affects the MTs and may induce magnetic dipoles in tubulins. It is proposed that cylindrical shape of MT could be the outcome of tubulin dipole–dipole interactions (49). Hence, such interactions would be established more powerful under the exposure of SMFs and the relative released energy would be increased. SMFs would induce torques on dipolar proteins such as tubulin, which could lead to oscillations of bound charges, rotation and reorganization of assembling tubulins (50). The ascending linear slope of MT elongation phase indicated that the rate of MT polymerization was also increased. Hydrolysis of GTP to GDP could supply enough energy for MT vibrations (19) and is essential for tubulin assembly. We propose that SMFs could provide a part of required energy for MT vibrations leading to increase collision plausibility of tubulin dimers which could consequently elevate the rate of MT polymerization and organization (51).

According to increasing of ANS fluorescence emissions, it seems that third structure of tubulin was changed. Tubulin dimers possess two hydrophobic regions which include 36 unpaired electrons (52). Hence, motion of delocalized electrons along MTs is possible and this mediates charge transfer in the tubulin structure (18). Transition of electrons between proteins results in conformational changes (53). Therefore, SMFs exerted magnetic forces on tubulin hydrophobic pockets leading to alteration of surrounding electron clouds. Spin of uncoupled electrons could orient toward MFs which would induce conformational changes. Hence, the hydrophobic regions of tubulin dimers were unpacked in a time-dependent manner. Since interactions of MT subunits are based on hydrophobic regions (54), tubulin–tubulin interactions would be strengthened by the exposure of SMFs.

The outcome of cell culture demonstrated that pyramidal forms of PC12 cells were found in the presence of SMFs but no significant changes were occurred in the cells viability. The alteration of cells shape and surface morphology is connected to cytoskeleton organization (55) including MT arrangements. Therefore, changes of MT polymerization and tubulin structure would alter the cell shape and size. On the other hand, each tubulin heterodimer binds to 18 Ca2+ (18, 36), thereby SMFs would affect either intracellular MTs structure directly or Ca2+ levels in the cells leading to morphological changes of the cells.

Conclusion

According to effects of SMFs on MT proteins, we propose that MT networks are involved in neuromagnetic communications in neocortex due to electromagnetic properties of MTs. Generated MFs by action-potential would assist MT dynamics, orientation and signal transduction in axons trough excitation of MT vibration, induction of magnetic dipoles in tubulin dimers, orientation and flow of unpaired electrons. Therefore, alterations of MFs in brains or exposure to exogenous MFs would affect

axonal MT structure, polymerization and orientation giving rise to changes in neural cells' shape and function, and decline of PC12 neuron-liked cells spines. Morphological changes of neural cells would disturb the synaptic relationships of neural networks. However, we suggest that usage of SMFs may promote tubulin self-assembly in patients with neurodegenerative disorders but SMFs should not be applied for long times.

Abbreviations

ANS, 8-anilino-1-naphthalenesulfonicacid; BSA, Bovine serum albumin; CNS, central nervous system; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid; EMF, electromagnetic field; GDP, guanosine 5´-diphosphate; GTP, guanosine 5´-triphosphate; MT, microtubule; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAAMF, neuronal activity-associated magnetic field; PIPES, piperazine-1,4-bis(2-ethanesulfonic acid); SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SMF, static magnetic field; TEM, transmission electron microscopy

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References

1. Verkhratsky A. Physiology of neuronal-glial networking. Neurochem Int. 2010 Nov;57(4):332-43.
2. Banaclocha MA. Architectural organisation of neuronal activity-associated magnetic fields: a hypothesis for memory. Med Hypotheses. 2004;63(3):481-4.
3. Fellin T. Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. J Neurochem. 2009 Feb;108(3):533-44.
4. Wikswo JP, Barach JP, Freeman JA. Magnetic field of a nerve impulse: first measurements. Science. 1980 Apr 4;208(4439):53-5.
5. Roth BJ, Wikswo JP, Jr. The magnetic field of a single axon. A comparison of theory and experiment. Biophys J. 1985 Jul;48(1):93-109.
6. Banaclocha MA. Are neuronal activity-associated magnetic fields the physical base for memory? Med Hypotheses. 2002 Nov;59(5):555-9.
7. Banaclocha MA. Neuromagnetic dialogue between neuronal minicolumns and astroglial network: a new approach for memory and cerebral computation. Brain Res Bull. 2007 Jun 15;73(1-3):21-7.
8. Martinez Banaclocha MA. Magnetic storage of information in the human cerebral cortex: a hypothesis for memory. Int J Neurosci. 2005 Mar;115(3):329-37.
9. Caudle RM. Memory in astrocytes: a hypothesis. Theor Biol Med Model. 2006;3:2.
10. Kirschvink JL, Kobayashi-Kirschvink A, Woodford BJ. Magnetite biomineralization in the human brain. Proc Natl Acad Sci U S A. 1992 Aug 15;89(16):7683-7.
11. Dobson J. Nanoscale biogenic iron oxides and neurodegenerative disease. FEBS Lett. 2001 May 4;496(1):1-5.
12. Kirschvink JL, Kobayashi-Kirschvink A, Diaz-Ricci JC, Kirschvink SJ. Magnetite in human tissues: a mechanism for the biological effects of weak ELF magnetic fields. Bioelectromagnetics. 1992;Suppl 1:101-13.
13. Banaclocha MA, Bokkon I, Banaclocha HM. Long-term memory in brain magnetite. Med Hypotheses. 2010 Feb;74(2):254-7.
14. Bokkon I, Salari V. Information storing by biomagnetites. J Biol Phys. 2010 Jan;36(1):109-20.
15. Mershin A, Kolomenski AA, Schuessler HA, Nanopoulos DV. Tubulin dipole moment, dielectric constant and quantum behavior: computer simulations, experimental results and suggestions. Biosystems. 2004 Nov;77(1-3):73-85.
16. Faber J, Portugal R, Rosa LP. Information processing in brain microtubules. Biosystems. 2006 Jan;83(1):1-9.
17. Hameroff SR, Watt RC. Information processing in microtubules. J Theor Biol. 1982 Oct 21;98(4):549-61.
18. Pokorny J, Hasek J, Jelinek F. Electromagnetic Field of Microtubules: Effects on Transfer of Mass Particles and Electrons. Journal of Biological Physics. 2005;31(3-4):501-14.
19. Pokorny J. Excitation of vibrations in microtubules in living cells. Bioelectrochemistry. 2004 Jun;63(1-2):321-6.
20. Downing KH, Nogales E. Tubulin structure: insights into microtubule properties and functions. Curr Opin Struct Biol. 1998 Dec;8(6):785-91.
21. Luduena RF, Shooter EM, Wilson L. Structure of the tubulin dimer. J Biol Chem. 1977 Oct 25;252(20):7006-14.
22. Nogales E. Structural insights into microtubule function. Annu Rev Biochem. 2000;69:277-302.
23. Iqbal K, Grundke-Iqbal I, Zaidi T, Merz PA, Wen GY, Shaikh SS, et al. Defective brain microtubule assembly in Alzheimer's disease. Lancet. 1986 Aug 23;2(8504):421-6.
24. Shevtsov PN, Shevtsova EF, Burbaeva G, Bachurin SO. Disturbed assembly of human cerebral microtubules in Alzheimer's disease. Bull Exp Biol Med. 2006 Feb;141(2):265-8.
25. Cappelletti G, Surrey T, Maci R. The parkinsonism producing neurotoxin MPP+ affects microtubule dynamics by acting as a destabilising factor. FEBS Lett. 2005 Aug 29;579(21):4781-6.
26. Stracke R, Bohm KJ, Wollweber L, Tuszynski JA, Unger E. Analysis of the migration behaviour of single microtubules in electric fields. Biochem Biophys Res Commun. 2002 Apr 26;293(1):602-9.
27. J. A. Tuszynski, J. A. Brown, E. Crawford, Carpenter EJ. Molecular Dynamics Simulations of Tubulin Structure and Calculations of Electrostatic Properties of Microtubules. Mathematical and Computer Modelling. 2005;41(10):1055-70.
28. Emura R, Ashida N, Higashi T, Takeuchi T. Orientation of bull sperms in static magnetic fields. Bioelectromagnetics. 2001 Jan;22(1):60-5.
29. Minoura I, Muto E. Dielectric measurement of individual microtubules using the electroorientation method. Biophys J. 2006 May 15;90(10):3739-48.
30. Havelka D, Cifra M. Calculation of the Electromagnetic Field Around a Microtubule. Acta Polytechnica. 2009;49:58-63.
31. J. Pokorny , F. Jelinek, V. Trkal , I. Lamprecht, Holzel R. Vibrations in Microtubules Journal of Biological Physics. 1997;23(3):171-9.
32. Hameroff S, Nip A, Porter M, Tuszynski J. Conduction pathways in microtubules, biological quantum computation, and consciousness. Biosystems. 2002 Jan;64(1-3):149-68.
33. Cifra M, Pokorny J, Havelka D, Kucera O. Electric field generated by axial longitudinal vibration modes of microtubule. Biosystems. 2010 May;100(2):122-31.
34. Vassilev PM, Dronzine RT, Vassileva MP, Georgiev GA. Parallel arrays of microtubules formed in electric and magnetic fields. Biosci Rep. 1982 Dec;2(12):1025-9.
35. Brown JA, Tuszynski JA. A review of the ferroelectric model of microtubules. Ferroelectrics. 1999;220(1):141-55.

36. Tuszyński JA, Hameroff S, Satarić MV, Trpisová B, Nip MLA. Ferroelectric behavior in microtubule dipole lattices: Implications for information processing, signaling and assembly/disassembly. Journal of Theoretical Biology. 1995;174(4):371-80.
37. Afrasiabi A, Riazi GH, Dadras A, Tavili E, Ghalandari B, Naghshineh A, et al. Electromagnetic fields with 217 Hz and 0.2 mT as hazardous factors for tubulin structure and assembly (in vitro study). J Iran Chem Soc. 2014;11(5):1295-304.
38. Williams RC, Jr., Lee JC. Preparation of tubulin from brain. Methods Enzymol. 1982;85 Pt B:376-85.
39. Miller HP, Wilson L. Preparation of microtubule protein and purified tubulin from bovine brain by cycles of assembly and disassembly and phosphocellulose chromatography. Methods Cell Biol. 2010;95:3-15.
40. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976 May 7;72:248-54.
41. Gaskin F, Cantor CR, Shelanski ML. Turbidimetric studies of the in vitro assembly and disassembly of porcine neurotubules. J Mol Biol. 1974 Nov 15;89(4):737-55.
42. Bhattacharyya B, Wolff J. The interaction of 1-anilino-8-naphthalene sulfonate with tubulin: a site independent of the colchicine-binding site. Arch Biochem Biophys. 1975 Mar;167(1):264-9.
43. Steiner M. Flourescence studies of platelet tubulin. Biochemistry. 1980 Sep 16;19(19):4492-9.
44. Greene LA, Tischler AS. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci U S A. 1976 Jul;73(7):2424-8.
45. Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, et al. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res. 1988 Sep 1;48(17):4827-33.
46. Repacholi MH, Greenebaum B. Interaction of static and extremely low frequency electric and magnetic fields with living systems: health effects and research needs. Bioelectromagnetics. 1999;20(3):133-60.
47. Valberg PA, Kavet R, Rafferty CN. Can low-level 50/60 Hz electric and magnetic fields cause biological effects? Radiat Res. 1997 Jul;148(1):2-21.
48. Dadras A, Riazi GH, Afrasiabi A, Naghshineh A, Ghalandari B, Mokhtari F. In vitro study on the alterations of brain tubulin structure and assembly affected by magnetite nanoparticles. J Biol Inorg Chem. 2013 Mar;18(3):357-69.
49. Schoutens JE. Dipole–Dipole Interactions in Microtubules. Journal of Biological Physics. 2005;31(1):35-55.
50. Repacholi MH. Low-level exposure to radiofrequency electromagnetic fields: health effects and research needs. Bioelectromagnetics. 1998;19(1):1-19.
51. Baulin VA, Marques CM, Thalmann F. Collision induced spatial organization of microtubules. Biophys Chem. 2007 Jul;128(2-3):231-44.
52. Mavromatos NE, Mershin A, Nanopoulos DV. QED-cavity model of microtubules implies dissipationless energy transfer and biological quantum teleportation. International Journal of Modern Physics B. 2002;16(24):3623-42.
53. Cavelier G. Short note: are electron-transport and electron-transfer involved in intracellular signaling? Med Hypotheses. 1995 Apr;44(4):261-2.
54. Hanssens I, Baert J, Van Cauwelaert F. Effect of guanine nucleotides on the hydrophobic interaction of tubulin. Biochemistry. 1990 May 29;29(21):5160-5.
55. Santoro N, Lisi A, Pozzi D, Pasquali E, Serafino A, Grimaldi S. Effect of extremely low frequency (ELF) magnetic field exposure on morphological and biophysical properties of human lymphoid cell line (Raji). Biochim Biophys Acta. 1997 Jul 24;1357(3):281-90.