Computational study on the binding affinity between microtubules and consciousness-altering substances

R. Pizzi, T. Rutigliano, A. Ferrarotti and M. Pregnolato

Abstract **-** Microtubules (MTs, protein polymers of tubulin) are very important in a number of cellular processes: they are part of cell cytoskeleton and are involved in cell division and intracellular transport. Their quantum properties inside neurons are also supposed to be involved in the consciousness process. A number of drugs are able to bind to tubulin and MTs and modify their activation states. To investigate the hypothesis of a possible correlation between conscious states and these molecular interactions we simulated the binding affinity between MTs and three psychotropic ligands, namely heroin, cocaine and LSD.
After preparing ligands using Molecular Dynamics, we applied docking procedures analyze the microtubule-neuropeptide complex. The study highlights that the drugs bind differently with respect to the control ligand, taxol. Moreover, the study shows that psychoactive ligands bind differently in MTs with respect to tubulin, confirming previous studies on the importance of the MTs conformation.

Keywords – binding, bioinformatics, cocaine, consciousness, docking, heroin, LSD, microtubules, Molecular Dynamics, tubulin.

I. INTRODUCTION

A. Background

Microtubules (MTs) are key constituents of all eukaryotic cells cytoskeleton. They are responsible of cell shape, they act in a variety of cell movements, provide platforms for intracellular transport of organelles and virus [1,2], and are also involved in cell division (mitosis and meiosis). MTs filaments are characterized by a tubelike structure, the building block is the protein called tubulin [3]. Microtubules are dynamic structures that undergo continual assembly and disassembly within the cell. This dynamic nature makes MTs sensitive to several pharmacological agents that are able to destroy or stabilize their structure [4]. In the last decade many theories and papers have been published concerning the biophysical properties of MTs. Their properties inside neurons are also supposed to be involved in the memory storage [5]. The hypothesis of MTs implication in coherent quantum states in the brain, evolving in some form of information transfer, must also be cited [6]. Since the nineties many theories and experiments have being developed and carried out that suggest an evident sensitivity of microtubules to electromagnetic waves and the possible role of this sensitivity in the consciousness process [7,8,9].

Pizzi et al. [10,11] evaluated some biophysical properties of MTs by means of specific physical measures of resonance and birefringence to assess the structural sensitivity of microtubules in presence of electromagnetic field. The experimental results highlighted a physical behaviour of MTs in comparison with tubulin: MTs react in a different way compared to tubulin. The dynamic simulation of MT and tubulin subjected to electromagnetic field was performed via Molecular Dynamics (MD) tools. The tubulin, despite its symmetric structure, seems to have different internal forces that tend to resist a dynamic stabilization. However, in the presence of electric field, although it tends to squash, it does not show any particular reaction. Instead, microtubules react sharply to electromagnetic fields both in the experimental tests and in the simulations. The same simulation performed with an ad-hoc self-organized Artificial neural network show a MTs dynamic organization much stronger than the tubulin one, and a dramatic increase in the spatial organization. Besides, MTs, despite their structural complexity, show a strong dynamic stability, which the electric field, after an initial transient, improves significantly [12].

The different behavior between microtubules and tubulin suggests that the tubular shape of MTs could be responsible of their peculiar properties, as in the case of carbon nanotubes, that have same size and shape of microtubules and exhibit analogous (quantum) properties due to their antenna-like spatial structure [13].

R. Pizzi is Senior Researcher at the Department of Computer Science of the University of Milan, Italy (corresponding author, tel. 0039 02 503 30072, e-mail rita.pizzi@unimi.it), T. Rutigliano is PhD Student of the same Department, M. Pregnolato is Associate Professor at the Department of Drug Sciences, University of Pavia, Italy., A. Ferrarotti is PhD Student of the same Department.

This paper aims to further investigate the interaction between tubulin, MTs and consciousness-related processes using a novel approach, namely by analyzing the binding of a set of consciousness-altering drugs to tubulin and MTs.

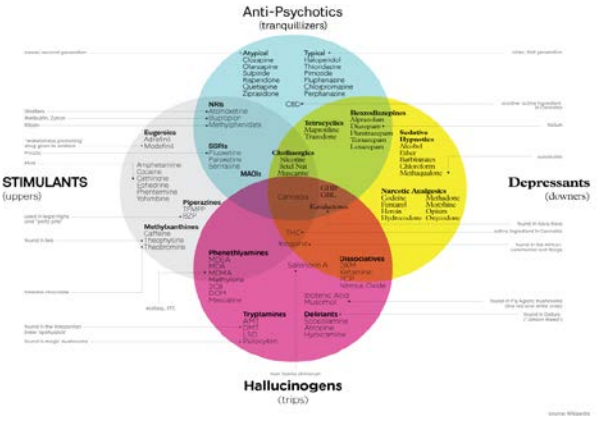


Fig.1 Psychoactive drugs.
Courtesy of Derek Snider

A number of drugs are able to bind to tubulin and modify its activation state [14,15]. In particular, we are interested in studying how Lysergic Acid Diethylamide (LSD-25), heroin (morphine diacetate) and cocaine (benzoylmethylecgonine), chosen as most representative among depressive, exciting and hallucinogen drugs respectively[16,17]) (Fig.1).

To explore the potential interaction between these psychoactive drugs and MTs we first performed a Molecular Dynamics (MD) procedure on the chosen molecular structures with conformation optimization in water medium.

Then we carried out a set of docking procedures between MTs and the optimized drug structures. Both the MD and the docking procedures will be widely explored in the following. Aim of these computational procedures is to identify binding sites for these drugs on tubulin and MTs, in such a way as to detect possible modifications due to potential conformational changes that occur as a result of the interaction between the protein and the substances. Such interactions may interfer with the biophysical and conformational properties of microtubules, causing possible biophysical effects at the CNS level.

II. Materials and Methods

A Molecular Dynamics

Structural bioinformatics is the branch of bioinformatics which is related to the analysis and prediction of the 3-dimensional structure of biological macromolecules such as proteins, RNA, and DNA. It deals with the comparison of overall folds and local motifs, principles of molecular folding, binding interactions and structure/function relationships, working both from experimentally solved structures (obtained e.g. by Nuclear Magnetic Resonance (NMR) or Circular Dichroism (CD) or X-rays) and from computational models. The two most widely used methods for atomic-level modelling are Monte Carlo statistical mechanics (MC) and molecular dynamics (MD) [18] . In our project we used the MD approach. In MD, new configurations are generated by application of the Newton equations of motion to all atoms simultaneously over a small time step to determine new atomic positions and velocities. In both cases, the overall force field controls and determines the evolution of the systems .

The atoms forming a protein have a potential energy E_{pot} generated by a force field: it is possible to calculate the energy of a macromolecule with a certain conformation considering the sum of the single energy contributions given by the covalent chemical bond and non-bond interactions: namely the electrostatic energy, calculated using the Coulomb potential, and the term due to the Van del Waals intermolecular dipole forces, calculated with the Lennard-Jones potential [19,20].

In MD, new configurations are generated by application of Newton's equations of motion to all atoms simultaneously over a small time step to determine the new atomic positions and velocities. The force field controls the total energy and forces, which determine the evolution of the systems. The protein explores the attraction basin to reach a minimum that corresponds to a stable position. MD provides detailed information on phenomena such as conformational changes of a protein or nucleic acid.

B Molecular Docking Simulation

Besides of MD, structural bioinformatics deals with molecular docking, a method that predicts the strength of association or binding affinity between two molecules, often the binding features of small ligands to a protein target. This makes docking important in the modern drug design. Most biological functions are mediated by interactions between proteins and ligands. A protein can interact with other proteins, with nucleic acids, with small ligands (eg. metabolites or ions), with more ligands simultaneously. The binding with a ligand can induce a conformational change that influences the activity or accessibility of other binding domains. The protein-ligand interaction is dictated mainly by the complementary nature of the two compounds: charged ligands tend to be attracted by regions of opposite charges, and the shape of the ligand is reflected by the shape of the binding site (steric complementarity). This methodology is an important application when structural information of the intermolecular complex is not available and already deposited in the Protein Data Bank (PDB) [21].

The purpose of an automatic molecular docking algorithm is to develop methods capable to predict the geometry of binding through a function that estimates the affinity between target and ligand: this feature is generally referred to as the score function. Different types of score functions have been implemented: force field based, knowledge based, consensus scoring etc. [22,23].

The main computational problem is that, in the process of molecular docking, a large number of conformational degrees

of freedom must be taken into account. Several algorithms have been developed for this purpose.

If the bond angles, bond lengths and torsion angles of the components are not modified at any stage of complex generation, we speak about *rigid body docking*. Docking procedures which permit conformational change, or *flexible docking* procedures, are computationally expensive and they must face the complex task to select small subset of possible conformational changes.

The rigid docking procedure considers the two interacting structures as rigids, taking into account only six translational and rotational degrees of freedom of the ligand with respect to the bigger molecule, that is considered fixed in space.

In this approach the choice of the ligand conformation is crucial, as it must correctly approach the other molecule in the intermolecular complex. This is the reason why we optimized the ligand structures with an ad-hoc MD procedure.

Most of the algorithms of molecular docking generate a large number of possible structures, which must then be evaluated in order to select for subsequent analysis a smaller, but representative set of conformations that could be the most likely similar to the real "docking mode ".

This is often realized using cluster analysis. Belonging to a cluster depends on how much the element under consideration is far from the cluster or close to it. When comparing different conformations, the most commonly used measure is the RMSD (root mean square distance) between pairs of atoms:

$$RMSD = \sqrt{\frac{\sum_{i=1}^{N_{atoms}} d_i^2}{N_{atoms}}}$$

where N_{atoms} is the number of atoms on which the RMSD is measured, and d_i is the distance between the atom coordinates of the two structures.

C Software

To model the molecules chosen for our study (heroin, cocaine and LSD) we used the Ascalaph Designer software version 1.8.44, completed by the package PC GAMESS / Firefly (BioMolecular modelling and MD) [24] [25] that yields an interface for the classical and quantum mechanics procedures.

The construction of the ligands chemical structures was performed using the Ascalaph Designer *ab-initio* Free Drawing. The next step was the optimization, i.e. the energy minimization, of all the built chemical structures (energy minimization algorithm: conjugate gradient method, stop conditions: gradient value = 0.001 and iteration number = 100). For the heroin molecule the value obtained for the energy minimization of the heroin molecule was E = 5.2133 Kcal/mol. For the cocaine molecule the final energy value was E = 39.408694 Kcal/mol. For the LSD molecule we obtained E = 74.4203 Kcal/mol.

After building energy optimized molecules, we performed the binding modelling using the HEX Protein Docking system [26,27,28,29], a molecular docking software that allows both calculation and 3D visualization. HEX is able to predict the binding between a protein and a ligand, considering the latter as a rigid body; the interaction between molecules takes place solely on the basis of their 3D shape and of their electrostatic complementarity. HEX is one of the few programs with a "built-in graphics" system useful to view the results, adopting modern graphics processing units (GPUs) to accelerate the process. In rigid docking the receptor molecule is considered fixed on the three-dimensional space and all the possible positions and orientations of the ligand in space are evaluated. The search procedure must take into account the six degrees of freedom: three translations and three rotations.

The first computationally efficient algorithm to determine the geometric complementarity between two molecular structures, able to solve the problem of rigid docking, was presented by Katchalski-Katzir et al. in 1992 [30]. This method consists of an automatic procedure that projects the molecule in a 3D grid, performing a distinction between surface and interior atoms. Then it calculates, using the Fourier transform, a correlation function that evaluates the overlapping degree of the molecular penetration relative to all the possible orientations of the molecule ligand.

HEX gets further and uses a FFT evolution called SPF (Spherical Polar Fourier). Each molecule is modelled in three dimensions using parametric functions that encode also the surface spatial potential distribution and are based on the expansion of spherical orthogonal functions. The *correlation* (or *overlap* as a function of translation/rotation operations) between a pair of 3D functions can be calculated using expressions which are similar to the conventional FFT docking methods.

Through this new approach it is possible to analyze in detail and quickly all the global features of a macromolecule protein, and represent it with a surface formed by spheres. The spheres represent both the spatial surface and the distribution of the potential, and through the research of complementarity of these surfaces, with a further analysis of the complex through an energy minimization, it is possible to define with extreme rapidity the possible interaction surfaces.

Finally a clustering procedure classifies all the possible solutions as explained above, ordering them in such a way that the first one is the most likely to be similar to the real biological docking.

The docking analysis was performed on two models, MTs and Tubulin. For the MTs structure we adopted a portion of the 12-protofilaments left-handed Microtubule model developed by the NANO-D research group at INRIA Grenoble-Rhone-Alpes [31] and, for comparative purposes, of the Tubulin structure: we chose the refined structure of alpha-beta Tubulin stabilized with taxol, Bos Taurus organism (PDB code: 1JFF). We always refer to the αβ Tubulin heterodimer, usually considered as one unit.

The HEX parameters chosen for the procedure were: Correlation type: shape + electrostatics; grid dimension: 0.6 å; range: 180 å; step size 7.5 å; solutions: 500; max clusters: 100; RMS threshold: 3.0 å.

III. Results

MTs are polymers of tubulin, and tubulin is a protein. Proteins are large biological molecules consisting of one or more long chains of amino acid residues. Proteins differ one from another primarily in their sequence of amino acids which usually results in folding of the protein into a specific three-dimensional structure that determines its activity and allows to perform its biological function. The folding is driven by a number of non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals forces, and hydrophobic packing. Proteins have four different levels of structure: 1) the primary structure of a protein refers to the linear sequence of amino acids that compose it; 2) the secondary structure refers to highly regular local sub-structures. Two main types of secondary structures are the alpha helix and the beta sheets, having a regular geometry depending on the conformation of the bonds between their CO and NH groups; 3) the tertiary structure refers to the three-dimensional structure. 4) the quaternary structure is the three-dimensional structure of a multi-subunit protein, depending on how the subunits fit together.

To understand the functions of the proteins at a molecular level, it is often necessary to determine their three-dimensional structure. A protein may undergo reversible structural changes in performing its biological function. The alternative structures of the same protein are referred to as different conformations, and transitions between them are called conformational changes. We analysed the ligand positions visualizing the protein structure in the ligand contact area, highlighting the protein conformational changes after the contact with the ligand.

Ligands (cocaine, LSD, heroin, taxol) were subjected to docking using HEX. We displayed each of the formed docking structures using Deep View [32,33]. MTs structures have always been displayed with the concave side facing the observer. In tubulin structures we made sure to keep always the same spatial orientation for conformations comparison.

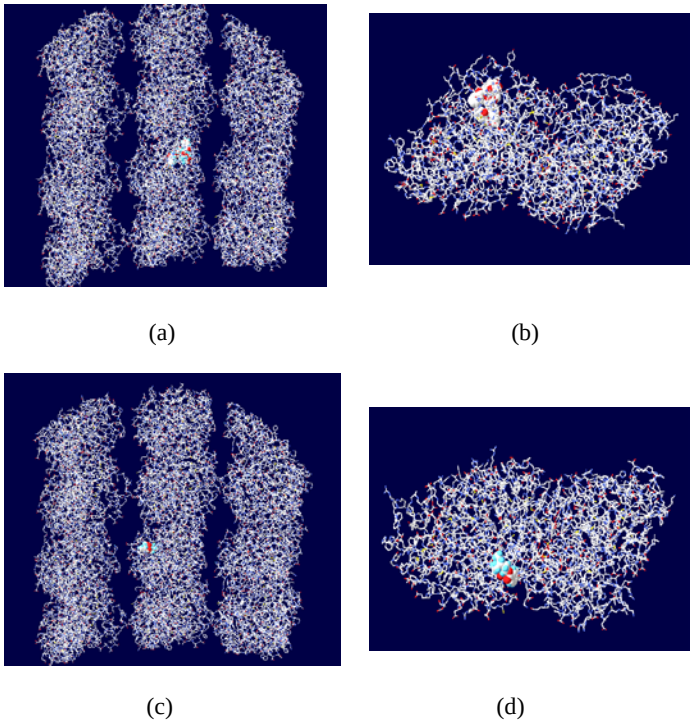
As a control ligand we used taxol (Paclitaxel), that is a mitotic inhibitor, devoid of psychotropic characteristics, and typically associated with tubulin in the databanks because of its stabilizing action [34]. From the complete labeled list of the groups (not shown) we could note that taxol is present only at the level of a single chain for each model, the chain called A in MTs and the chain called B in tubulin. Taxol in MTs is wrapped both in alpha helices and beta sheets (Fig.3a). Taxol in tubulin is in contact with alpha helices (Fig.3b), it is positioned on the opposite surface with respect to the other ligands (Fig.2). Taxol is completely incorporated in the structures and shows a quite different behaviour in docking conformation.

We observed that in MTs cocaine and heroin are close to a kind of niche, perhaps the access way. They are displayed in a similar docking area in MTs (Fig 2c, e): in fact, observing the protein secondary structure we can visualize three beta sheets on the ligands background (Fig.3e,g). LSD position is again in such docking area but it shows a shifted position compared to cocaine and heroin ligands (Fig 2g): in Fig.3g it is possible to note the different positioning. All these three ligands are docked to the concave face of MTs, an interesting hint to the ability of the MT to realize its transport function for this kind of substances. All these three ligands are docked in between the two monomers α,β in tubulin (Fig. 2).

Analysing the protein secondary structure we can clearly visualize that there is no correspondence between positions of the ligands in MT and tubulin: the example of taxol was previously reported; a second example is LSD, where in tubulin contacts beta sheet (Fig. 3h) and in MT contacts a alpha helix (Fig. 3g), and so on for the other ligands and structures. Alpha helices are more superficial of beta sheets, therefore those ligands that make contact with beta sheets penetrate better the structure, as in the case of cocaine in MT (Fig. 3c).

The analysis of residues in the neighbourhood of the binding sites is reported in Fig. 4, Table 1 and Table 2.

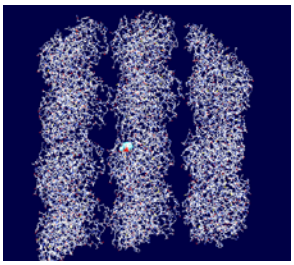
Here it is better shown that cocaine and heroin have the same binding site (Fig. 4 a,c). They are able to change the protein conformation of MT in fact there is a distorsion of secondary structure(Fig. 4 a,c). The approach cannot compare directly aminoacid positions between tubulin and MT, however we can analyse the ligand position on the basis of protein chain: from the complete labeled list of the groups (not shown) it is possible to evaluate that, while cocaine and heroin are present in the MT structure only within the chain A, in tubulin the structures are present in the transition zone between chain A and B.



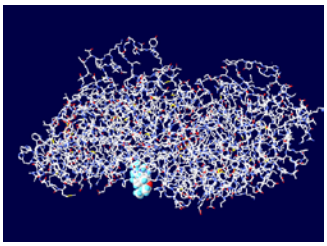
(a) (b)

(c) (d)

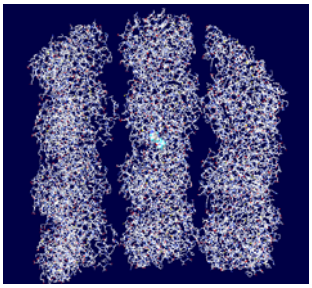
Fig.2 (a,b,c,d) MTs structures on the left panel, tubulin structures on the right panel
(a) taxol ligand in MT; (b) taxol ligand in tubulin ;
(c) cocaine ligand in MT; (d) cocaine ligand in tubulin.



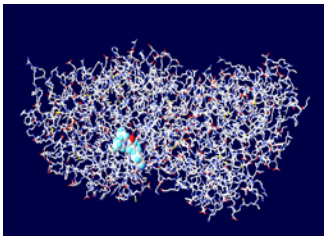
(e)



(f)

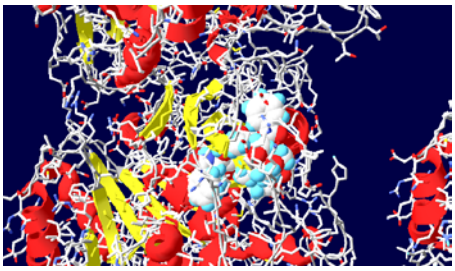


(g)

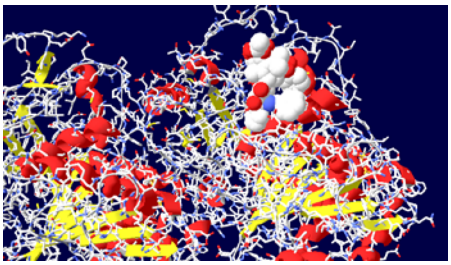


(h)

Fig.2 (e,f,g,h)
(e) heroin ligand in MT; (f) heroin ligand in tubulin
(g) LSD ligand in MT; (h) LSD ligand in tubulin

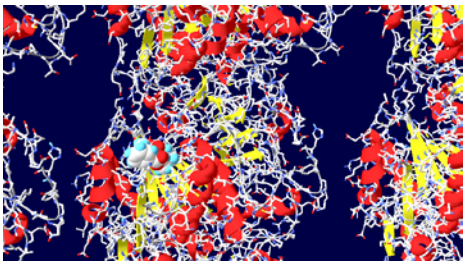


(a)

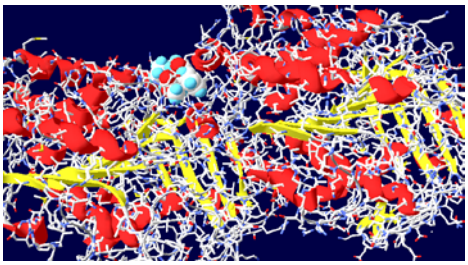


(b)

Fig.3 (a,b)
Protein secondary structures highlighted: in red alpha helices, in yellow beta sheets.MTs structures on the upper panel , tubulin structures on the lower panel.
(a) taxol ligand in MT; (b) taxol ligand in tubulin.

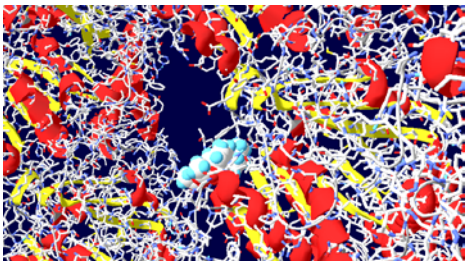


(c)

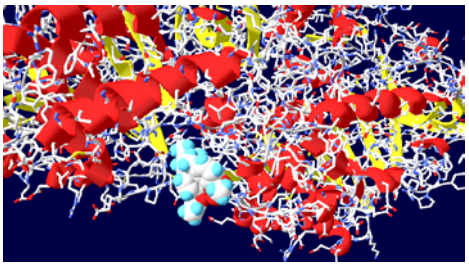


(d)

Fig.3 (c,d)
(c) cocaine ligand in MT; (d) cocaine ligand in tubulin.

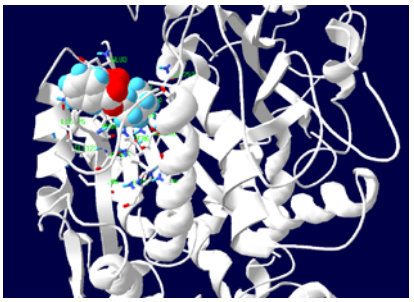


(e)

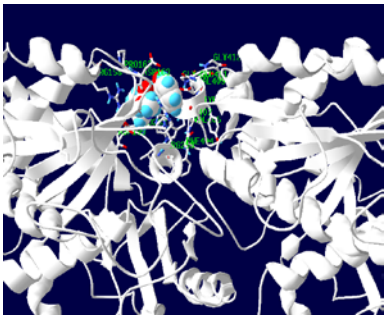


(f)

Fig.3 (e,f)
(e) heroin ligand in MT; (f) heroin ligand in tubulin;

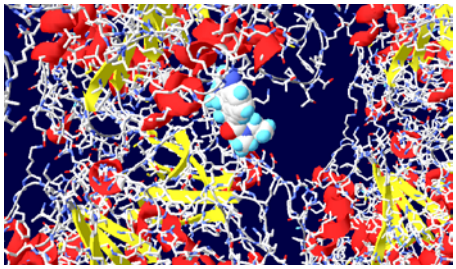


(a) cocaine radius in MT

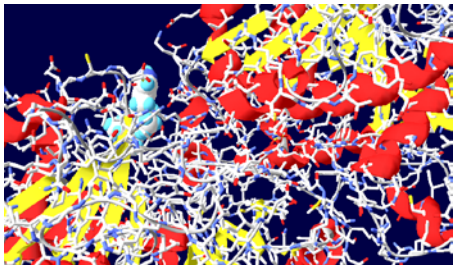


(b) cocaine radius in tub

Fig.4 (a,b) Aminoacid residues selected within a 8 Angstrom radius

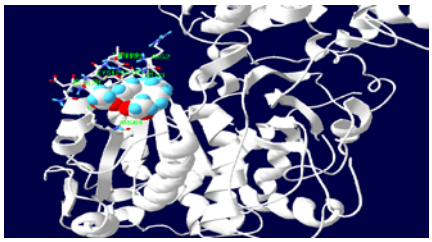


(g)

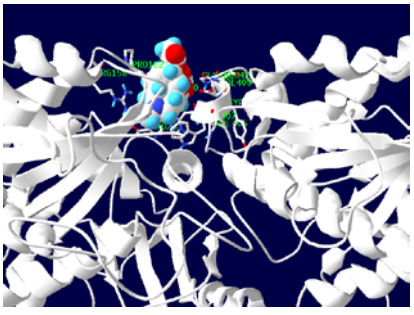


(h)

Fig.3 (g,h)
(g) LSD ligand in MT; (h) LSD ligand in tubulin.

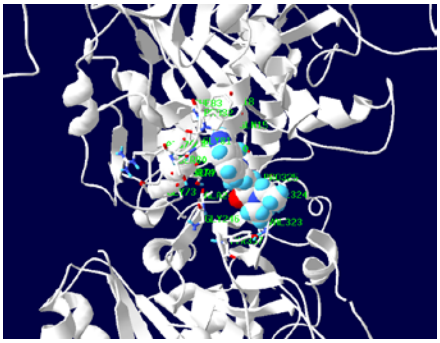


(c) heroin radius in MT

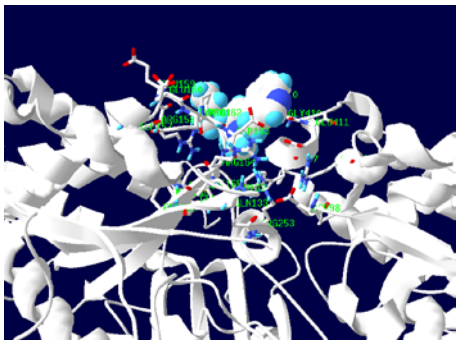


(d) heroin radius in tub

Fig.4 (c,d) Aminoacid residues selected within a 8 Angstrom radius



(e) LSD radius in MT



(f) LSD radius in tub

Fig.4 (e,f) Aminoacid residues selected within a 8 Angstrom radius

Table 1 Group list within a radius of 8 å, in MT

ligands	Aminoacid residues list in MT structure
taxol	LEU318-TYR319-ARG320-GLY321-ASP322-VAL323-ASP327-GLY354-ASN356 GLU358-PRO360-VAL371-GLN372-ARG373-ALA374-VAL375-CYA376
cocaine	LEU125-ALA126-GLN128-CYS129-THR130-GLY131-LEU132-GLN133-ARG243
heroin	ILE5-ARG64-ASP120-ARG121-ILE122-ARG123-LYS124-LEU125-ALA126-
LSD	ASP127-GLN128-CYS129-THR130-GLY131-LEU132-PHE135-TYR161- GLN11-ASN14-GLN15-ILE16-ALA18-LYS19-GLY73-THR74-MET76-ASP76- SER77-VAL78-ARG79-SER81-PRO82-PHE83-GLY84-GLY246- ALA247-VAL324-PRO325

Table 2 Group list within a radius of 8 å, in tubulin

ligands	Aminoacid residues list in tubulin structure
taxol	LYS19-PHE20-VAL23-ILE24-GLU27-LEU227-ASN228-HIS229-LEU230-VAL231 SER232-ALA233-THR234-MET235-SER236-GLY237-VAL238-PHE272- MET302-PRO360-THR376
cocaine	ASP98-ALA100-ALA102-ARG105-THR109-HIS406-THR407-TYR408-GLY410- GLU411-PRO162-ASP163-ARG164-ILE165-ASP199-ARG253-ALA256-VAL257-
heroin	VAL405-HIS406-TRP407-TYR408-GLY410-GLU411-ARG158-PRO162-
LSD	ASP98-ARG105-TRP407-GLY410-GLU411-LEU132-GLN133-GLY134-PHE135- ILE157-ARG158-GLU159-TYR161-PRO162-ASP163-ARG164-ILE165-MET166- ASP199-ARG253

On the basis of these data we can conclude that the binding process varies according to the different target structures.

The validity of the results brought by the docking procedures is also supported by the fact that the first hundred solutions found by HEX are virtually identical, confirming that the first solution chosen on the basis of the previously mentioned cluster algorithm, that we considered in our analysis, is realistically similar to the natural conformation.

IV. Conclusions

We have shown that the three chosen ligands, which play different roles in altering the state of consciousness, after the docking procedure appear to be positioned differently on the MT. In particular LSD shows a completely different position with respect to the other two ligands. The control structure, taxol, is positioned quite separately from the three psychotropic structures.

We can group ligands according to the docking mode, dividing them in three groups: the first composed by taxol alone, the second one including cocaine and heroin and the third one including LSD alone.

The role of taxol is to stabilize the polimerization of MTs, and the different positions of the other ligands suggest that they must have a functional role that is different from stabilization. Up to now MTs have not been considered as targets for psychotrope substances, but we can now hypothesize that they may play not just the role of storage proteins but also an active role in the binding of the psychotrope drugs.

Cocaine is a powerful nervous system stimulant, heroin is an opioid analgesic, LSD is a psychedelic drug inducing psychological effects, which can include altered thinking processes, synesthesia, altered sense of time and spiritual experiences [35]. Cocaine has a stimulant role, heroin a depressant role, but they don't alter the perception of reality as LSD: the fact that cocaine and heroin show the same area of docking and a different area with respect to LSD could be an interesting hint of the role of MTs in the consciousness process, that needs further studies to be disclosed.

This hypothesis could be enhanced in the future by extending our analysis to other similar psychoactive drugs and verifying if their docking position will confirm to be similar to the positions of the already examined substances.

Furthermore, our *in silico* experimental results highlight a different behaviour of MTs in comparison with tubulin. We conclude that, as already shown in our previous works [10,11,12], the MT tubular structure may have an additional functional role that cannot be found in the free tubulin structure.

In the light of the numerous theories that see the MTs structure active in the consciousness process (among the others [36,37,38]), this study suggests an interaction between MT surface and consciousness-altering drugs, proposing a further contribution to the open and evolving worldwide research on the functional role of MTs in the consciousness process.

REFERENCES

[1]J.S. Hyams and C.W. Lloyd, *Microtubules*. Wilmington: Wiley-Liss, 1994, vol. 13.

[2]F. Abaitua, R.N. Souto, H. Browne, T. Daikoku and P. O'Hare, "Characterization of the herpes simplex virus (HSV) -1 tegument protein VP1-2 during infection with the HSV temperature-sensitive mutant tsB7" *J. Gen. Virol.*, vol. 90, pp. 2353-2363, 2009.

[3]T. Kremser, K. Hasenpusch-Theil, E. Wagner, D.Buttgereit and R. Renkawitz-Pohl, " Expression of the 13 3 tubulin gene (8 Tub6OD) in the visceral mesoderm of Drosophila is dependent on a complex enhancer that binds Tinman and UBX T", *Molecular and general Genetics*, vol. 262, pp. 643-658, 1999.

[4]B.R. Hearn, S.J. Shaw and D.C. Myles, " Microtubule targeting agents", *Comprehensive Medicinal Chemistry*, vol. II, no. 7, pp. 81-110, 2006.

[5]T.J Craddock, J.A. Tuszynski, S. Hameroff, "Cytoskeletal signaling: is memory encoded in microtubule lattices by CaMKII phosphorylation?", in *PLoS Comput Biol*, vol. 8, no. 3, 20012 pp. e1002421.

[6]S. Hameroff and R. Penrose, "Orchestrated reduction of quantum coherence in brain microtubules: A model for consciousness", *Math. Comput. Simul.*, vol. 40, 1996, pp. 453-480, 1996.

[7]R. Oldenbourg, E. D. Salmon, P. T. Tran, "Birefringence of Single and Bundled Microtubules", *Biophysical Journal*, vol. 74, pp. 645–54, 1998.

[8]A.G. Michette, N. Mavromatos, K. Powell, M. Holwill and S.J. Pfauntsch, " Nanotubes and microtubules as quantum information carriers," in *Proc. SPIE*, vol. 522, 2004, pp. 5581.

[9]X.R. Huang and R.W. Knighton, "Microtubules contribute to the birefringence of the retinal nerve fiber layer", *Invest. Ophthalmol. Vis.Sci.*, vol. 46, no. 12, pp. 4588–93, 2005.

[10] R. Pizzi and S. Fiorentini, "Artificial Neural Networks Identify the Dynamic Organization of Microtubules and Tubulin Subjected to Electromagnetic Field", in *Proc. WSEAS Int. Conf. Recent Advances in Applied Computer Science*, Genova, 2009.

[11] R. Pizzi, G. Strini, S. Fiorentini, V. Pappalardo and M. Pregnolato, "Evidences of new biophysical properties of microtubules", in *Focus on artificial neural networks*, Hauppauge:Nova Science, 2010.

[12] R. Pizzi, S. Fiorentini, G. Strini and M. Pregnolato, "Exploring structural and dynamical properties of Microtubules by means of artificial neural networks", in *Complexity Science, Living Systems and Reflexing Interfaces: New Models and Perspectives*, New York: IGI Global, 2012.

[13] Y. Wang, K. Kempa, B. Kimball, J.B. Carlson, G. Benham, W.Z. Li, T. Kempa, J. Rybczynski, A. Herczynski and Z.F. Ren, "Receiving and transmitting lightlike radio waves: Antenna effect in arrays of aligned carbon nanotubes", *Appl. Phys. Lett.*, vol. 85, no. 13, pp. 2607–9, 2004.

[14] B.R. Hearn, S.J. Shaw and D.C. Myles, "Microtubule targeting agents", *Comprehensive Medicinal Chemistry II*, vol. 7, no. 3, pp. 81-110, 2006.

[15] M.A. Jordan and L. Wilson, " Microtubules as a target for anticancer drugs" , *Nature Reviews Cancer*, vol. 4, pp. 253-265, 2004.

[16] C. Lüscher and M.A. Ungless, " The mechanistic classification of addictive drugs", *PLoS Medicine*, vol. 3, no. 11, pp. e437, 2006.

[17] A. Verdejo-Garcia, O. Contreras-Rodríguez, F. Fonseca, A. Cuenca, C. Soriano-Mas, J. Rodriguez, R. Pardo-Lozano, L. Blanco-Hinojo et al. , "*Functional alteration in frontolimbic systems relevant to moral judgment in cocaine-dependent subjects", Addict Biol*, vol. 19, no. 2, pp. 272-281, 2014.

[18] W.L. Jorgensen, Julian Tirado-Rives and J., ": Monte carlo vs molecular dynamics for conformational sampling," *J.Phys.Chem.*, vol. 100, no. 34, pp. 14508–14513, 1996.

[19] T. Schlick, *Molecular Modeling and Simulation: An Interdisciplinary Guide*. New York:Springer-Verlag, 2002.

[20] A.D.J. MacKerell, ": Empirical force fields for biological macromolecules: Overview and issues," *Journal of Computational Chemistry*, vol. 25, pp. 1584-1604, 2004.

[21] 21. RCSB Protein Data Bank - RCSB PDB**.** Available: http://www.rcsb.org

[22] A.R. Leach, : Molecular modelling: principles and application, Prentice Hall, 2001.

[23] J. Gasteiger and T. Engel, : An introduction to chemoinformatics (Structural databases and descriptors), Weinheim:Wiley -VCH, 2003.

[24] A. Granovsky, *Firefly version 8*. Available: http://classic.chem.msu.su/gran/firefly/index.html

[25] M.W. Schmidt, K.K. Baldridge, J.A. Aboatz, S.T. Elbert, J. Gordon, J.H.K. Jensen, M. S., N. N., K. A., S. Su, T.L. Windus, M. Dupuis and J.A. Montgomery, ": General atomic and molecular electronic tructure system," *J.Comput*, vol. 14, pp. 1347-1363, 1993.

[26] D.W. Ritchie and Venkatraman V., ": Ultra-fast FFT protein docking on graphics processors," *Bioinformatics*, vol. 26, pp. 2398–405, 2010.

[27] HEX Manual. Available: http://www.loria.fr/~ritchied/HEX/

[28] R. Pizzi, T. Rutigliano, A. Ferrarotti and M. Pregnolato ", Computational prediction of binding affinity between psychotropic drugs and neural cytoskeleton elements," in *Proc. NAUN 8th Int. Conf. on Applied Mathematics, Simulation, Modelling*, Florence, 2014, pp. 132-138.

[29] R. Pizzi, T. Rutigliano, P. Guadalupi and M. Pregnolato, "Molecular cooperation to reinforce immune response during carcinoma: A structural bioinformatics analysis", in *Proc.NAUN 8th Int. Conf. on Applied Mathematics, Simulation, Modelling*, Florence, 2014, pp. 346-353.

[30] E. Katchalski-Katzir, I. Shariv, M. Eisenstein, A.A. Friesem, C. Aflalo and L.A. Vakser, "Molecular surface recognition: Determination of geometric fit between proteins and their ligands by correlation techniques", *Proc.Natl.Ac.Sc*, vol. 89, pp. 2195–9, 1992.

[31] INRIA NANO-D. Available: http://nano-d.inrialpes.fr/

[32] N. Guex, M.C. Peitsch, "SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling", *Electrophoresis*, vol. 18, pp. 2714-2723, 1997.

[33] SWISS pdb VIEWER. Available: http://www.expasy.org/spdbv/

[34] J. Lowe, H. Li , K.H. Downing, E. Nogales, " Refined structure of alpha beta-tubulin at 3.5A resolution", *J.Mol.Biol*, vol. 313, pp.1045-1057, 2001.

[35] World Health Organization, *Neuroscience of psychoactive substance use and dependence*, pp. 89 , 2004.

[36] S. Sahu, S. Ghosh , K. Hirata, D. Fujita, A. Bandyopadhyay, "Multilevel memory switching properties of a single brain Microtubule", *Appl. Phys. Lett.*, vol. 102, pp. 123701 , 2013.

[37] T. Craddock, D. Friesen, J. Mane, S. Hameroff , J.A. Tuszynski, "The Feasibility of Quantum Coherent Effects in Microtubules and Their Potential Role in Neuron Function", in *Proc. Towards the Science of Consciousness*, Tucson AZ, 2014, pp.202-203.

[38] N.J. Woolf, A. Priel, J.A.Tuszynski, Nanoneuroscience: Structural and Functional Roles of the Neuronal Cytoskeleton in Health and Disease. Heidelberg: Springer Verlag, 2010.