Identification of 6-hydroxi-nicotine as a novel neuroprotectant with antioxidant properties

ALEXANDRU IOAN CUZA UNIVERSITY of IAŞI www.uaic.ro

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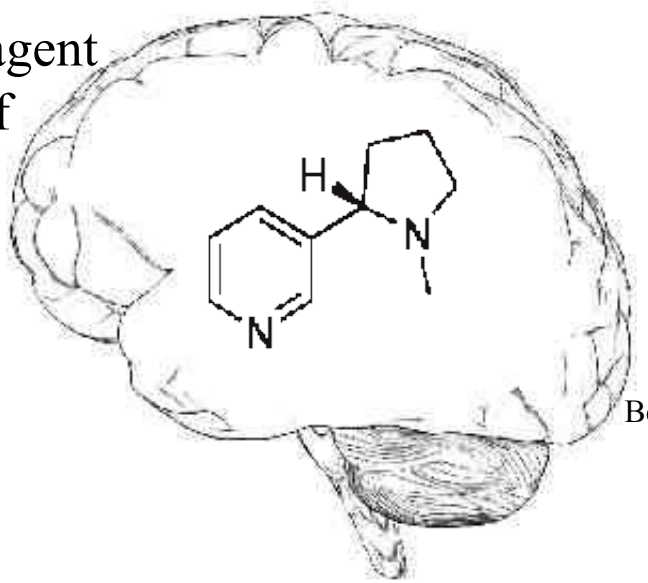
Nicotine as a potential neuro-protective drug

- anti-oxidant effects at low concentrations
- cognition-enhancing agent
- well-known agonist of nicotinic acetylcholine receptors (nAChR)

Murray and Abeles, *Aging & Mental Health*. 2002, 6, 129–138.

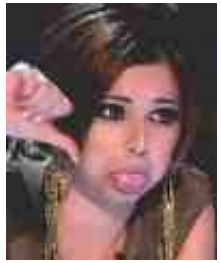


The good things!!!

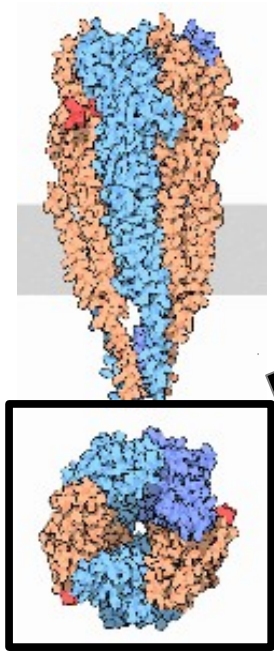
- short half-time (about 2 hours)
- proven negative effects on various other organs such as lungs
- linked to cigarettes and the negative publicity associated with smoking

Beccafusco, *Mol Interv*. 2004 Oct;4(5):285-95.

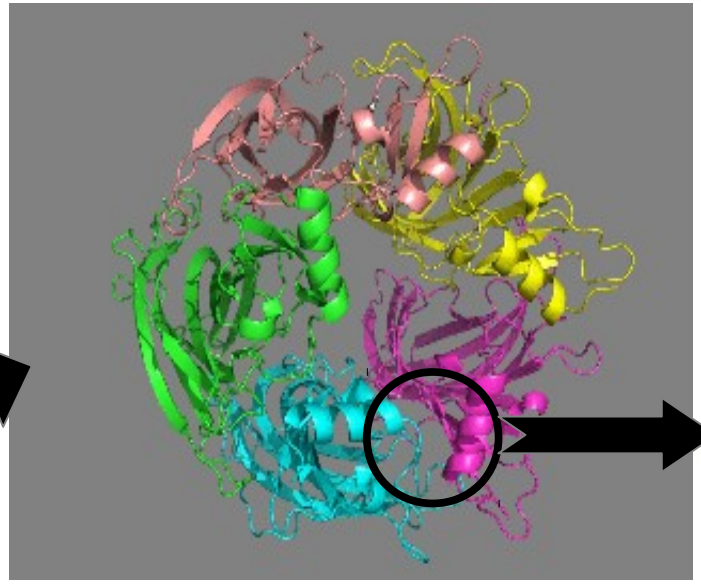
The bad things!!!



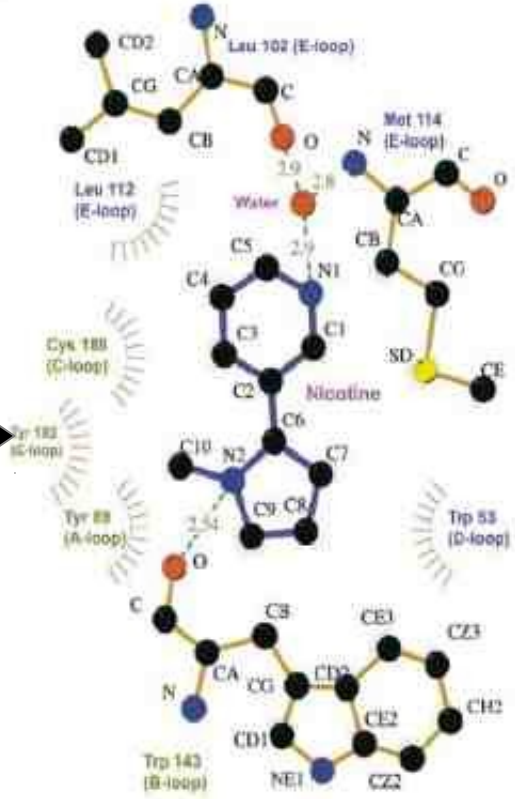
AChBP structure and nicotine binding



Acetylcholine receptor nAChR PDBID 2BG9



Acetylcholine binding protein AChBP PDBID 1UW6



doi:10.1016/j.jmb.2004.12.031 J. Mol. Biol. (2005) 346, 967–989

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Refined Structure of the Nicotinic Acetylcholine Receptor at 4 Å Resolution

Nigel Unwin

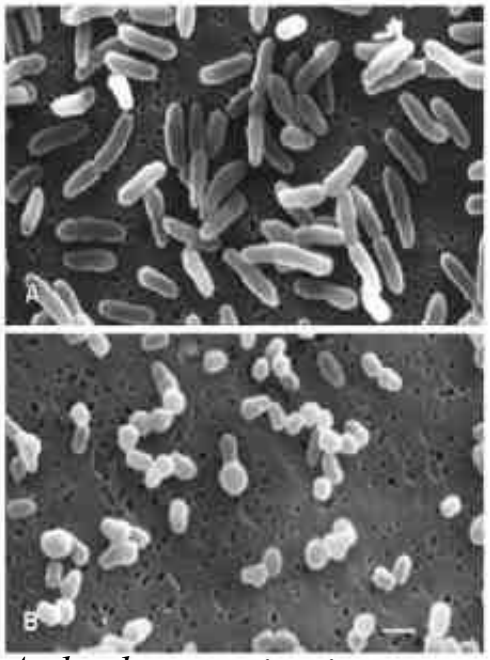
Neuron, Vol. 41, 907–914, March 25, 2004, Copyright ©2004 by Cell Press
ELSEVIER

Nicotine and Carbamylcholine Binding to Nicotinic Acetylcholine Receptors as Studied in AChBP Crystal Structures

Patrick H.N. Celie,[1] Sarah E. van Rossum-Fikkert,[1] Willem J. van Dijk,[1] Katjuša Brejc,[1,3] August B. Smit,[2] and Titia K. Sixma[1,*]

2–5 binding sites at selec
sponse to agonist bindi
opened. LGICs are involv

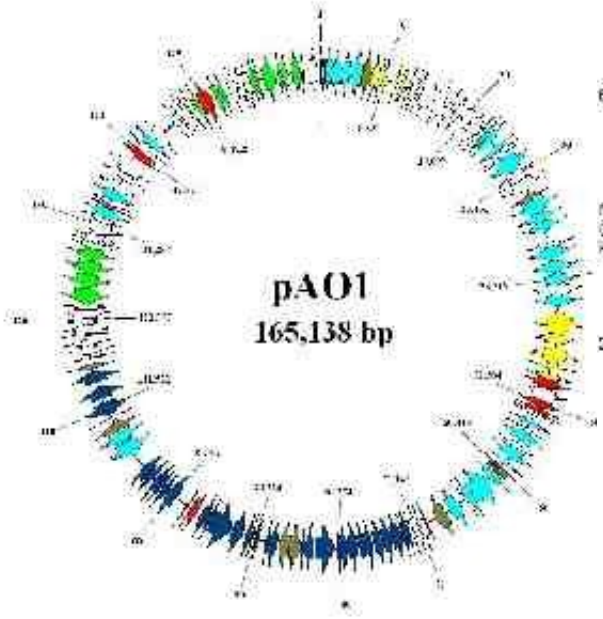
Nicotine and *Arthrobacter nicotinovorans* pAO1

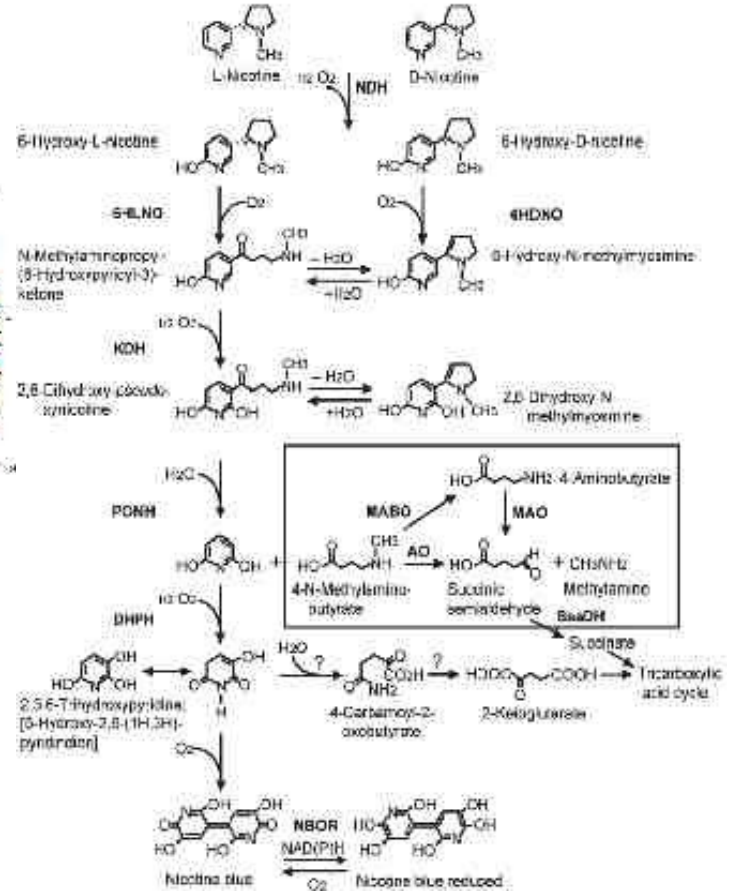


Arthrobacter nicotinovorans

under the electron microscope

Gram positive actinobacterium
Isolated from nicotine rich soil





Appl Microbiol Biotechnol (2006) 69: 493–498
DOI 10.1007/s00253-005-0226-0

MINI-REVIEW

Roderich Brandsch

Microbiology and biochemistry of nicotine degradation

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An NAD(P)H-Nicotine Blue Oxidoreductase Is Part of the Nicotine Regulon and May Protect *Arthrobacter nicotinovorans* from Oxidative Stress during Nicotine Catabolism

Marius Mihasan, Calin-Bogdan Chiribau, Thorsten Friedrich, Vlad Artenie, and Roderich Brandsch

Purpose of the study and experimental design

Identification of potential neuro-protective agents with biotechnological applications derived from the *A. nicotinovorans* metabolites

A. Computational screening of all the *A. nicotinovorans* nicotine metabolites by:

- *in-silico* docking with nAChBP – AutoDock and ADT, Olson Laboratory, U.S.A. (Morris et. al., 1998, *J. Comp. Chem*.19: 1639-1662)
- QSAR evaluation of the anti-oxidant properties (Rastija et. al., 2009, *Eur. J. Med. Chem*. 44, 400–408)

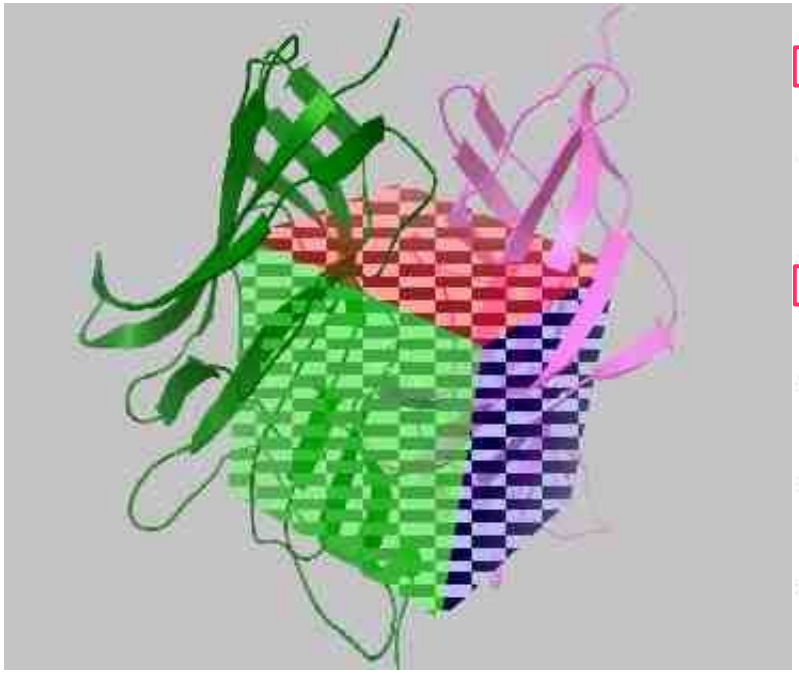
B. *In-vivo* evaluation of the identified compounds on:

- *s*hort-term memory by means of Y-maze task
- working and reference memory by means of radial arm-maze task
- oxidative stress levels in the temporal lobe measuring the extent of some lipid peroxidation products like malondialdehyde (MDA) and defense enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX)

Computational results

***In-silico* targeted docking** – *rigid receptor* subunits A and B from PDB ID 1UW6

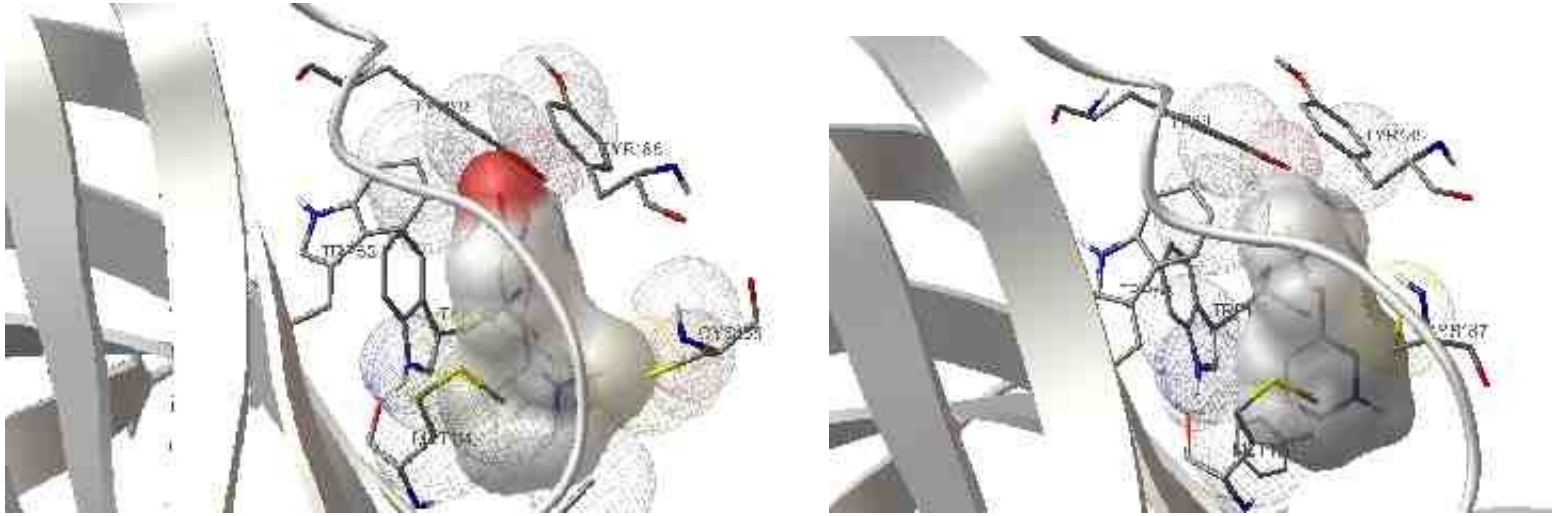
- *targeted region* - a cube of aprox. 150 Å^3 centered on Tyr143
- *flexibile ligands* – 3D structures obtained from Pub Chem database



Tested Compound	Binding Energy	Ligand Efficiency	RMSD*
L-Nicotine	-6.17	-0.51	0.2 Å
CID 89594	-6.17	-0.51	0.25 Å
	-6.12	-0.51	0.29 Å
D-Nicotine	-6.42	-0.54	0.58 Å
pseudonicotine	-6.21	-0.52	0.62 Å
CID 89594	-6.12	-0.51	0.69 Å
6-hydroxy-L-Nicotine	-6.66	-0.51	0.19 Å
CID 439383	-6.61	-0.51	0.21 Å
	-6.57	-0.51	0.25 Å
6-hydroxy-D-Nicotine	-6.51	-0.5	0.75 Å
CID 439886	-6.45	-0.5	0.79 Å
	-6.13	-0.47	0.76 Å
6-hydroxy-N-methylmyosmine	-5.23	-0.37	1.26 Å
CID 9543125	-5.04	-0.36	1.29 Å
	-4.85	-0.35	1.35 Å
2,6-dihydroxy-N-methylmyosmine	-5.23	-0.37	1.29 Å
CID 9543125	-5.04	-0.36	1.36 Å
	-4.85	-0.35	1.35 A

The binding pose of 6-hydroxy-L-Nicotine (A) and nicotine (B) as depicted by *in-silico* docking experiments

(image created with UCSF Chimera, Pettersen *et. al.,* *J Comput Chem.* 2004 Oct;25(13):1605-12)



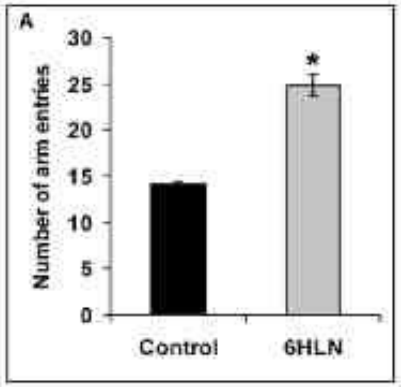
Tested Compound	Molar refractivity	Van der Waals volume	gmin	IC50
6-hydroxy-L-Nicotine	48.5532	179.67	0.609	43.09
L/D-Nicotine	48.6942	159.98	-0.023	54.54
Cotinine	50.1631	166.14	0.230	46.52

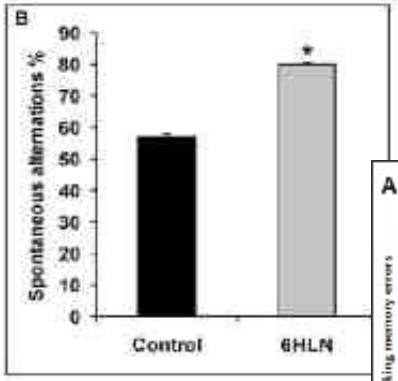
QSAR descriptors used for the calculation of IC50

In-vivo effects of 6-hidroxy-L-nicotine on rats cognitive functions

Animals and drug administration

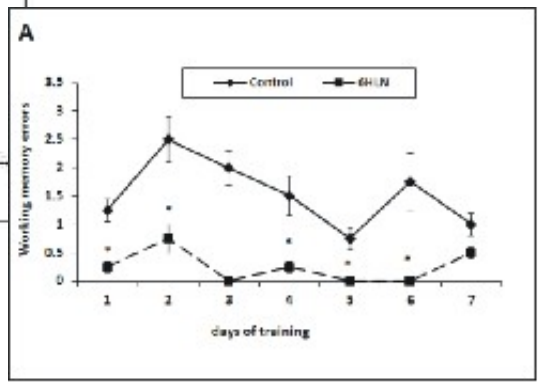
- male Wistar rats (3-4 months old)
- 6-hidroxy-L-nicotine was injected intraperitonealy, 0.3 mg/kg b.w, daily, for 7 consecutive days. Control animals received i.p. an equal volume of sterile saline (1 ml/kg b.w.)
- rats were trained after 7 continuous days of 6-hidroxy-L-nicotine administration.

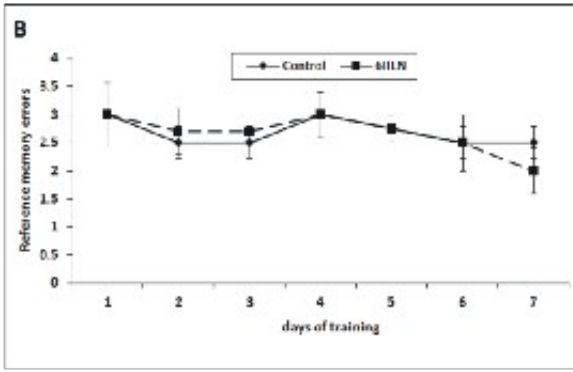




Radial arm-maze task

Values are mean ± S.E.M. (n=10 animals per group), *p<0.0001 vs. control group



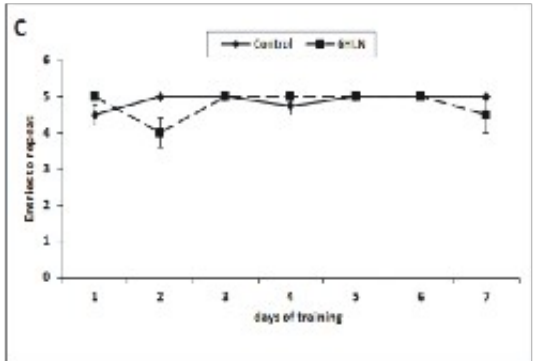


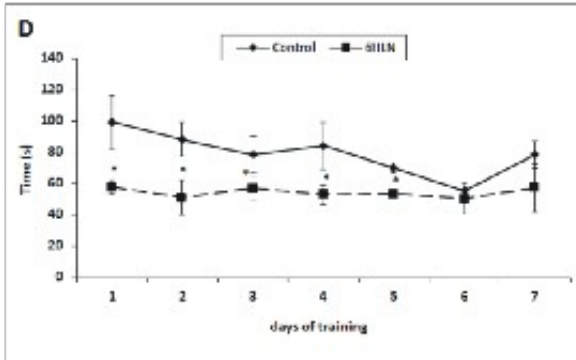
Y-maze task

Values are mean ± S.E.M. (n=10 animals per group), *p<0.0001 vs. control group

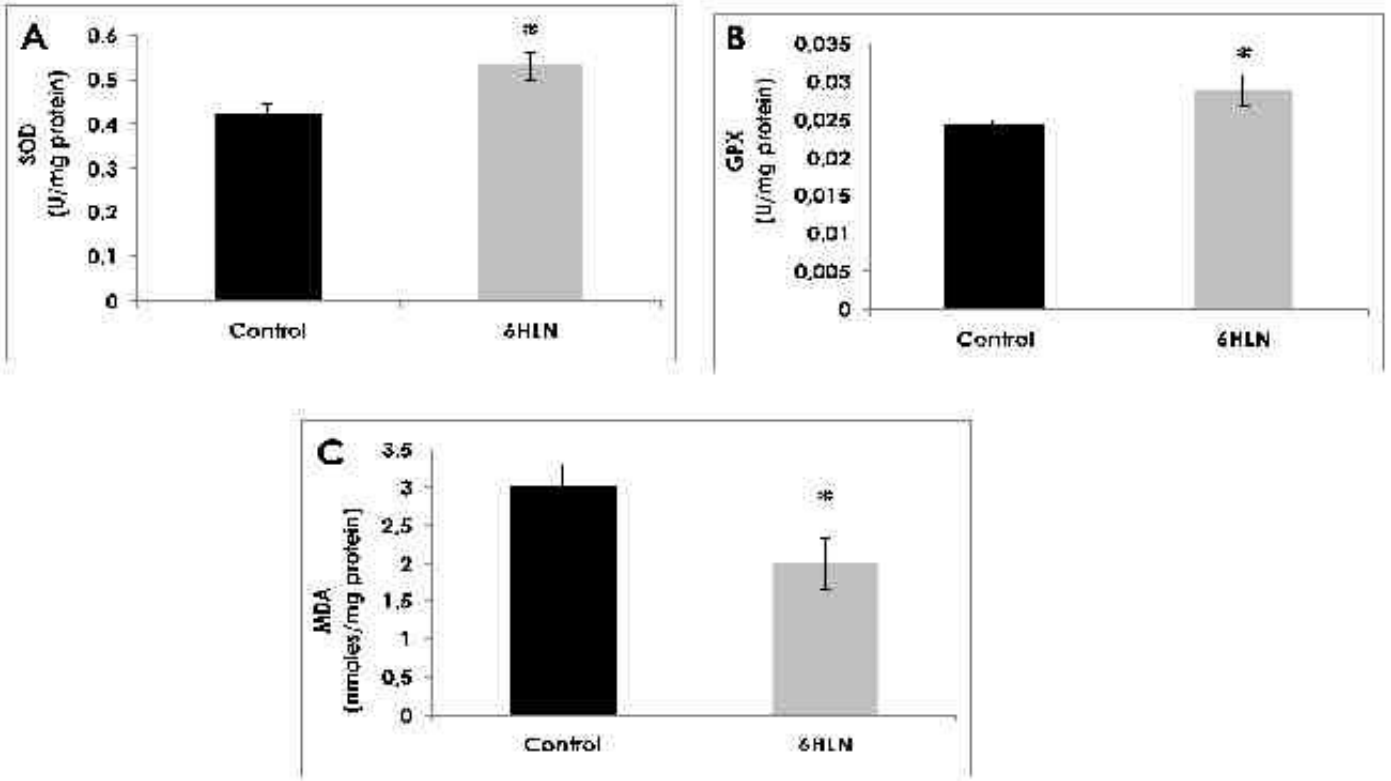

AUTHOR'S PROOF

fct6-hydroxy-L-nicotine from *Arthrobacter nicotinovorans* sustain spatial memory formation by decreasing brain oxidative stress in rats





In-vivo effects of 6-hidroxy-L-nicotine on rat brain oxidative status functions



Values are mean ± S.E.M. (n= 10 animals per group), *p<0.05 vs. control group


AUTHOR'S PROOF

fet-hydroxy-l-nicotine from *Arthrobacter nicotinovorans* sustain spacial memory formation by decreasing brain oxidative stress in rats

What next ?

- is 6-hidroxy-L-nicotine able to cross the blood-brain barrier?

- does 6-hidroxy-L-nicotine still retain the systemic toxicity of nicotine?

- taking into account that nAchRs exist in various pentameric subtypes (Wu and Lukas, 2011, *Biochemical Pharmacology* 82, 800–807), what is specificity of this compound in regard to nAchR subunits and what is the relevance of this specificity for neurodegenerative dizorders (e.g. Alzheimer's disease)?

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- *Arthrobacter nicotinovorans* manipulations

Assist. Prof. **Lucian Hritcu, PhD**
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- animal tests and assessment of cognitive functions

Prof. **Vlad Artenie, PhD**
Biology Faculty, A.I. Cuza University of Iasi

- fruitful talks on enzyme assays and oxidative stress

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