Defining the high affinity nicotinic receptor-associated proteome

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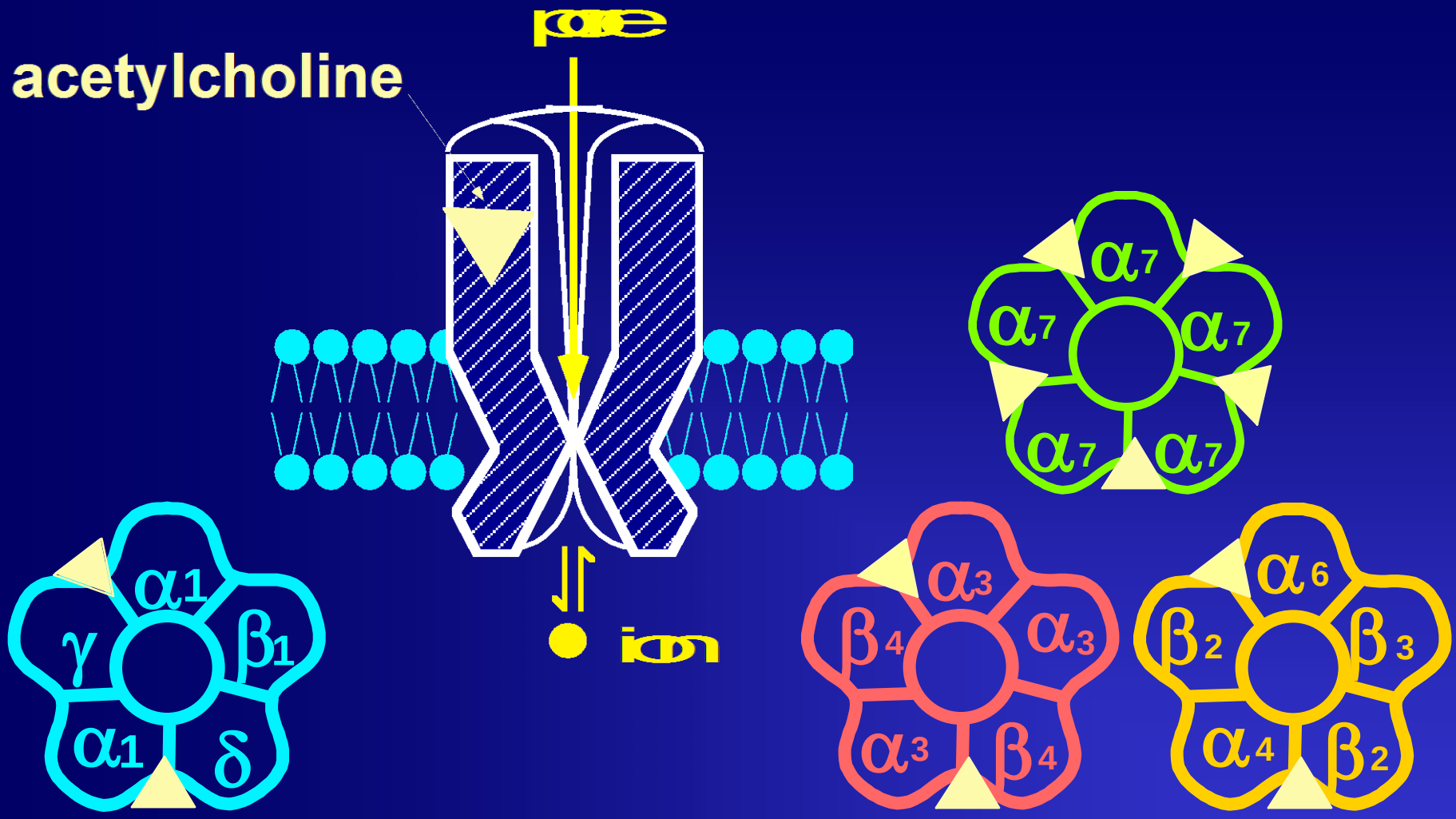
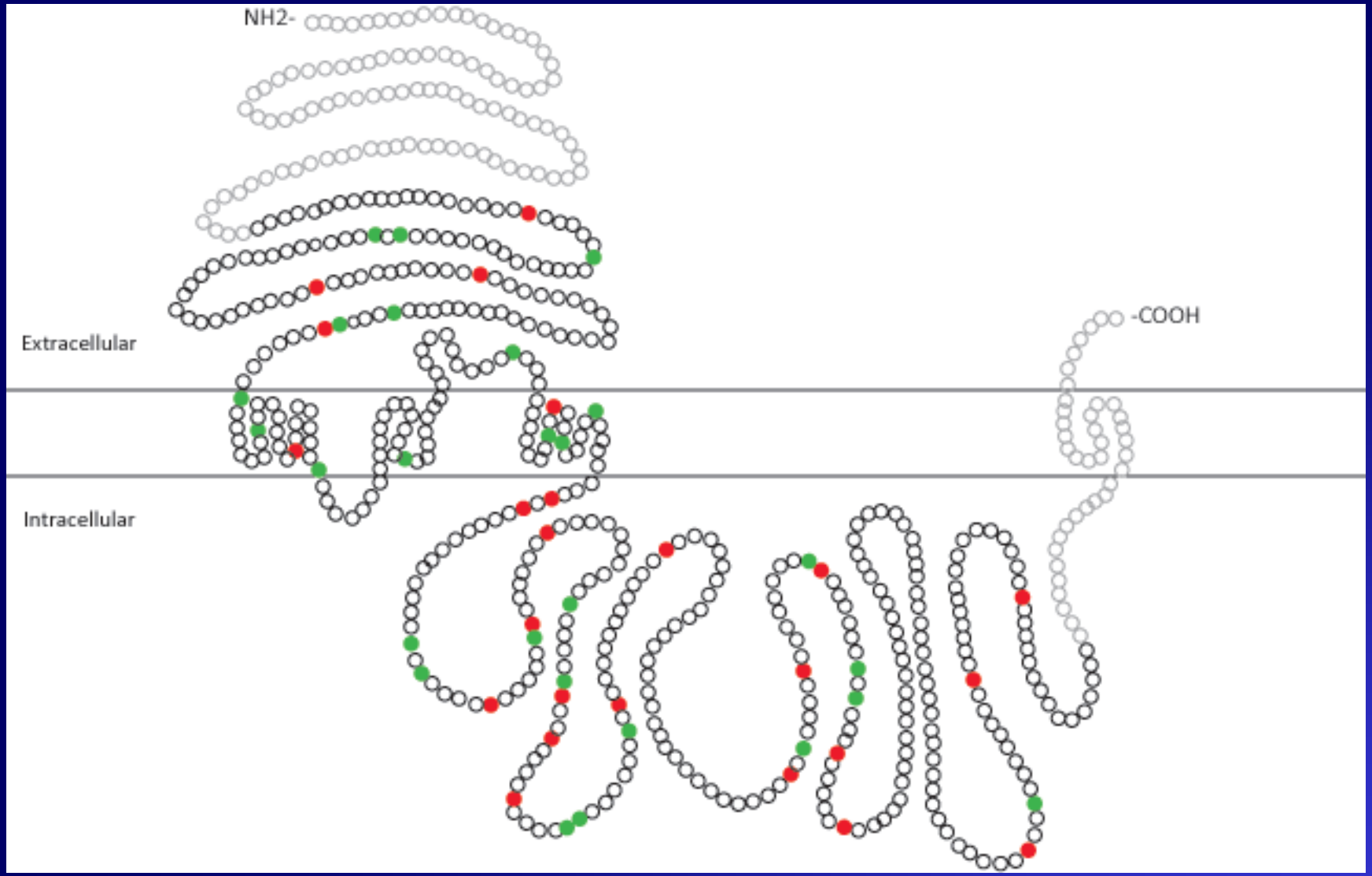
Structure of nicotinic ACh receptors

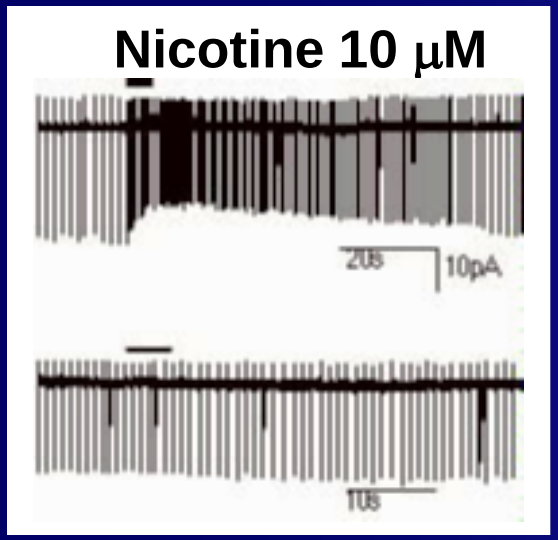


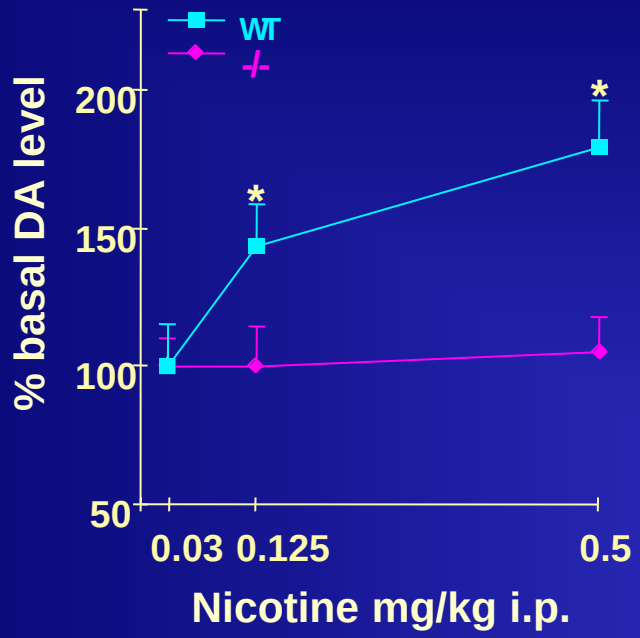
Structure of nicotinic ACh receptors

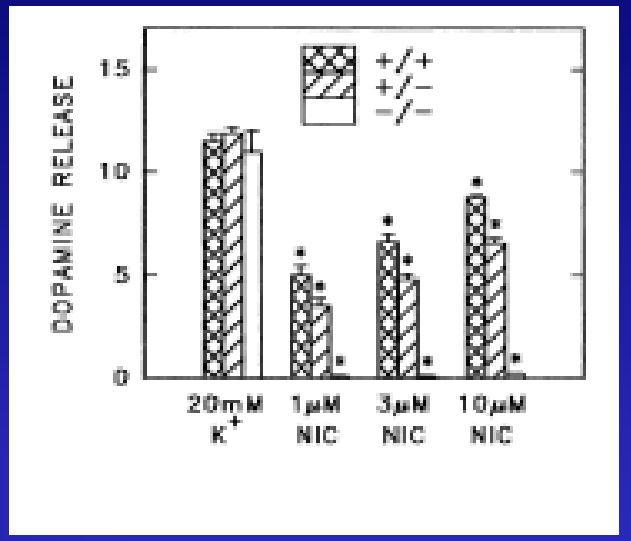


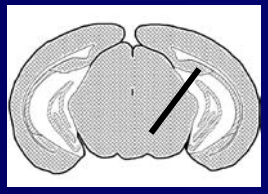
Xie et al, Biol Psych, 2010

Nicotine does not stimulate dopamine release in β2 knockout mice





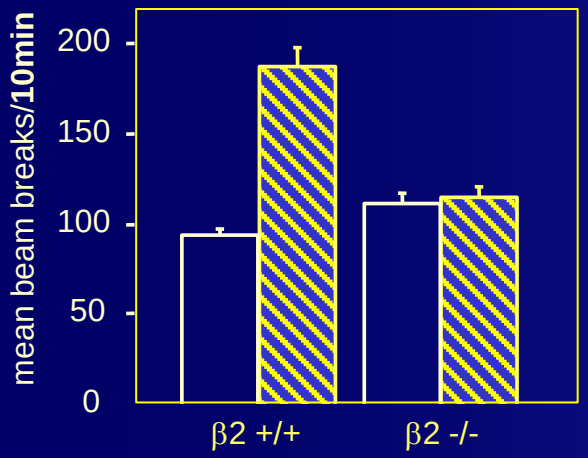


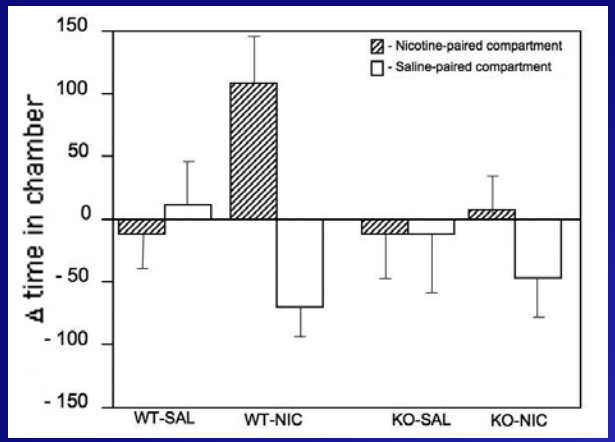


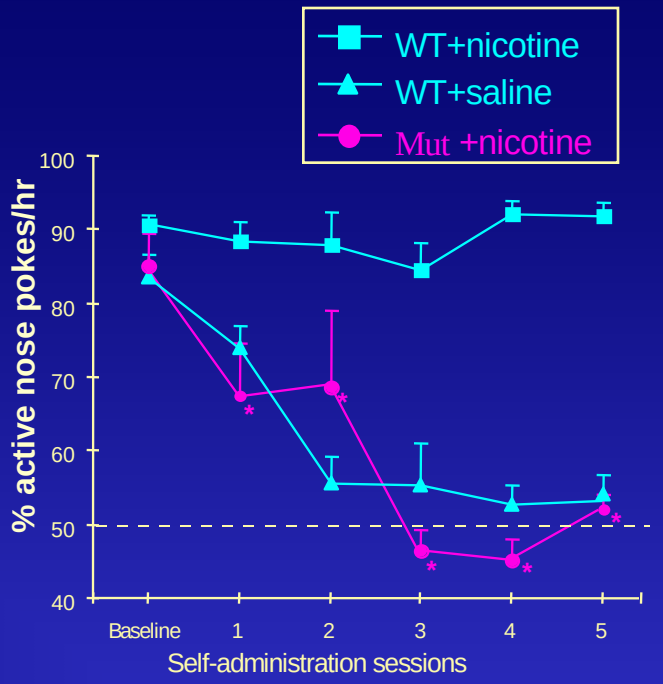
Picciotto et al, Nature, 1998

Grady et al, J Neurochem, 2001

…and does not support behaviors related to addiction







Locomotion

Place preference

Self-administration

King et al, Neuropharm. 2004

Brunzell et al, NPP. 2009

Picciotto et al, Nature, 1998

Transgenic expression of β2 in VTA rescues nicotine-induced locomotion (Mineur et al).

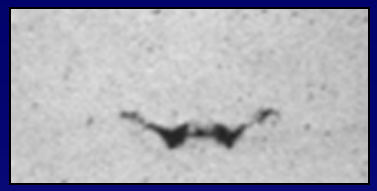
Viral-vector rescue of β2 in VTA rescues nicotine self administration (Maskos, et al).

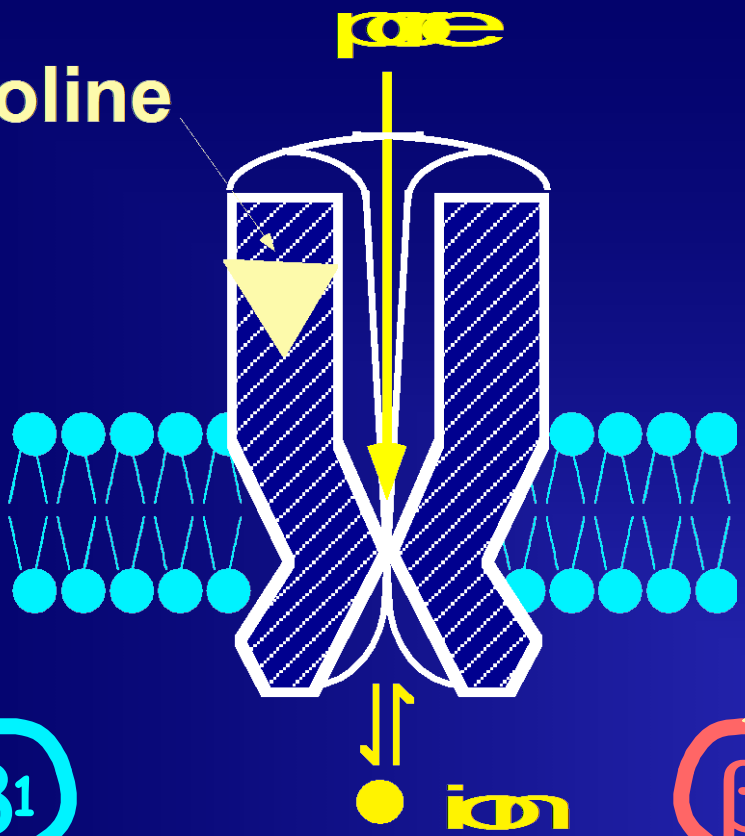
Expression of hypersensitive α4 or α6 nAChRs increases sensitivity to nicotine place preference (Tapper et al, Drenan et al).

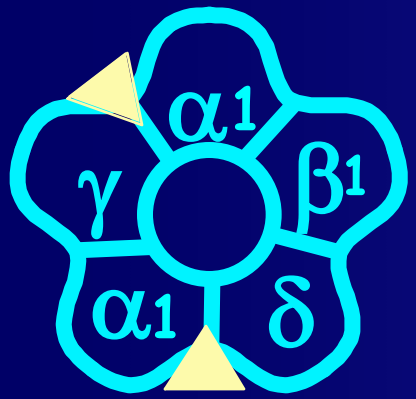
Knockout of α4 in TH-positive neurons abolishes nicotine place preference (McGranahan, et al).

α4/α6/β2 nAChRs in VTA are sufficient for nicotine reinforcement

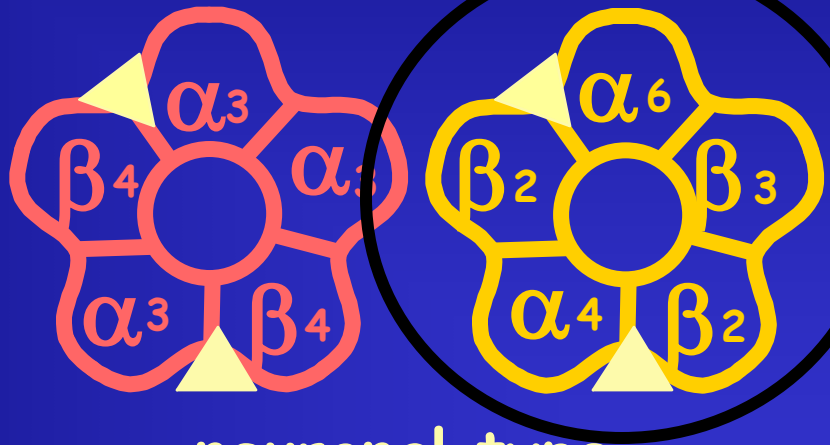
nAChRs involved in nicotine reinforceme





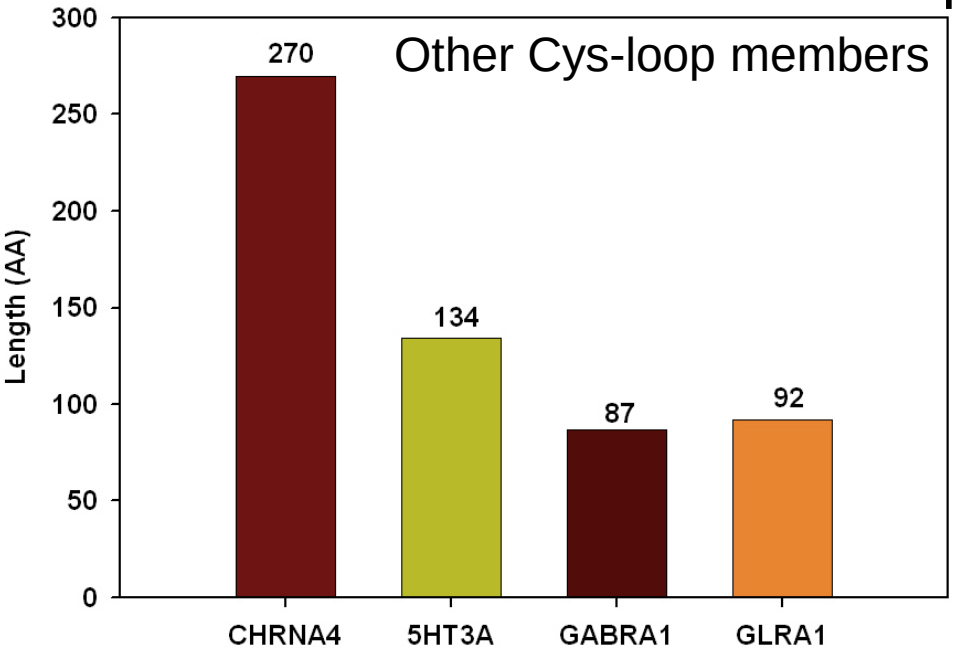


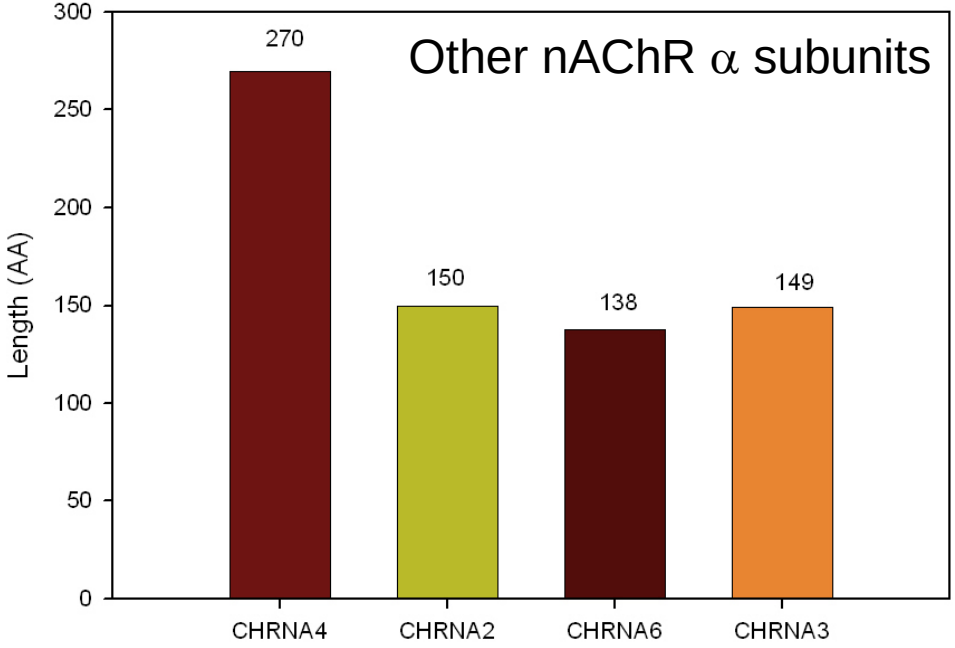
muscle type
nicotinic receptor



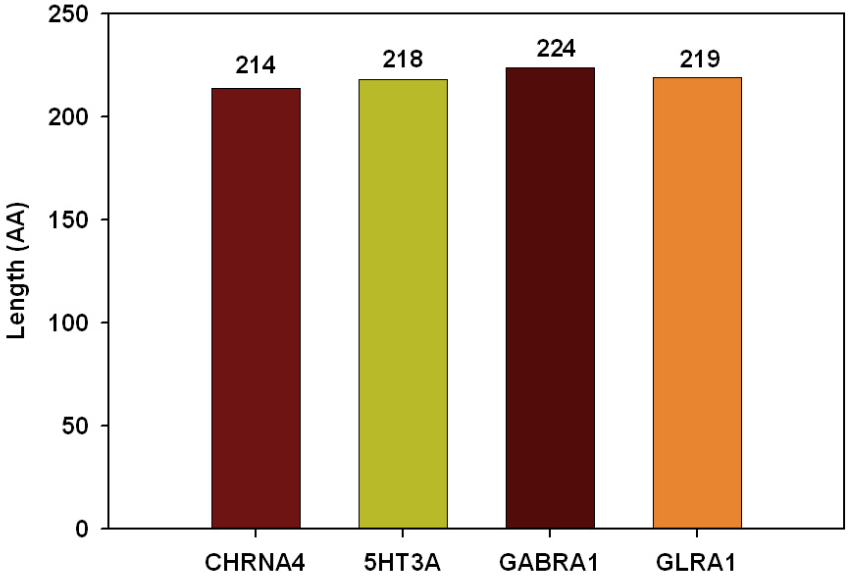
neuronal type
nicotinic receptors

M3-M4 Loop Length

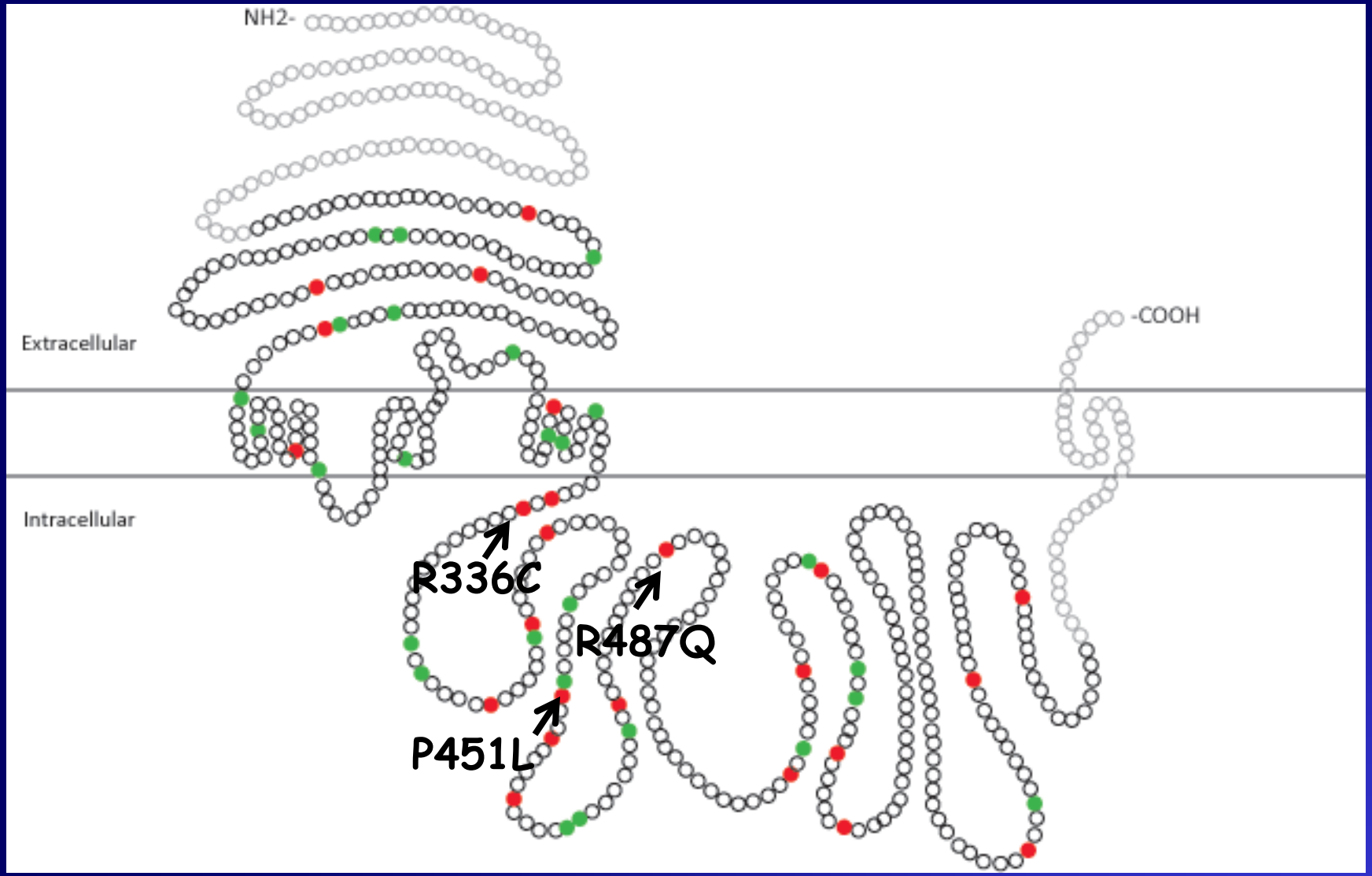




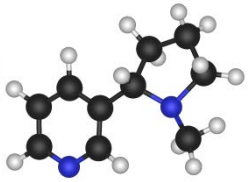
Extracellular N-Terminal Domain



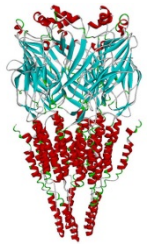
Polymorphisms in the α4 nAChR subunit



Xie et al, Biol Psych, 2010

CHRNA4 Variants




Rare Nonsynonymous Variants in Alpha-4 Nicotinic Acetylcholine Receptor Gene Protect Against Nicotine Dependence

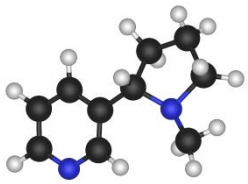
Pingxing Xie, Henry R. Kranzler, Michael Krauthammer, Kelly P. Cosgrove, David Oslin, Raymond F. Anton, Lindsay A. Farrer, Marina R. Picciotto, John H. Krystal, Hongyu Zhao, and Joel Gelernter

- All missense mutations appearing at conserved residues in the M3-M4 intracellular loop

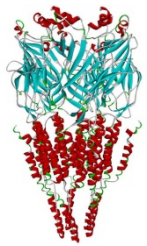
- Cursory search of disrupted eukaryotic linear interaction motifs (ELM) narrowed focus

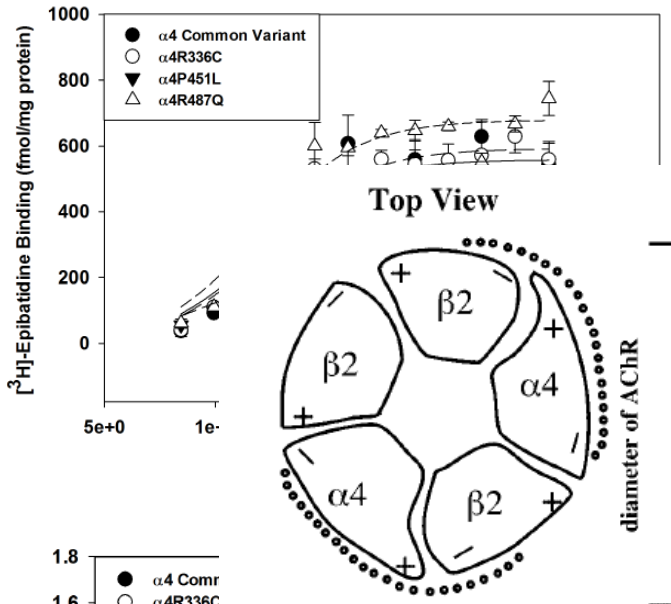
- Tested effects on:

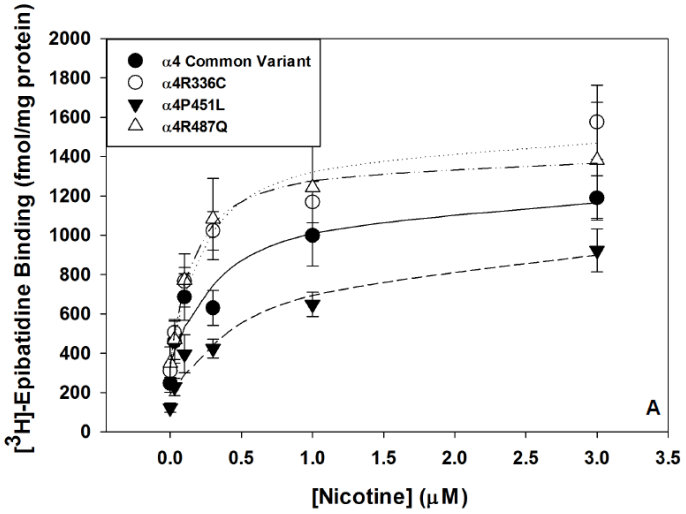
Receptor assembly/expression in HEK293 cells
Agonist-evoked responses in Xenopus oocytes
Interactome from immunoprecipitated receptor complexes

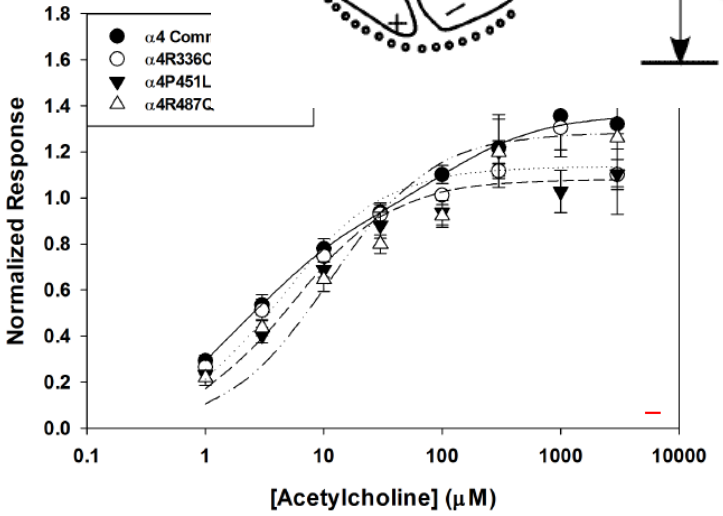



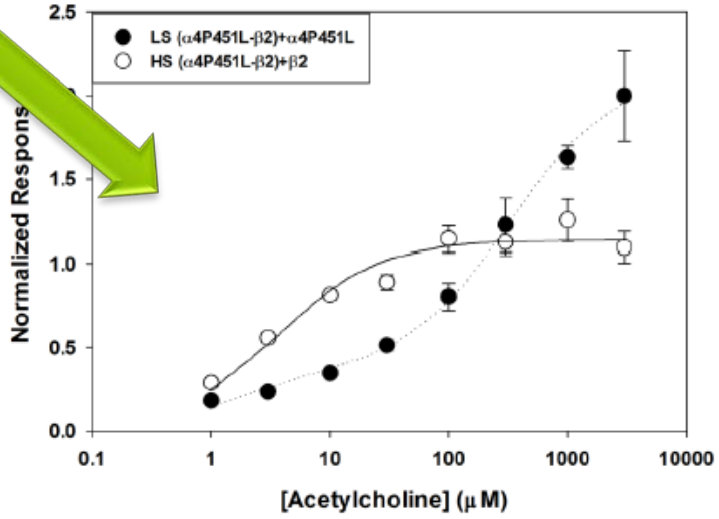
CHRNA4 Variants

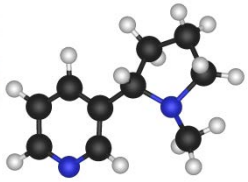


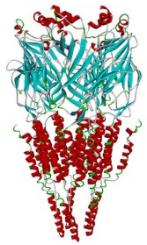






CHRNA4 Variants

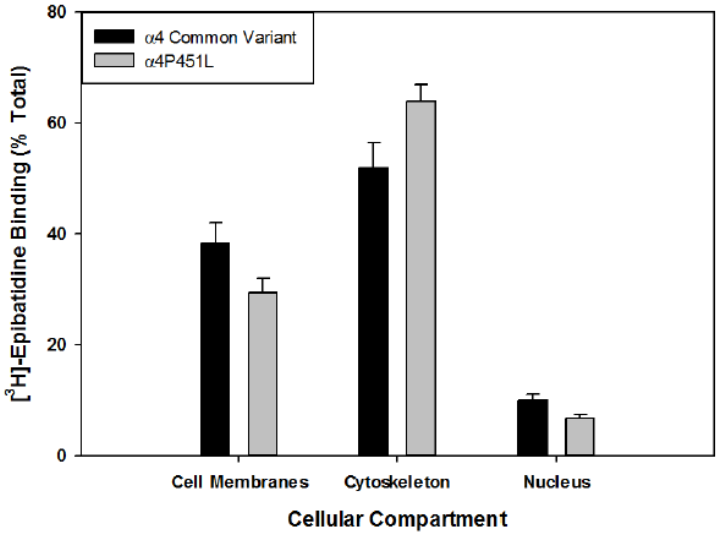



Enrichment of phosphorylated proteins prior to LC-MS/MS protein ID effectively selects for mature pentamers vs retained intracellular intermediates

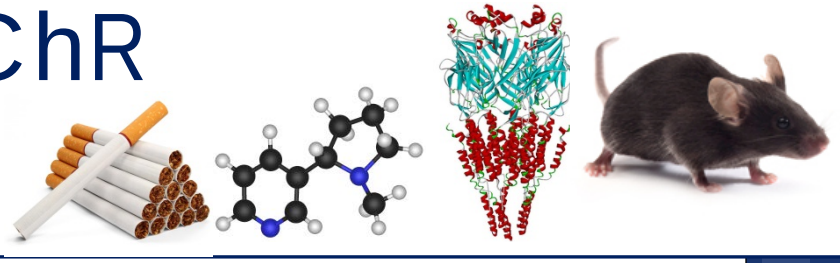
Identified interactomes vary considerably across α4 rare variants

α4P451L recruits importin isoforms and Reduces 14-3-3 chaperone binding, yet no difference in nuclear fraction binding sites is found

Additional variation in associated proteins is awaiting further validation with other model systems



Defining the α4β2* nAChR Interactome



- Studies of nAChR interactomes and regulation requires a quantitative, unbiased, high-throughput method for discovery-phase examinations.
- Integrating iTRAQ label-based quantitative proteomics with transgenic manipulation of the target protein.

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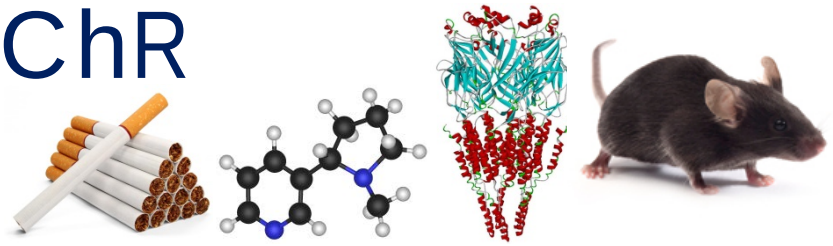
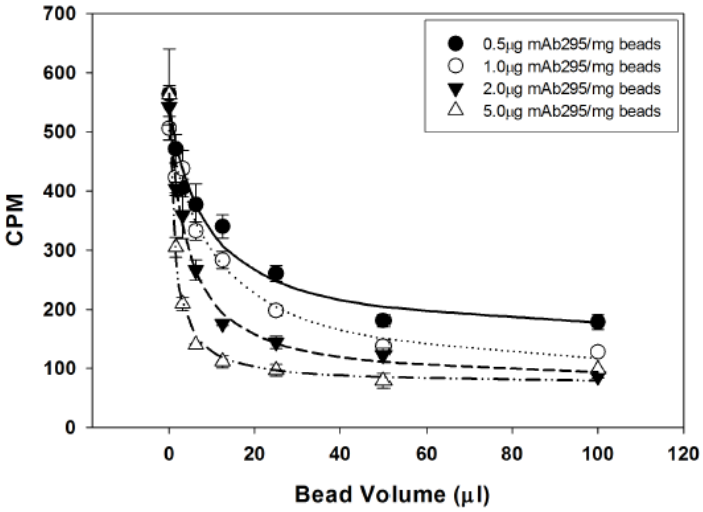
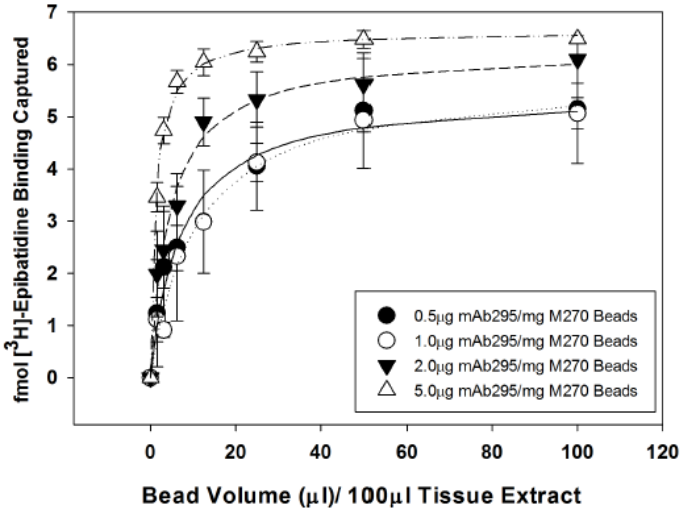
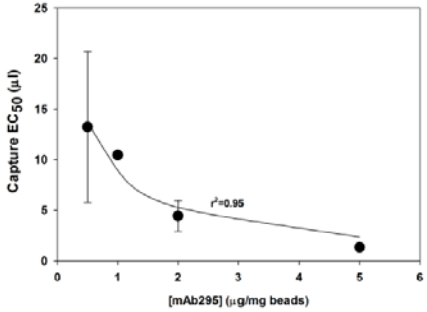






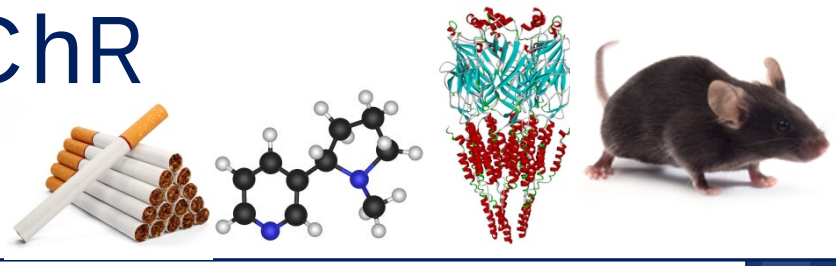
[mAb295] (μg/mg beads)	Maximal Capture of [³H]-Epibatidine Binding Sites (fmol/0.1ml)	½ Maximal Capture Bead Volume (μl of 10mg/ml)	Capture Efficiency (% Total)
0.5	5.7±0.6	13.2±7.5	78.1±10
1.0	5.8±0.3	10.5±0.04	85.2±3
2.0	6.3±0.4	4.5±1.5	86.8±5
5.0	6.6±0.1	1.3±0.19	88.3±2

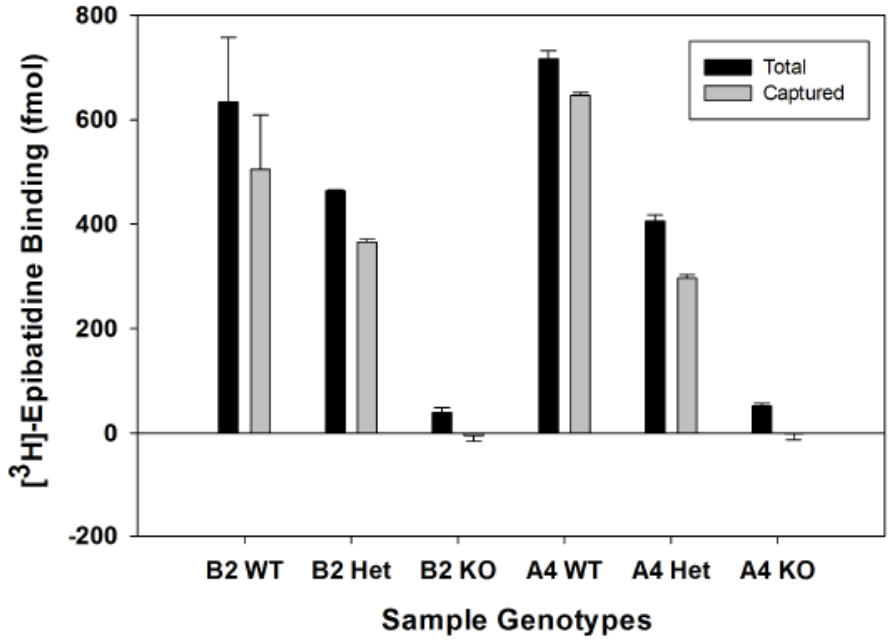


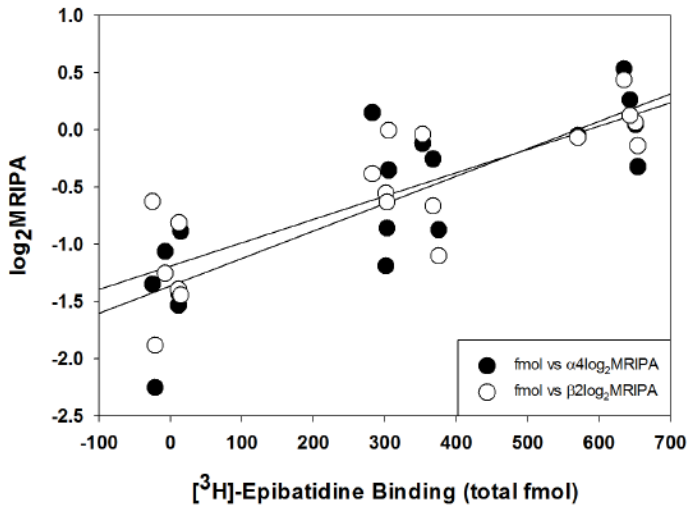
M270-immobilized mAb295:

- Produces near-complete capture of solubilized β2* nAChRs
- Optimal conditions are achieved with 5μg mAb/mg beads, used at 10% total sample volume.

Defining the α4β2* nAChR Interactome

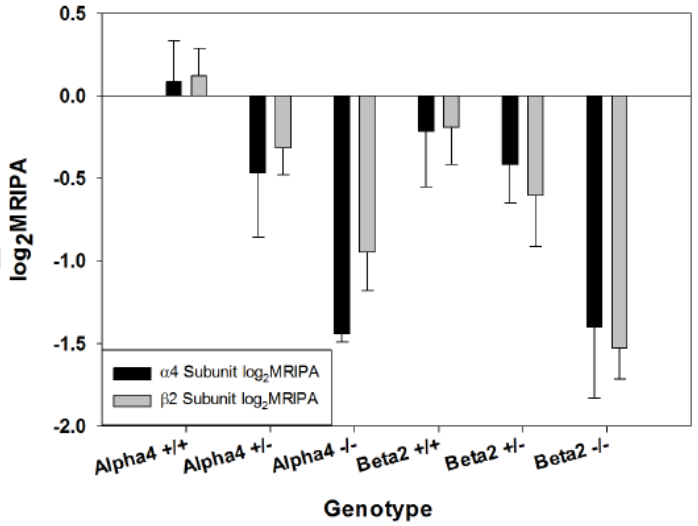






Immunopurified β2* nAChR Complexes:

- ✓ Follow expected gene-dose expression
- ✓ Correlate well with iTRAQ quantitation
- ✓ Confirm earlier reports of subunit inter-dependence



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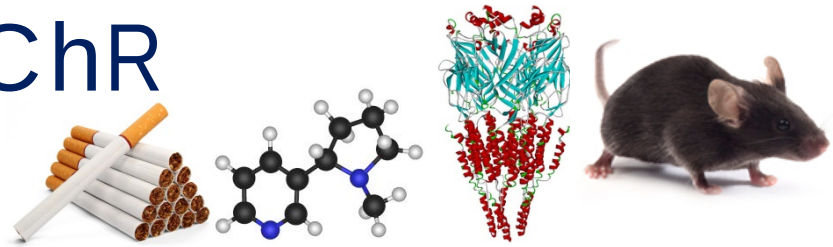
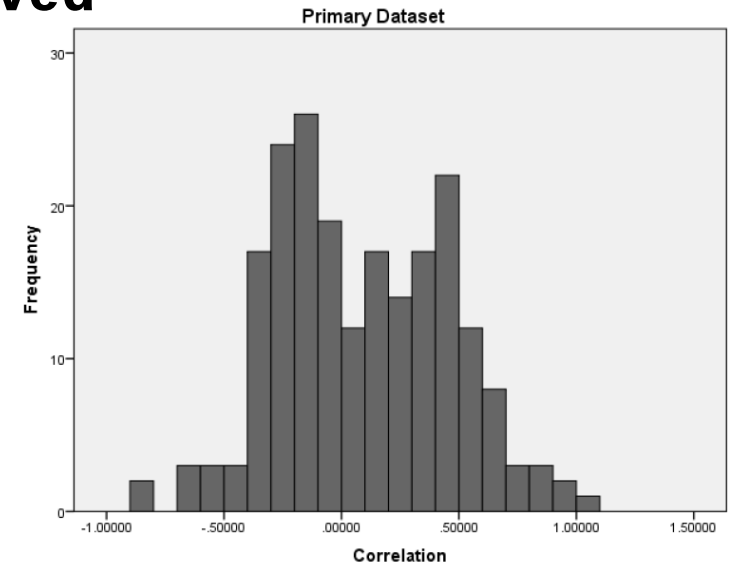
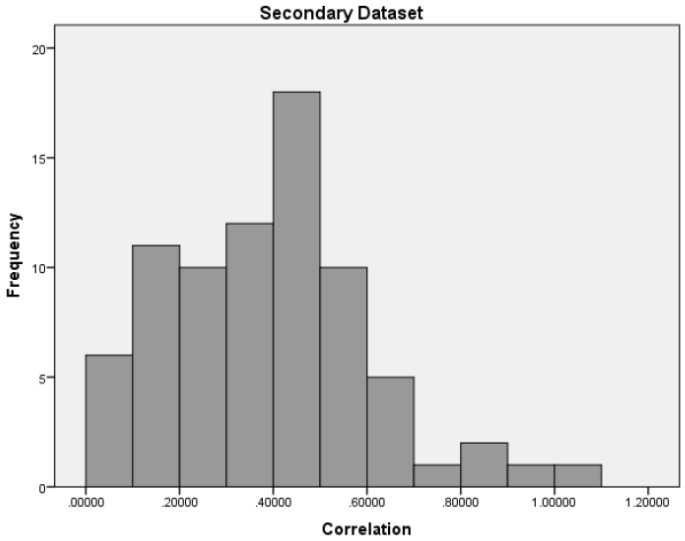


Initially identified 208 proteins:

- **Frequency distribution was bimodal**
- **Indicated multiple processes involved**

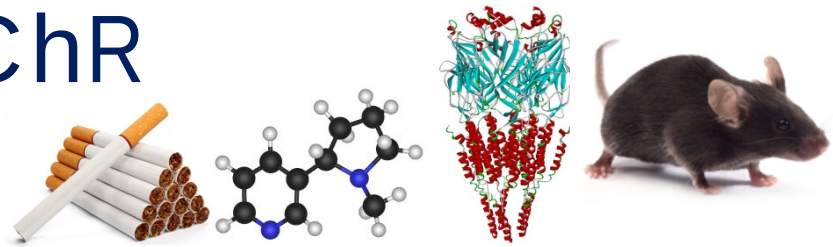




After correcting for cell compartment (based on UniProt assignment):

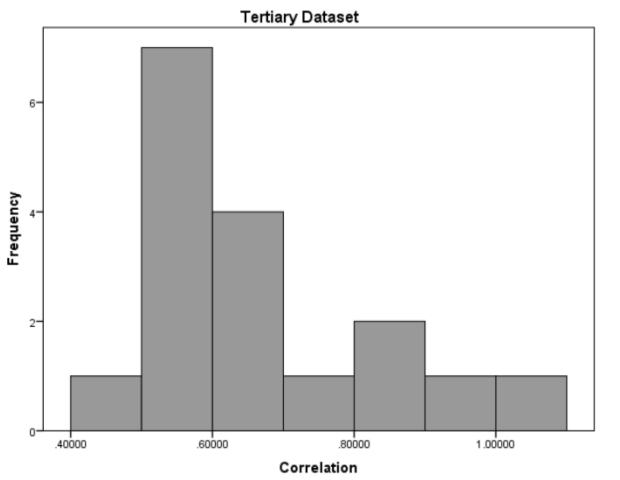
- **List decreased to 98 proteins**
- **Unimodal distribution**

Defining the α4β2* nAChR Interactome

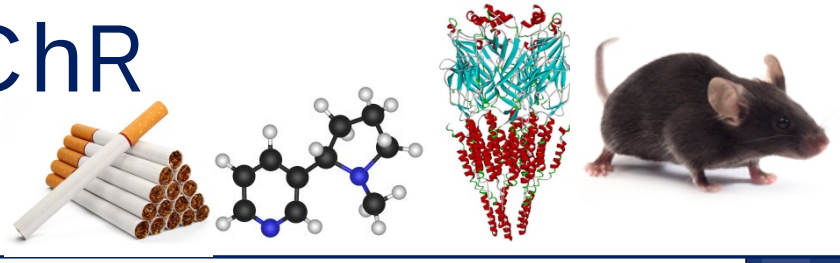


Correlation	N	F	p	PROTEIN	UniProtKB Accession	Cellular Compartment	Previously Identified?	Molecular Function
0.748	12	20.268	0	Glial fibrillary acidic protein	P03995	Cytoplasm	No	protein binding; structural molecule activity
0.858	18	44.542	0	Neuronal acetylcholine receptor subunit alpha-4	O70174	Cell junction	No	transporter activity; signal transducer activity; protein binding; neurotransmitter binding; amine binding
1	18	-	0	Neuronal acetylcholine receptor subunit beta-2	Q9ERK7	Cell junction	No	transporter activity
0.652	18	11.844	0.003	Neurofilament light polypeptide	P08551	Growth cone	No	protein binding; structural molecule activity
0.645	18	11.404	0.004	Actin-related protein 3	Q99JY9	Cytoplasm	No	nucleotide binding; protein binding
0.637	18	10.904	0.004	Calcium/calmodulin-dependent protein kinase type II subunit alpha	P11798	Cytoplasm	No	transferase activity; nucleotide binding; protein binding
0.917	18	21.235	0.01	Calcium/calmodulin-dependent protein kinase type II subunit gamma	Q923T9	Sarcoplasmic reticulum membrane	No	transferase activity; nucleotide binding; protein binding
0.57	18	7.681	0.014	F-actin-capping protein subunit alpha-2	P47754	Cytoplasm	No	protein binding
0.562	18	7.386	0.015	Thyroid hormone receptor-associated protein 3	Q569Z6	Nucleus	No	nucleotide binding; protein binding
0.665	12	7.933	0.018	Transcriptional activator protein Pur-alpha	P42669	Nucleus	No	nucleic acid binding; translation regulator activity; protein binding
0.539	18	6.563	0.021	Ectonucleotide pyrophosphatase/phosphodiesterase family member 6	Q8BGN3	Cell membrane	No	catalytic activity; hydrolase activity
0.519	18	5.884	0.027	Spectrin beta chain, brain 1	Q62261	Cytoplasm	No	protein binding; lipid binding; structural molecule activity
0.856	6	11.009	0.029	Ras-related protein Rap-1A	P62835	Cell membrane	No	hydrolase activity; protein binding; nucleotide binding
0.512	18	5.695	0.03	Myosin-10	Q61879	Cytoplasm	No	protein binding; nucleotide binding; hydrolase activity; motor activity
0.506	18	5.496	0.032	Myelin proteolipid protein	P60202	Cell membrane	No	structural molecule activity; protein binding
0.502	18	5.378	0.034	Spectrin alpha chain, brain	P16546	Cytoplasm	Yes	protein binding; ion binding
0.493	18	5.149	0.037	Tubulin beta-3 chain	Q9ERD7	Cytoplasm	No	hydrolase activity; nucleotide binding; structural molecule activity; protein binding; peptide binding

Further filtering based on correlation with internal standard (β2 nAChR subunit) yielded 17 proteins



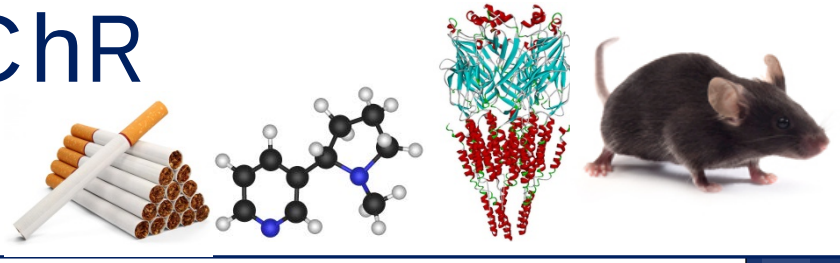
Defining the α4β2* nAChR Interactome



What did we learn?

- iTRAQ sensitivity is equivalent to pharmacological methods for nAChR quantitation
- α4 and β2 subunit expression is highly interdependent
- The majority of ID'd proteins did not follow linear association with β2
- Low-abundance nAChR subunits (α5,α6) will require pre-enrichment for successful ID

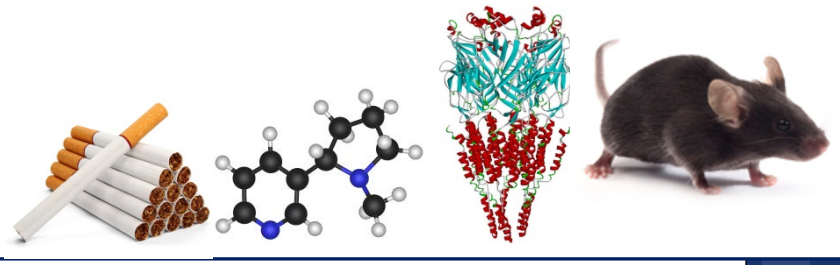
Defining the α4β2* nAChR Interactome



Perhaps most importantly:

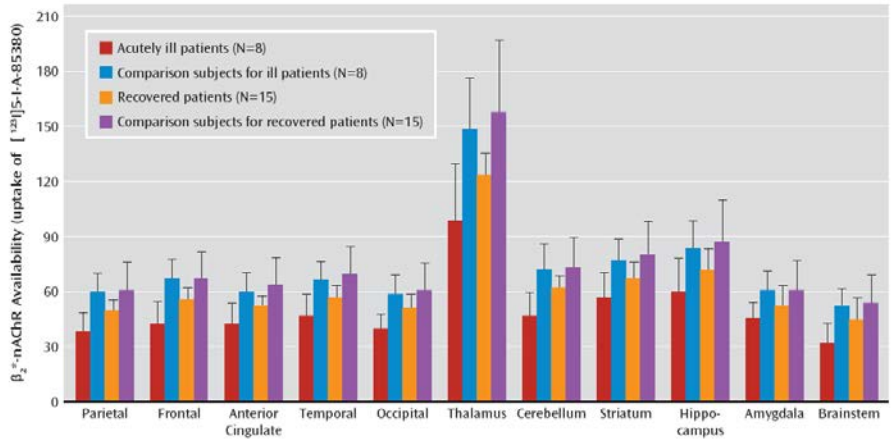
- Coupling iTRAQ with gene-dose dependent expression of a target protein and immuno-affinity purification is a viable workflow for the ID of high-value targets for future study/validation.

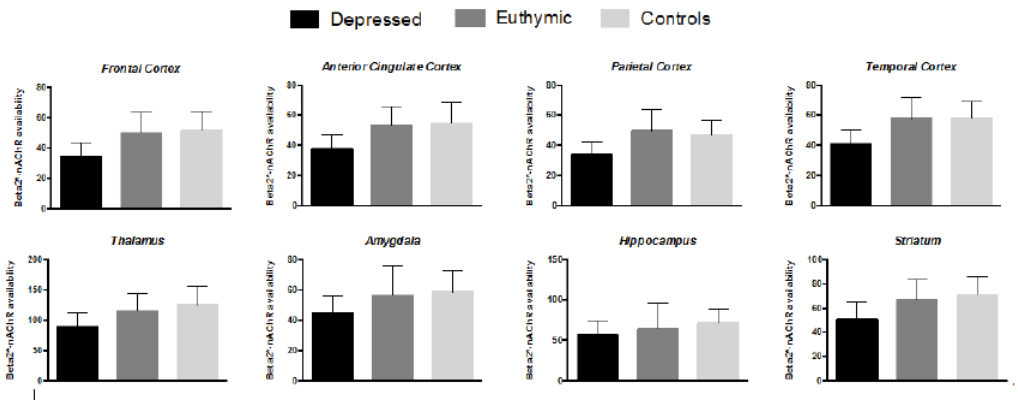
nAChRs, Smoking, and Bipolar Disorder



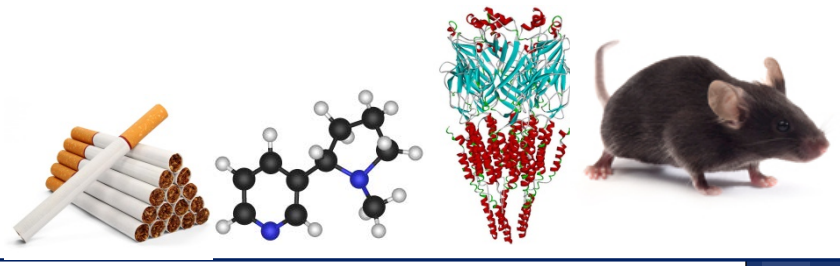
nAChRs in Major Depression and Bipolar Disorder (BPD)

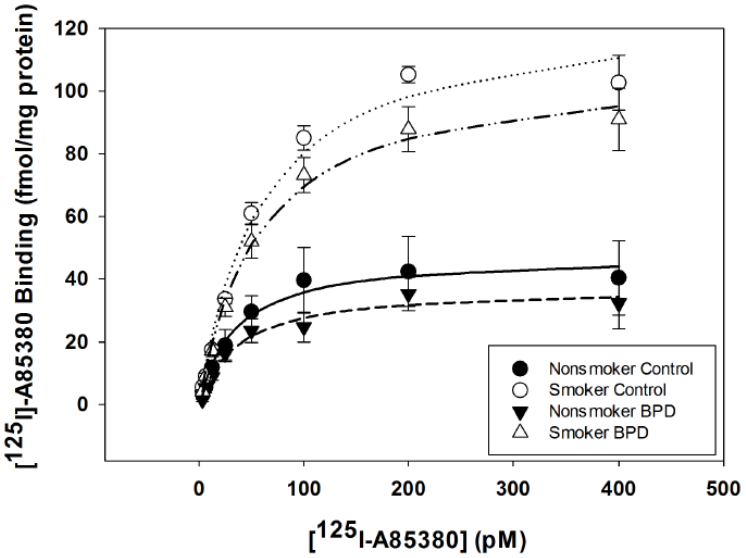
β2* nAChR occupancy by [^{123}I]-A85380 is decreased in MD and BPD measured by *In vivo* SPECT



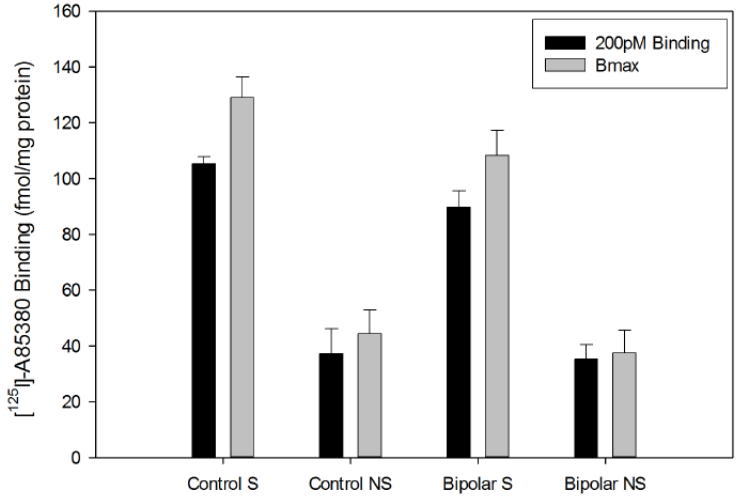


nAChRs, Smoking, and Bipolar Disorder





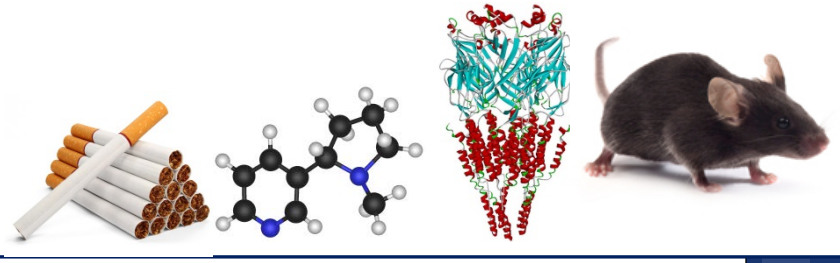
Saturation of [^{125}I]-A85380 binding to postmortem tissue homogenates



Estimated Bmax and bound fmol at 200pM [^{125}I]-A85380 are equivalent

Degree of upregulation by smoking status hints at a discrepancy between Control and BPD

nAChRs, Smoking, and Bipolar Disorder



Control group: 127 proteins with significant smoking effect (51 up, 76 down)

BPD group: 135 proteins with significant smoking effect (50 up, 85 down)

59 proteins with significant BPD x Smoking interaction by ANOVA

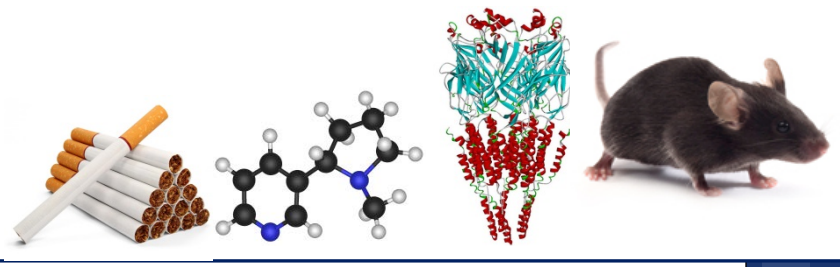
Some proteins of note in control samples:

- 14-3-3 isoforms, CamKII and HSP variants are downregulated by smoking

- VILIP-1, NCAM1, synaptotagmin, and β-adducin are upregulated by smoking

Additional samples will augment and validate preliminary findings

Future Aims



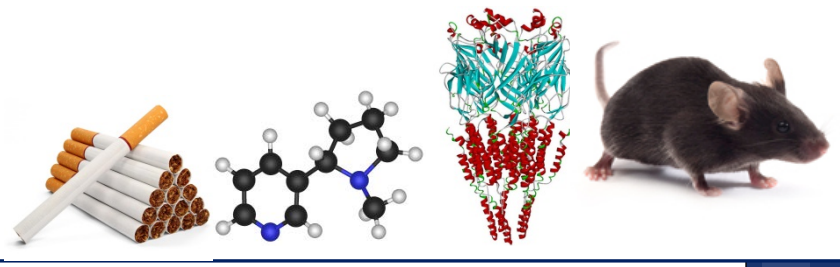
iTRAQ/nAChR transgenic project:

- Adding α4/β2 double-het group
- Cortical vs thalamic nAChRs
- Saline vs chronic nicotine groups

BPD nAChR project:

- Adding ‘n’ to label-free quantitation experiments
- Attempting stoichiometry estimations

Acknowledgments



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