Cotinine and 6-hydroxy-L-nicotine mitigate the memory deficits and oxidative stress induced by brain infusion of $A\beta_{25-35}$ in rats

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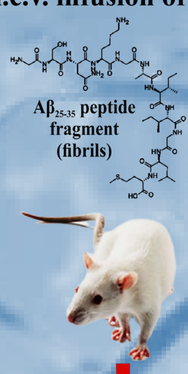
ABSTRACT

Animal model of AD induced by i.c.v. infusion of $A\beta_{25-35}$ peptide

$A\beta_{25-35}$ peptide fragment (fibrils)



Cognitive impairment

Brain oxidative stress

Chronic treatment

NICOTINE (NIC)
Stimulates nAChRs => improve memory, attention and learning
Cardiovascular and addictive side-effects
Potentially lethal to humans (LD50 0.5 – 1 mg/kg)
Scaffold for developing new drugs for AD

COTININE (COT)
Cognitive enhancer in transgenic mouse model of AD
Positive Allosteric Modulator (Echeverria & Zeitlin, 2012)
Inhibitor of Aβ1-42 aggregation (Echeverria et al., 2011)
Reduce oxidative stress (de Aguiar *et al.*, 2013)
100x less toxic than NIC

6-HYDROXY-L-NICOTINE (6HLN)
Improve memory and learning in normal rats and animal model of AD
Reduce oxidative stress (Hritcu *et al.*, 2013)
Might stimulate nAChRs (Mihasan *et al.*, 2013)

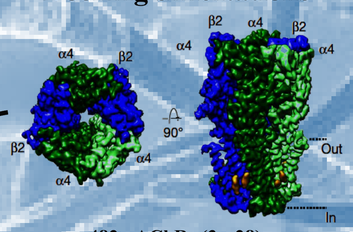
Attenuated memory deficits and decreased brain oxidative stress.

COT & 6HLN bind preferentially and with higher energy than NIC to α4-β2 over the α4-α4 interface of α4β2 nAChRs.

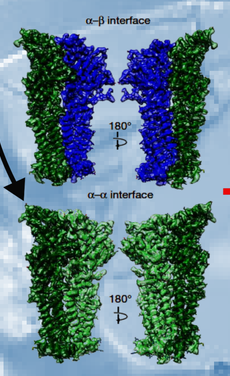
COT & 6HLN might represent new neuropharmacological agents in AD.

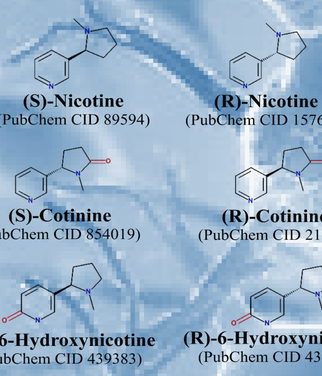
METHODS

Docking simultations



α4β2 nAChRs (3α:2β)
(PDB ID 6CNK, 3.9 Å, Walsh *et al.*, 2018)





(S)-Nicotine (PubChem CID 89594)
(R)-Nicotine (PubChem CID 157672)
(S)-Cotinine (PubChem CID 854019)
(R)-Cotinine (PubChem CID 21907)
(S)-6-Hydroxynicotine (PubChem CID 439383)
(R)-6-Hydroxynicotine (PubChem CID 439886)

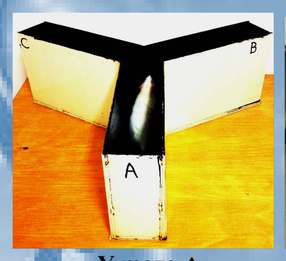
***In silico* tools**

Autodock 4
AutoDock Tools v1.5.6
PyMOL v.2.2.3

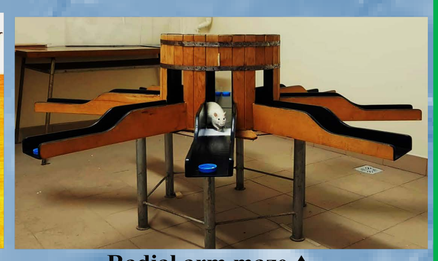
Frog v2.14
UCSF Chimera v1.13.1
DeepView-Swiss-PdbViewer v4.1.0

Tracking software
ANY-maze v6.1
(Stoelting Co., Woods Dale USA)

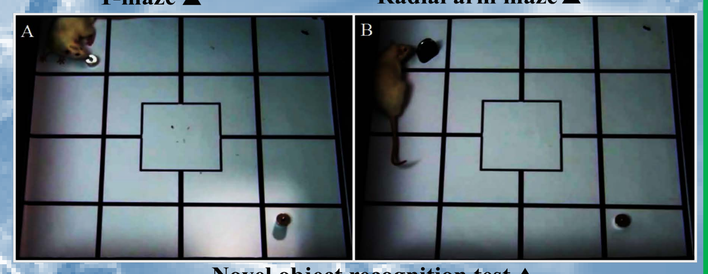
Behavioral tasks



Y-maze ▲



Radial arm maze ▲



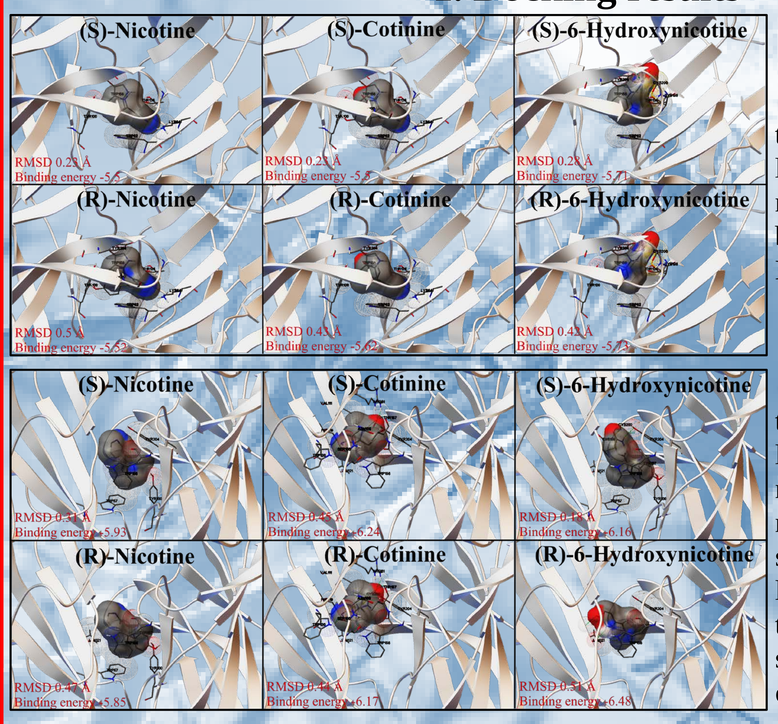
Novel object recognition test ▲
A) Familiarization and **B)** Testing session

Biochemistry

Biochemical parameter	Reference
Catalase (CAT) activity	Sinha, 1972
Superoxide-dismutase (SOD) activity	Winterbourn et al., 1975
Level of reduced glutathione (GSH)	Salbitani et al., 2015
Level of protein carbonylation	Oliver et al., 1987

RESULTS & DISCUTIONS

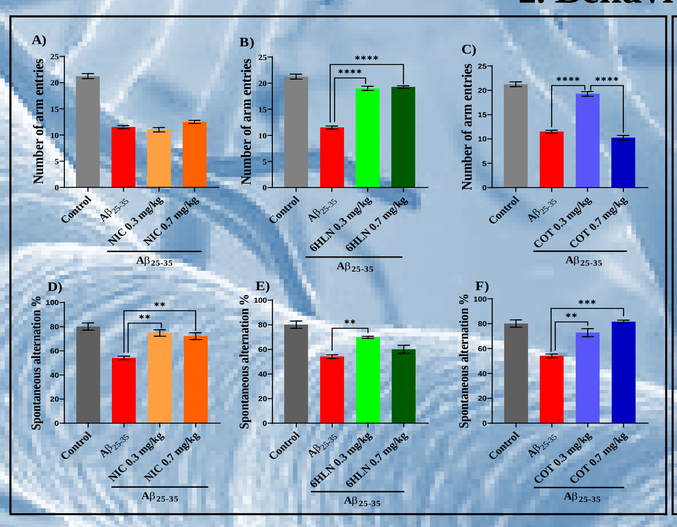
1. Docking results



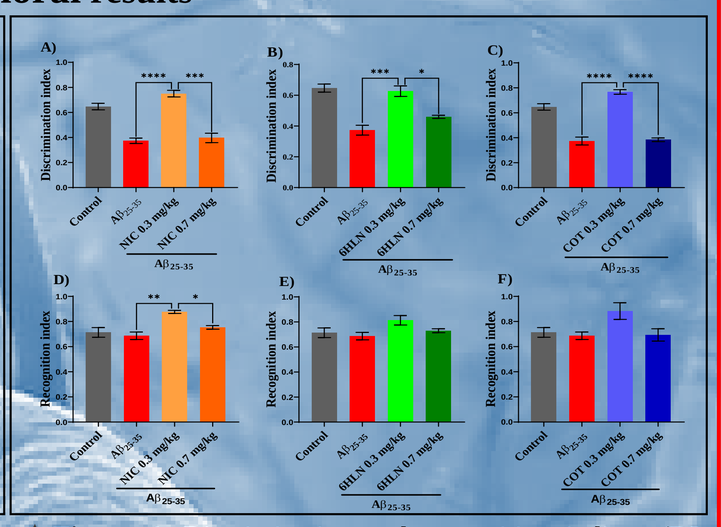
◀ **Figure 1.** Docking results for the best binding pose of the tested ligands at the α4-α4 site of α4β2 nAChRs. 6HLN shows a better binding potential than COT and NIC at α4-α4 site.

◀ **Figure 2.** Docking results for the best binding pose of the tested ligands at the α4-β2 site of α4β2 nAChRs. At the α4-β2 site was noticed a similar trend as at α4-α4 site, but the binding energy of the ligands was smaller suggesting that their affinity towards α4-β2 site was higher than α4-α4 site of α4β2 nAChRs.

2. Behavioral results

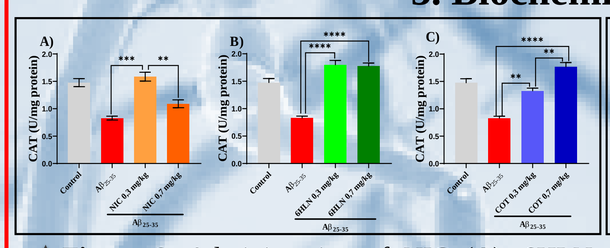


▲ **Figure 3.** Within Y-maze, 6HLN (0.3 and 0.7 mg/kg) and COT (0.3 mg/kg) improve locomotor activity (**B**,**C**) by increasing the number of arm entries and, alongside NIC, enhance spatial recognition memory (**D**,**E**,**F**) by increasing the spontaneous alternation %. For Tukey *post hoc* - ****p<0.0001, ***p=0.0003, **p=0.0047.

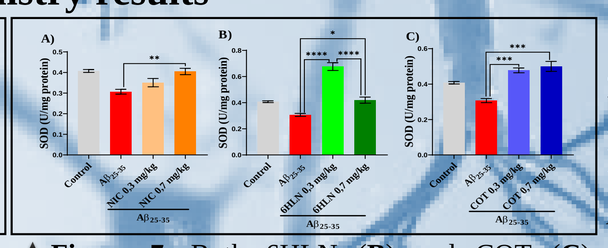


▲ **Figure 4.** Discrimination and recognition indices (DI and RI) obtain from retention phase in NOR test. The dose of 0.3 mg/kg of NIC (**A**), 6HLN (**B**) and COT (**C**) improve recognition memory in $A\beta_{25-35}$-treated rats by increasing DI. Only NIC (0.3 mg/kg) was able to significantly improve RI (**D**) although a similar trend was also observed for 6HLN (**E**) and COT (**F**). For Tukey *post hoc* - ****p<0.0001, ***p=0.0008, **p=0.0054, *p=0.0407.

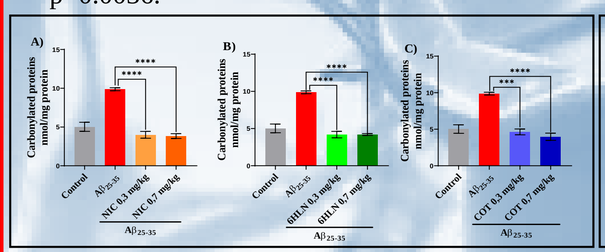
3. Biochemistry results



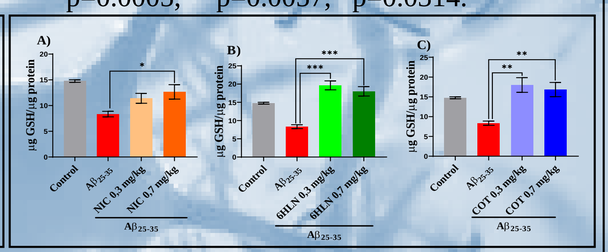
▲ **Figure 6.** Administration of NIC (**A**), 6HLN (**B**) and COT (**C**) to $A\beta_{25-35}$-treated rats restored the CAT specific activity in the hippocampus. For Tukey *post hoc* - ****p<0.0001, ***p=0.0006, **p=0.0036.



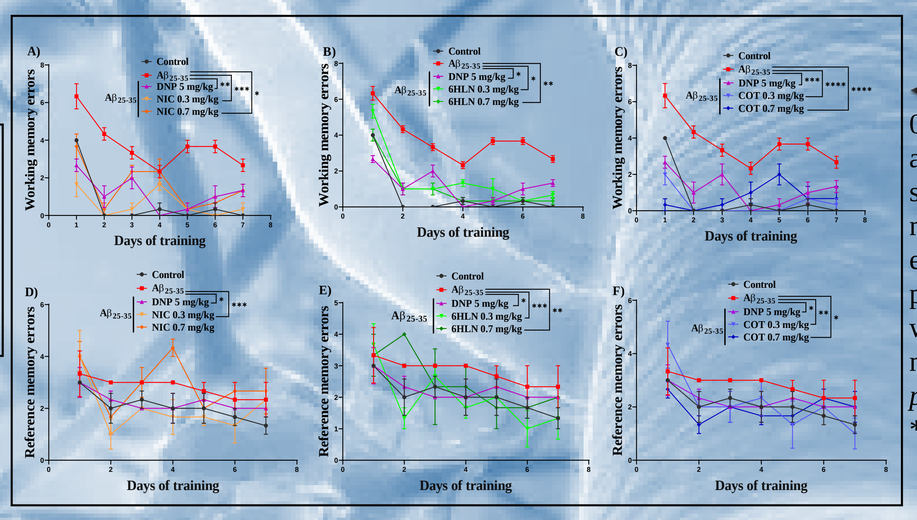
▲ **Figure 7.** Both 6HLN (**B**) and COT (**C**) significantly increase the specific activity of SOD in the hippocampus of the rats infused i.c.v. with $A\beta_{25-35}$. For Tukey post hoc - ****p<0.0001, ***p=0.0005, **p=0.0057, *p=0.0314.



▲ **Figure 8.** Both doses of NIC (**A**), 6HLN (**B**) and COT (**C**) significantly reduced the level of carbonylated proteins in the hippocampus of the $A\beta_{25-35}$-treated rats. For Tukey *post hoc* - ****p<0.0001, ***p=0.0002



▲ **Figure 9.** NIC (**A**), 6HLN (**B**) and COT (**C**) restored the level of GSH in the hippocampus of the $A\beta_{25-35}$-treated rats. For Tukey *post hoc* - ***p=0.0004, **p=0.0081, *p=0.382



◀ **Figure 5.** Administration of 0.3 and 0.7 mg/kg of NIC (**A**,**D**), 6HLN (**B**,**E**) and COT (**C**,**F**) to $A\beta_{25-35}$-treated rats significantly improves the spatial memory in RAM task. The treatments enhance short- and long-term memory performances by decreasing the working memory errors and reference memory errors respectively. For Tukey *post hoc* - ****p<0.0001, ***p=0.0009, **p=0.0074, *p=0.0121.

CONCLUSIONS

➢ Injection of 6HLN and COT in $A\beta_{25-35}$-treated rats resulted in significant improvement of memory function and in a decrease of the oxidative stress in the rat hippocampus.

➢ COT and 6HLN bind preferentially and with higher energy than NIC to α4-β2 compared to α4-α4 interface of α4β2 nAChRs.

➢ 6HLN and COT could represent a viable therapeutic alternative to improve cognitive symptoms in AD.

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