5-HT$_4$R and *5-HT$_6$R*, serotonin receptors involved in Alzheimer's Disease, form a functional heterodimer in the central nervous system

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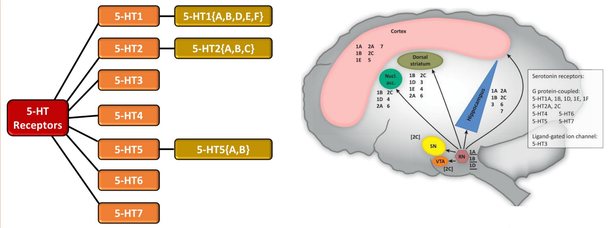
Abstract

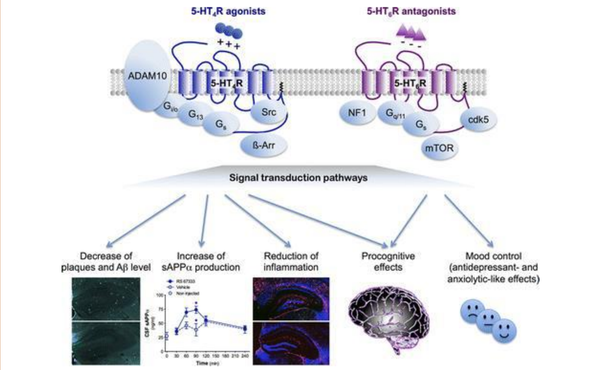
It has long been hypothesized that lower levels of serotonin (5-HT), the neurotransmitter that regulates happiness, sleep and appetite, may be a driving force behind the development of Alzheimer's Disease (AD) and memory loss. Among active serotonin receptors, 5-HT$_{1A(B)}$R, 5-HT$_4$R, and 5-HT$_6$R have been shown to be involved early in cognitive decline, suggesting they could be targets for AD drugs. But despites numerous attempts, development of drugs targeting these receptors failed at stage 3 of pre-clinical trials, the latest being STARSHINE, STARBEAM, and STARBRIGHT. It is well known that serotonin receptors, like most GPCRs, achieve their function through homo/heterodimerization or high order oligomerization. We sought to find if serotonin receptors involved in Alzheimer's Disease (AD) heterodimerize and investigate the functional consequences.

Serotonin Receptors in the CNS



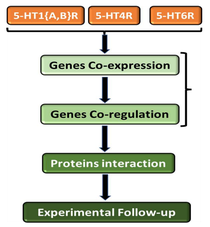
- Addiction, Aggression
- Learning, Memory, Mood
- Anxiety, Appetite, sleep
- Cognition, Imagination

Low level of serotonin, driving force behind AD



- Several serotonin receptors have been implicated in AD: 5-HT$_{1A(B)}$R, 5-HT$_4$R, 5-HT$_6$R, 5-HT$_7$R

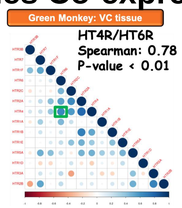
Method overview



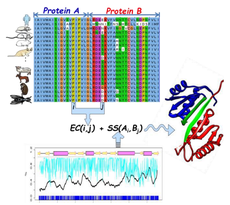
Genomic/Epigenomic data

	Species	Type	Tissue	GEO_ID
data_GM	Green Monkey	RNA-Seq	- dorsolateral prefrontal cortex - visual cortex	GSE108282
data_RM	Rhesus Macaque	RNA-Seq	-dorsolateral prefrontal cortex - visual cortex	GSE108282
data_BB	Baboon	RNA-Seq	64 tissues	GSE98965
data_MM	Mouse CK-p25	scRNA-Seq	Microglia	GSE103334
data_SM	Mouse AD-transgenic	scRNA-Seq	Immune cells	GSE98969
data_HS	Human (AD patients)	RNA-Seq Chip-Seq	Prefrontal cortex	GSE53697

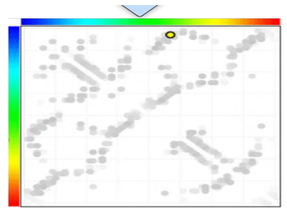
Genes Co-expression



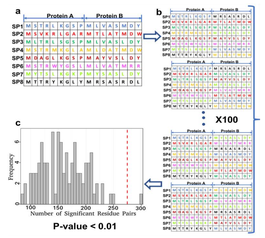
Proteins interaction



Evolutionary Couplings



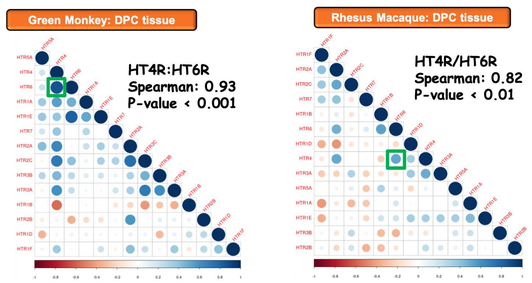
Statistical Significance



Method: we used published RNA-Seq, scRNA-Seq, and Chip-Seq data of various tissues in the Central Nervous System (CNS) of model animals including Mouse CK-p25, Mouse AD-transgenic, Green Monkey, Rhesus Macaque, Baboon as well as human post-mortem AD and healthy brains to study the co-expression and co-regulation of genes encoding 5-HT receptors. Then using Evolutionary Coupling (EC) methods, we investigated the heterodimerization of these receptors and estimate their statistical significance through simulated phylogeny

5-HT$_4$R and 5-HT$_6$R are co-expressed in the CNS

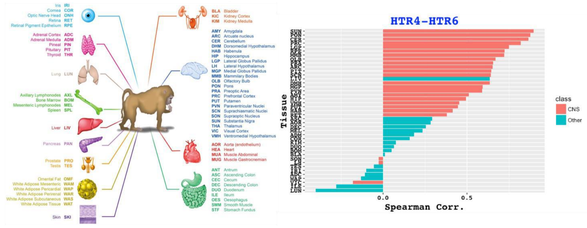
data_GM	19 (7 - 32 years)	RNA-Seq	- dorsolateral prefrontal cortex (DPC)	
Data_RM	25 (3 - 33 years)		- visual cortex (VC)	



Green Monkey: Serotonin receptors mRNA co-expression

data_BB	12 Males 6 - 7 years	RNA-Seq	64 Tissues 22 brain regions	GSE98965

Tissues were collected every 2 hours across the 24-hour day

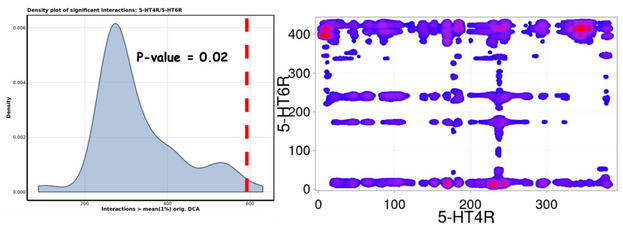


Results are similar across species, platforms and techniques

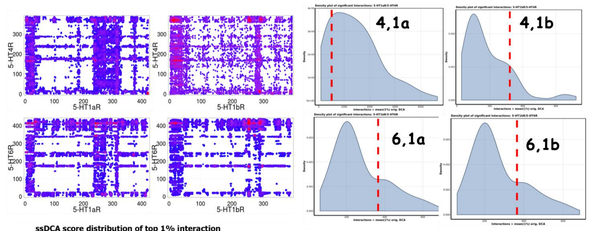
Baboon: 5-HT$_4$R and 5-HT$_6$R mRNA are mainly co-expressed in the CNS

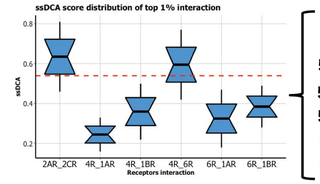
5-HT$_4$R and 5-HT$_6$R for a functional heterodimer

361 pairs of 5-HT$_4$R and 5-HT$_6$R across vertebrates
5-HT$_4$R: 388 AA; 5-HT$_6$R: 440 AA; MSA conservation: 70-90%



Protein Co-evolution suggests 5-HT$_4$R/5-HT$_6$R dimerize





5-HTr involved in AD

5-HT$_4$R:5-HT$_6$R	H
5-HT$_4$R:5-HT$_{1A}$R	✖
5-HT$_4$R:5-HT$_{1B}$R	✖
5-HT$_6$R:5-HT$_{1A}$R	✖
5-HT$_6$R:5-HT$_{1B}$R	✖

5-HT$_4$R/5-HT$_6$R heterodimerization is likely functional

Results

We found that: 5-HT$_4$R and 5-HT$_6$R are **expressed in the same brain region**, have **co-regulated gene expression** in both normal and AD subjects, and the corresponding proteins are coevolving, leading to the hypothesis that **they form a functional heterodimer**. We used the Direct Coupling Analysis follows by local convolution of Evolutionary Scores to assess the heterodimerization and found **the interaction to be functional**. Through simulated phylogeny, the interaction was deemed significant with a p-value = 0.02.

RIP serotonin? Not so fast ...

Pharmacology: previous drugs always target one receptor, agonist 5-HT$_4$R or antagonist 5-HT$_6$R. We hypothesize that finding and targeting the dimer interface could have a better outcome.

Conclusion

We used computational methods to show that 5-HT$_4$R and 5-HT$_6$R form a functional heterodimer in the CNS and predict the most likely interfaces. As both proteins are involved in AD, drugs targeting the dimer, instead of individual monomers, may have better therapeutic outcomes.

References

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