UNIVERSITY OF MICHIGAN

Changes in the Brain during Chronic Exposure to Nicotine

May 2010

Inadvertent therapeutic effects of chronic nicotine

Henry Lester

- Nicotine Addiction
- Parkinson's Disease
- ADNFLE

- Behavior
- Circuits
- Synapses
- Neurons
- Intracell.
- Binding
- Nic vs ACh
- Proteins
- RNA
- Genes

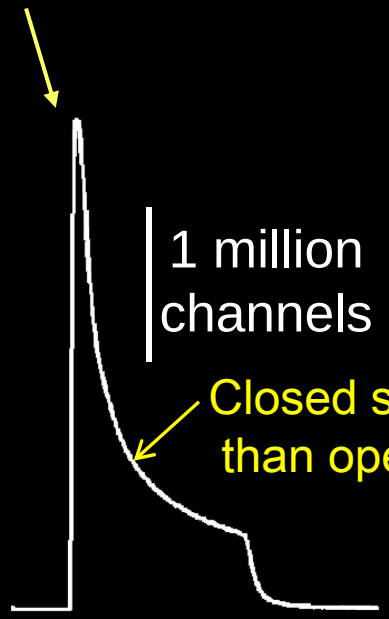
The nicotine video

Produced for Pfizer to explain varenicline (Chantix) to physicians

This summarizes knowledge in ~ 2004.

"physical" addiction vs "psychological" addiction.

Desensitization and "Upregulation"



Nicotine Addiction

Parkinson's Disease

ADNFLE

Behavior

Circuits

Synapses

Neurons

Intracell.

Binding

Nic vs ACh

Proteins

RNA

Genes

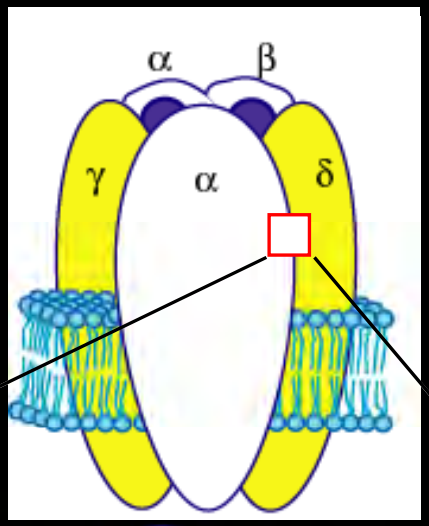
Nicotine and ACh act on many of the same receptors, but . . .

1. Nicotine is highly membrane-permeant. ACh is not.
   Ratio unknown, probably > 1000.

2. ACh is usually hydrolyzed by acetylcholinesterase (turnover rate ~10^4 /s.) In mouse, nicotine is eliminated with a half time of ~ 10 min.
   Ratio: ~10^5

3. EC_{50} at muscle receptors: nicotine, ~400 μM; ACh, ~ 45 μM.
   Ratio, ~10. Justified to square this because nH = 2. Functional ratio, ~100.
   For nicotine, EC_{50}(muscle) / EC_{50}(α4β2) = 400
   We studied this difference (next 4 slides)

The AChBP interfacial “aromatic box” occupied by nicotine (Sixma, 2004),
Probed functionally by unnatural amino acid mutagenesis



H-bond 12-fold tighter binding vs muscle

Additional H-bond to non-α subunit

αY198
C2

αW149
B

αY93
A

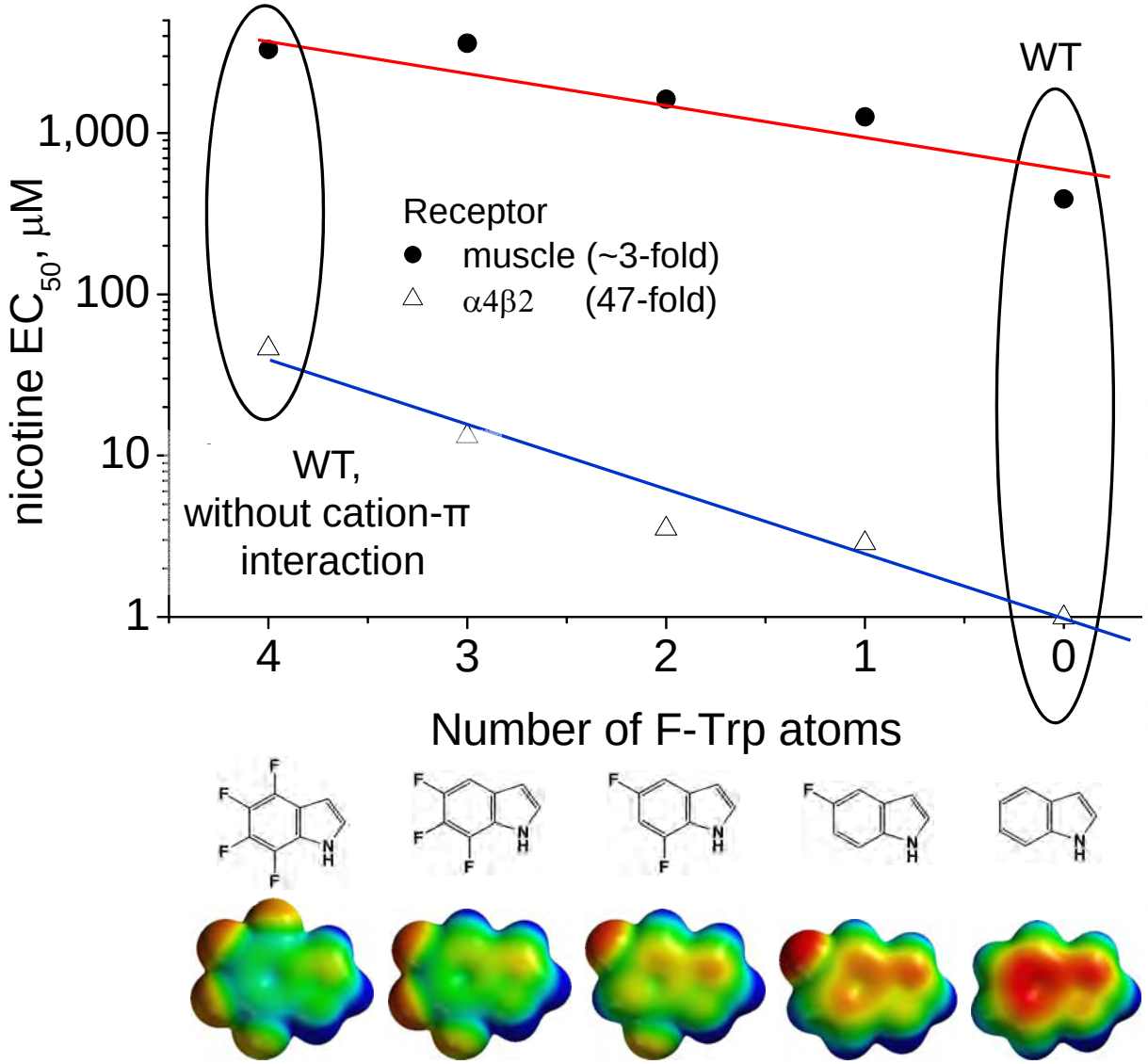
αY190
C1

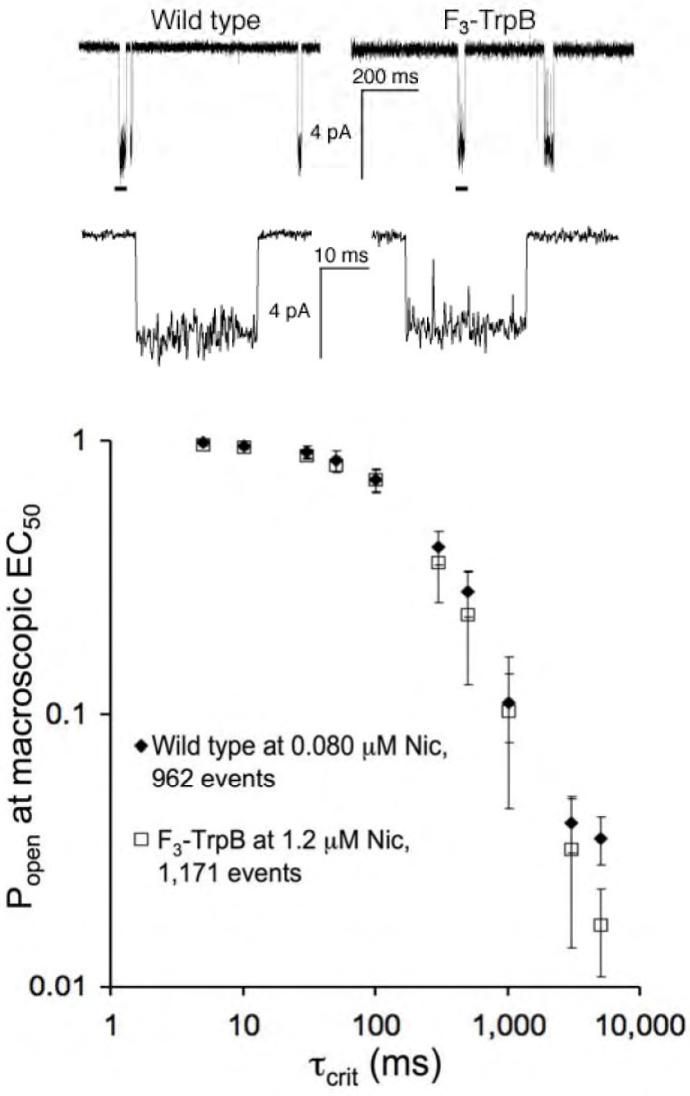
non-αW55
D

(Muscle Nicotinic numbering)

Cation-π interaction
16-fold tighter binding vs muscle

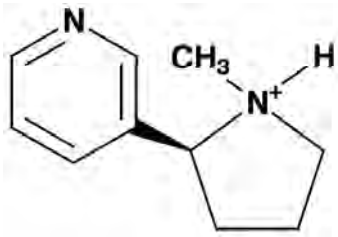
Nicotine makes a stronger cation-π interaction with Trp B
at α4β2 receptors than at muscle receptors;
this partially explains α4β2 receptors' high binding affinity for nicotine.

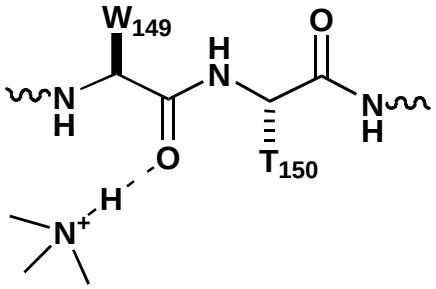


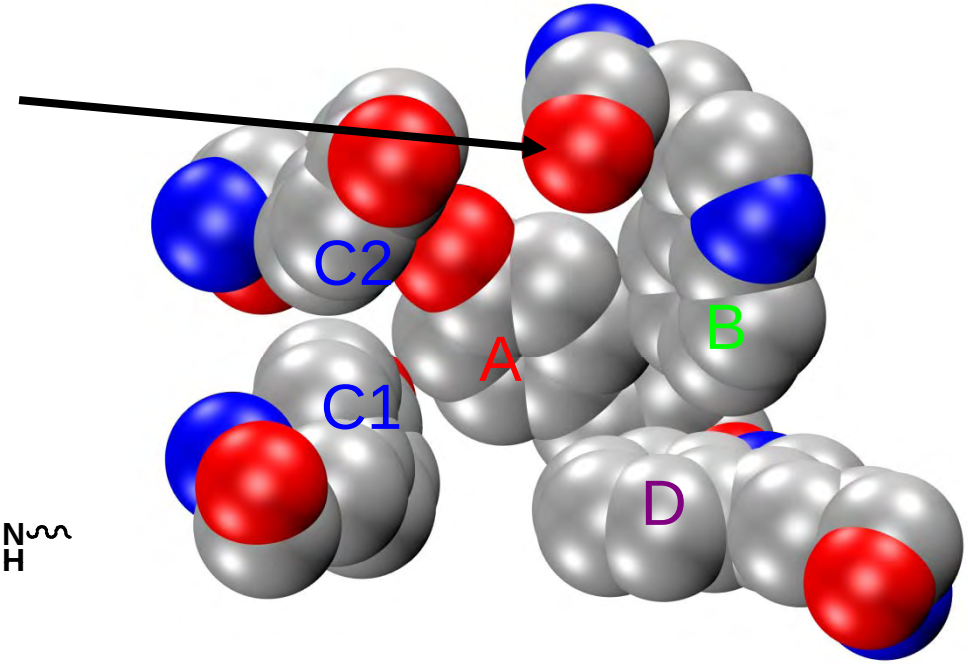


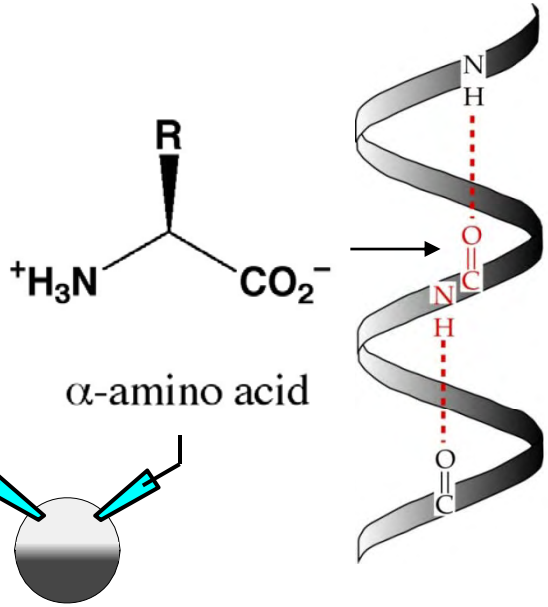
Nicotine makes a stronger H-bond to a backbone carbonyl at α4β2 than at muscle receptors: Shown by amide to ester substitution:

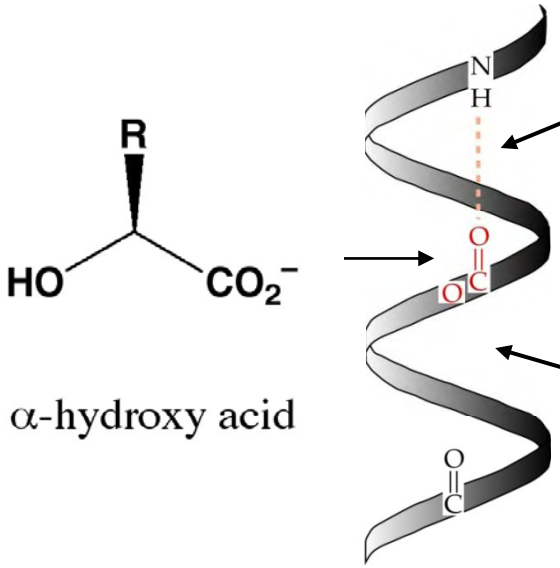
Fold EC_{50} increase	
α4β2	20
muscle	1.5











Weaker hydrogen bond

Deleted hydrogen bond

Nicotine EC50 values:

Muscle nAChR	single component	~ 400 μM
α4β2	two components	~ 1 μM, ~200 μM

Underlying the 400-fold higher nicotine sensitivity

of

neuronal vs muscle receptors:

Factor of ~16 for the cation-π interaction;

Factor of ~ 12 for the H-bond;

16 x 12 = 192. We still can't explain a factor of 400/192 ~ 2.

Xiu et al, Nature 2009

Possible molecular mechanism #1 for changes with chronic nicotine:

Signal transduction triggered by a ligand-gated channel

receptor

G protein

i | q | s | t

effector

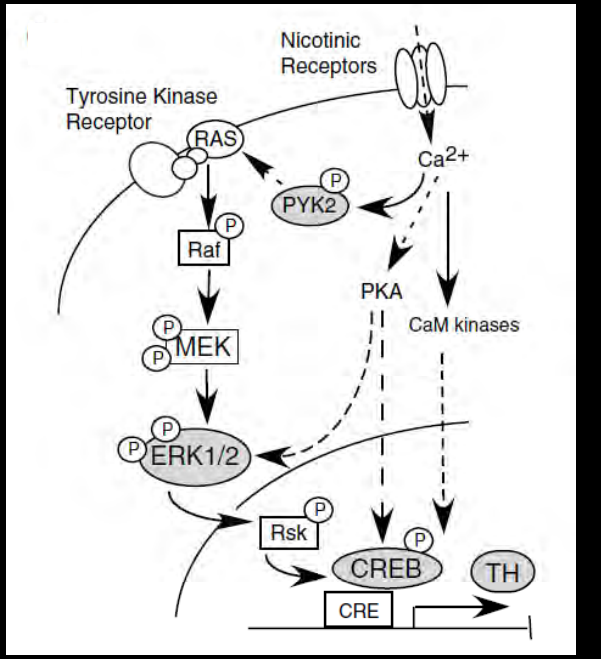
channel | enzyme

intracellular messenger

Ca^{2+} | cAMP

kinase

phosphorylated protein



Brunzell, Russell, & Piccotto, 2003

NMDA receptors

and

nAChRs

are highly permeable to Ca^{2+}

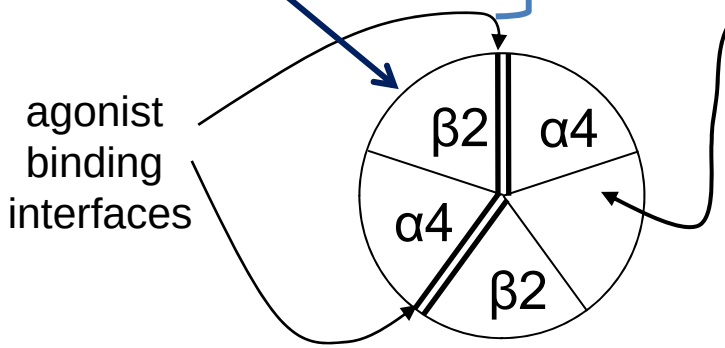
as well as to Na^{+}.

Possible Mechanism #2 for changes with chronic nicotine: Upregulation with chronic nicotine (1983: Marks & Collins; Schwartz and Kellar)

- Brain Region
- Cell type: DA vs GABA vs Glut
- Subcellular Compartment: Axon terminal vs Somatodendritic
- Pharmacological subtype: low vs high affinity
- Detailed stoichiometry: subunit composition

Chronic nicotine upregulates nAChRs
We now appreciate that upregulation displays specificity at all levels studied

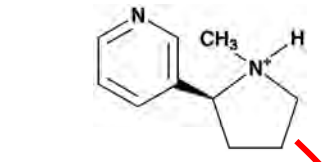
- Behavior
- Circuits
- Synapses
- Neurons
- Intracell.
- Binding
- Nic vs ACh
- Proteins
- ~~RNA~~
- Genes

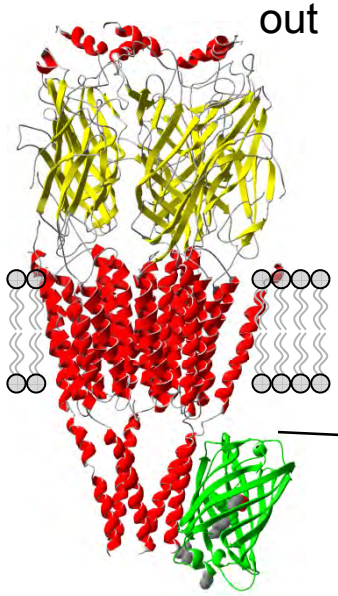


auxiliary subunit

	sensitivity	up-regulated?
α4	Low	Probably not
β2	High	Yes, but selectively
α5	High	Unknown

The SePhaChARNS mechanism: Upregulation occurs because Nicotine is a Selective Pharmacological Chaperone of Acetylcholine Receptor Number and Stoichiometry





Endoplasmic reticulum

Golgi complex

Early endosome

Plasma membrane

COPI

ERGIC

Clathrin

COPII

COPI

nAChR

nAChR

Secretory vesicle

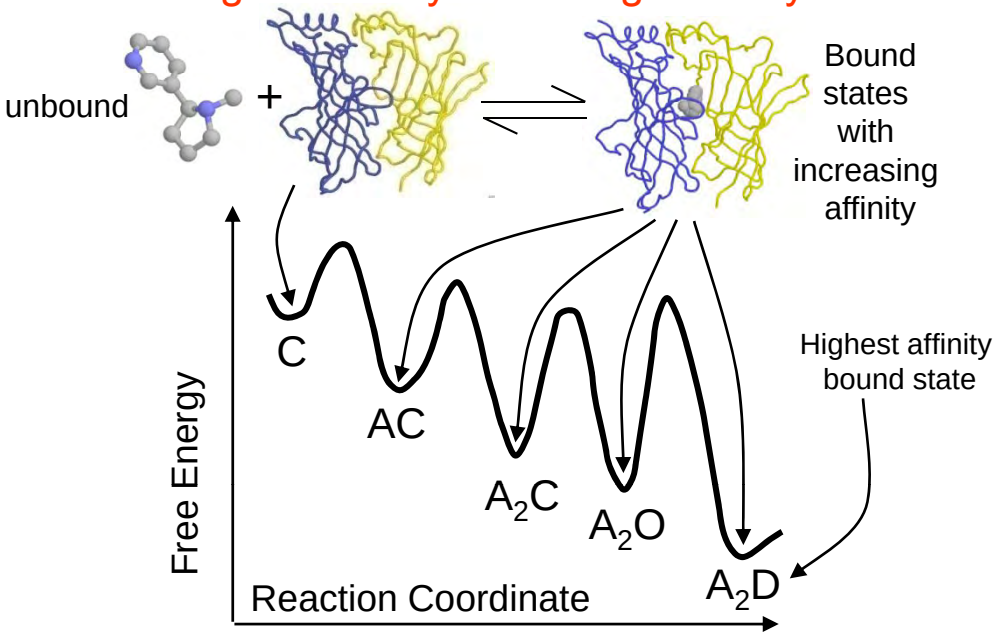
Secretory pathway

Pharmacological chaperoning: upregulation starts here (Oversimplification)

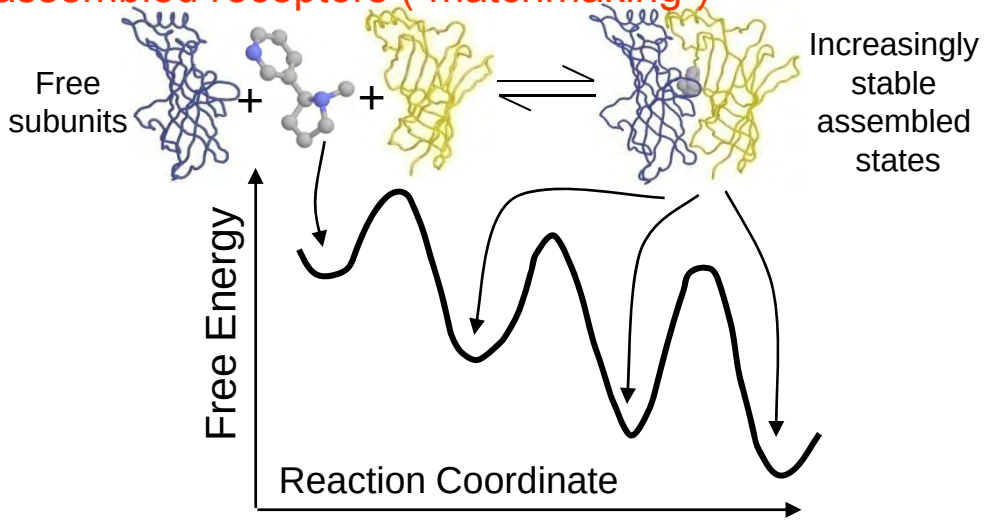
Early LTP / Opioids: regulation starts here (Oversimplification)

SePhaChARNS: Thermodynamics

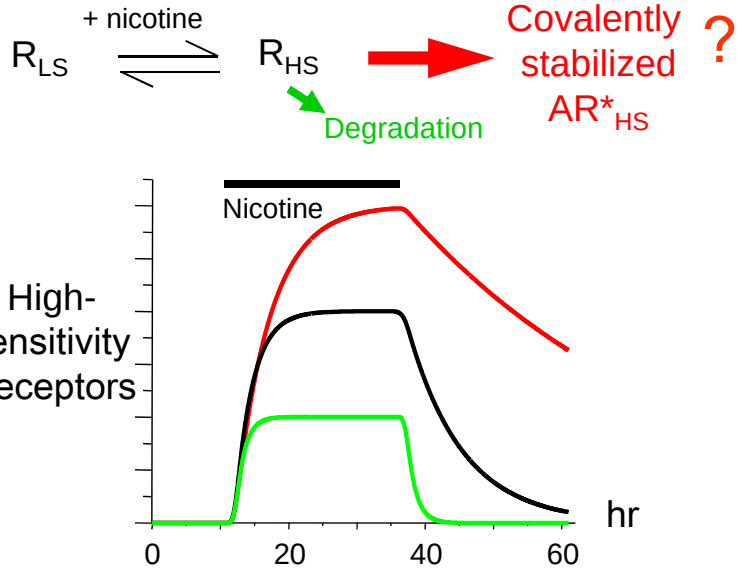
#1. Binding eventually favors high-affinity states



#2. Nicotine binds to subunit interfaces, favoring assembled receptors ("matchmaking")

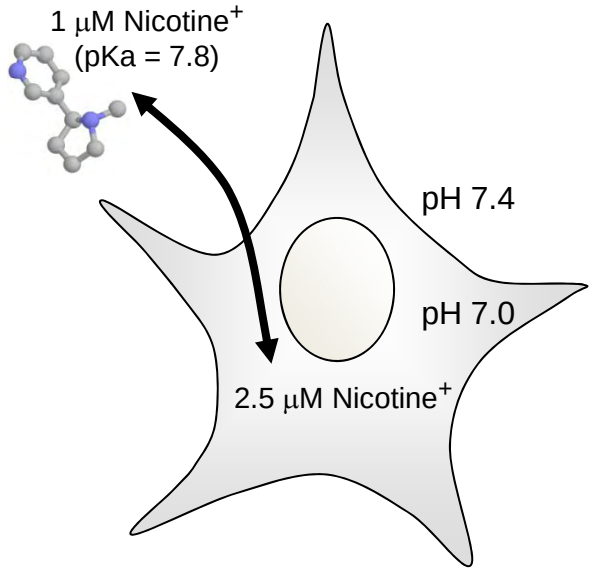


. . . . But
#3 reversible events may be amplified by covalent bonds

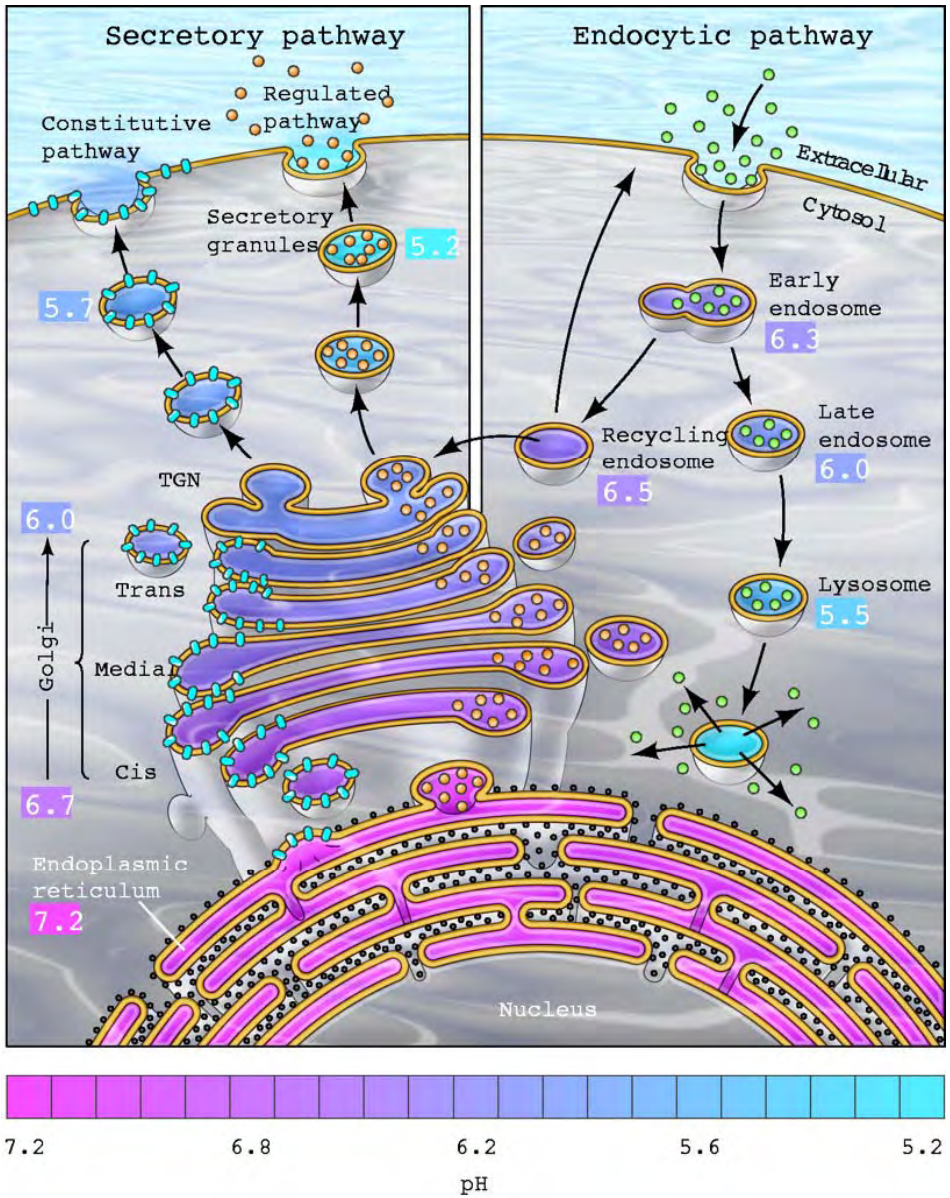


Thermodynamics of SePhaChARNS
#4. Acid trapping may keep intracellular nAChRs desensitized

Nicotine accumulates slightly in cytoplasm



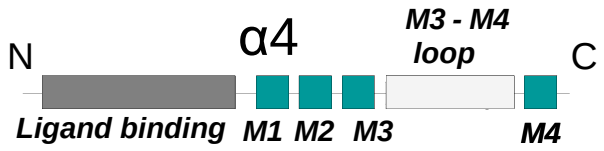
. . . and then markedly in intracellular organelles.

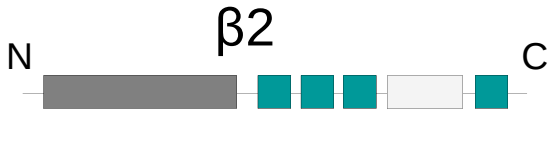


P. Paroutis, N. Touret, S Grinstein (2004) Physiology 19: 207-215

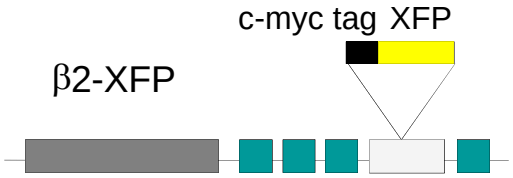
nicotine+/nicotine: 10 30 100 300

Fluorescent AChRs for localization and Förster resonance energy transfer (FRET)



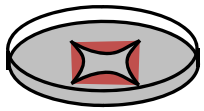


Raad Nashmi
Rahul Srinivasan



FRET pairs
(m = monomeric)

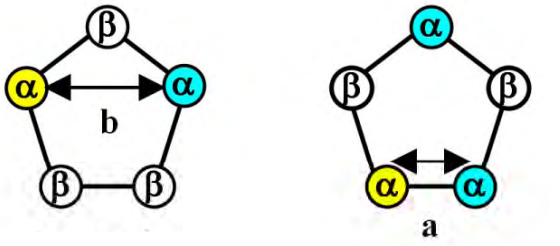
XFP =	mCerulean	ECFP	mEGFP	mEYFP	mVenus	mCherry



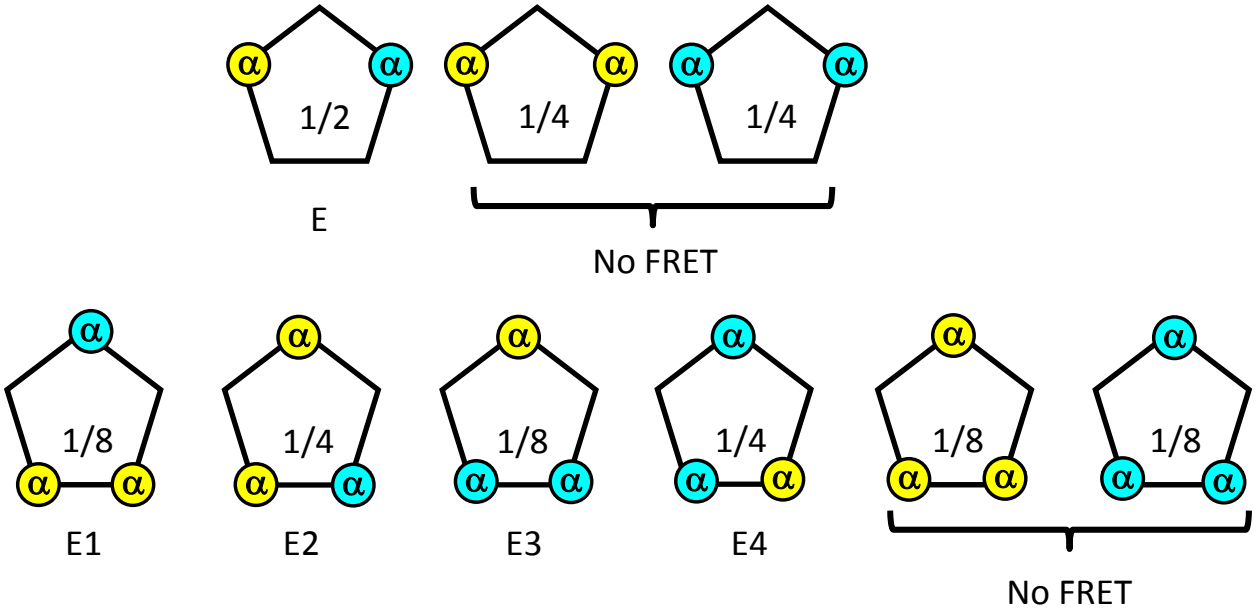
Neuro2a

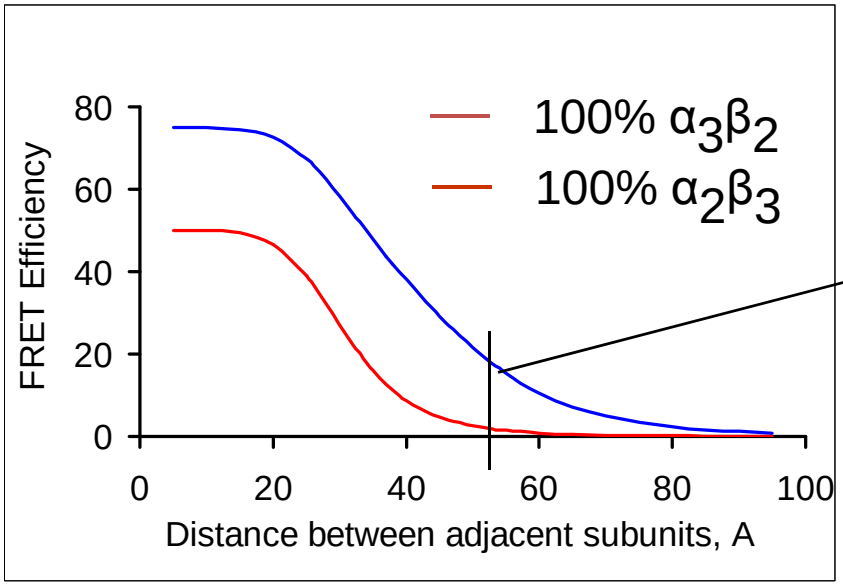
Theory of FRET in pentameric receptors with $\alpha_n\beta_{(5-n)}$ subunits

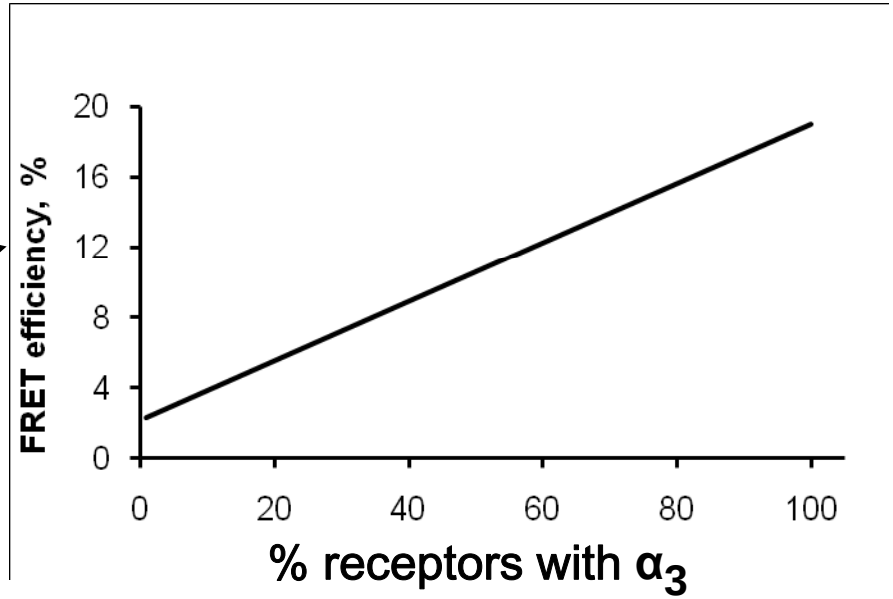
50% α-CFP, 50% α-YFP



b/a =1.62; 1.62^{-6} = 0.055





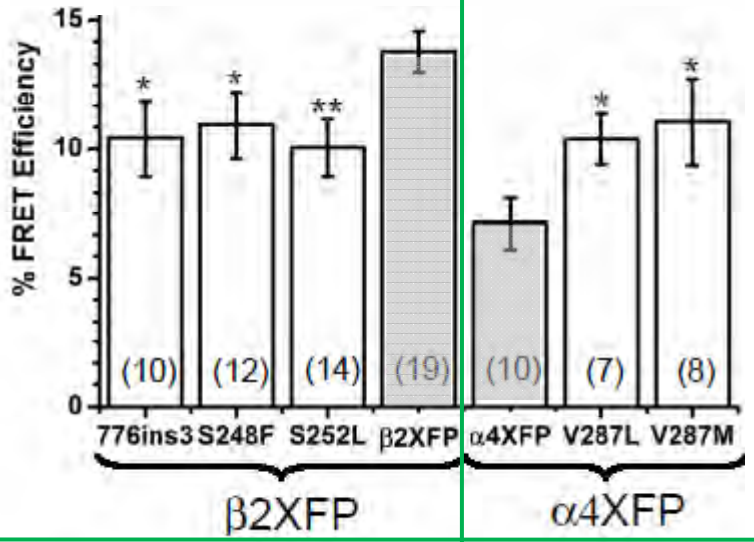


Autosomal Dominant Nocturnal Frontal Lobe Epilepsy: Five M2 Domain Mutations Cause Excess Intracellular $(\alpha4)_3(\beta2)_2$ Stoichiometry

We studied the α4 subunit mutations

We studied the β2 subunit mutations

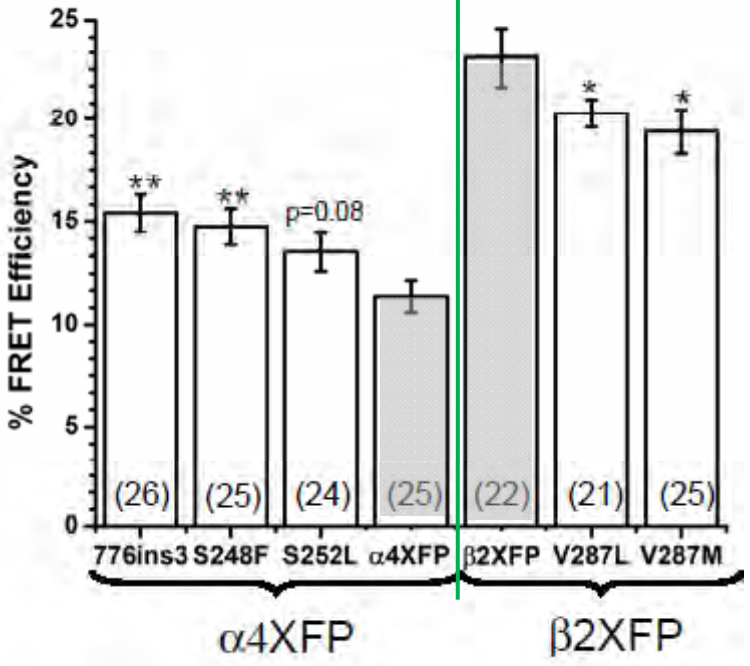
WT



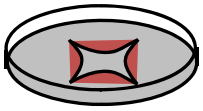
We placed the FRET pair in the WT subunits

In the two cases tested, these differences are abolished by 24-48 h incubation in 1 μM nicotine.

Son et al, Mol Pharm 2009

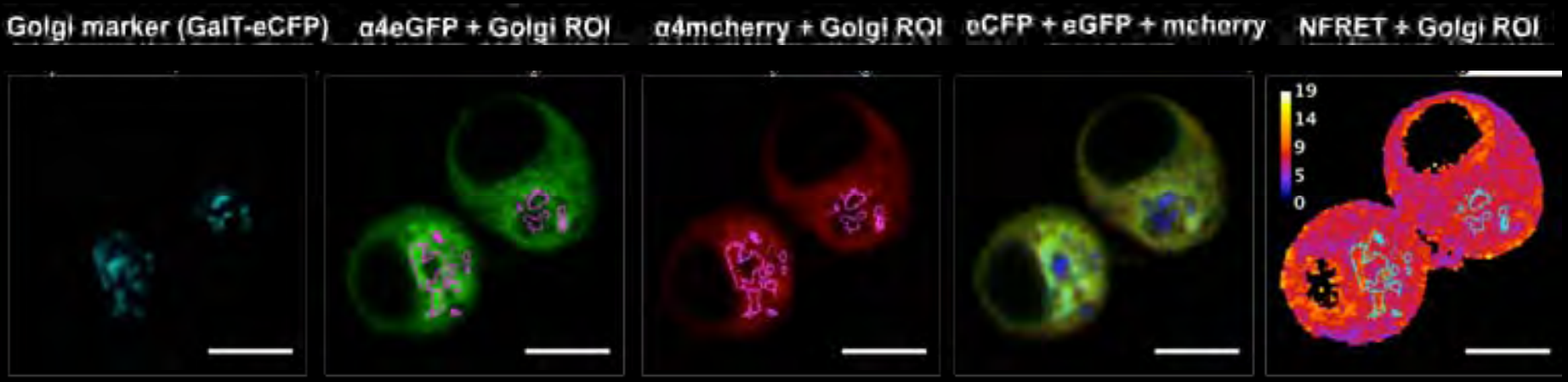


We placed the FRET pair in the mutant subunits

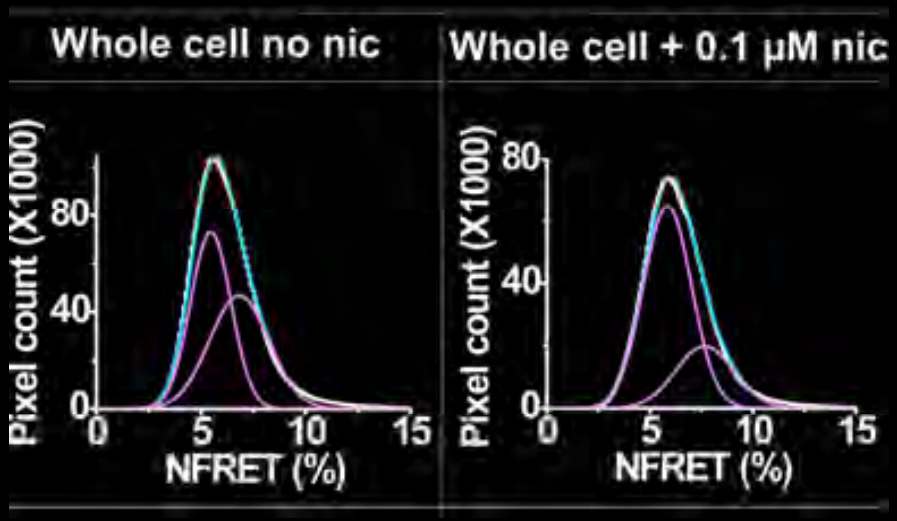


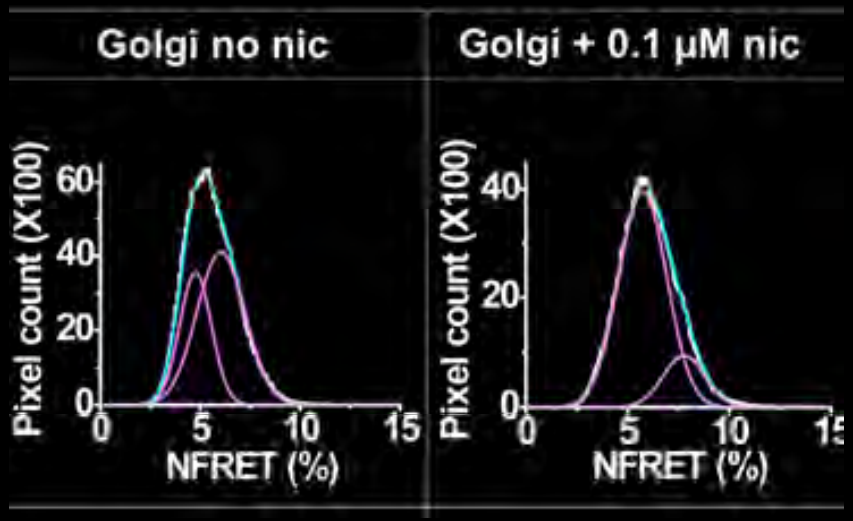
Neuro2a

Pixel-by-pixel NFRET measurements:
48 h incubation in nicotine shifts nAChR stoichiometry: $\alpha4_3\beta2_2 \rightarrow \alpha4_2\beta2_3$

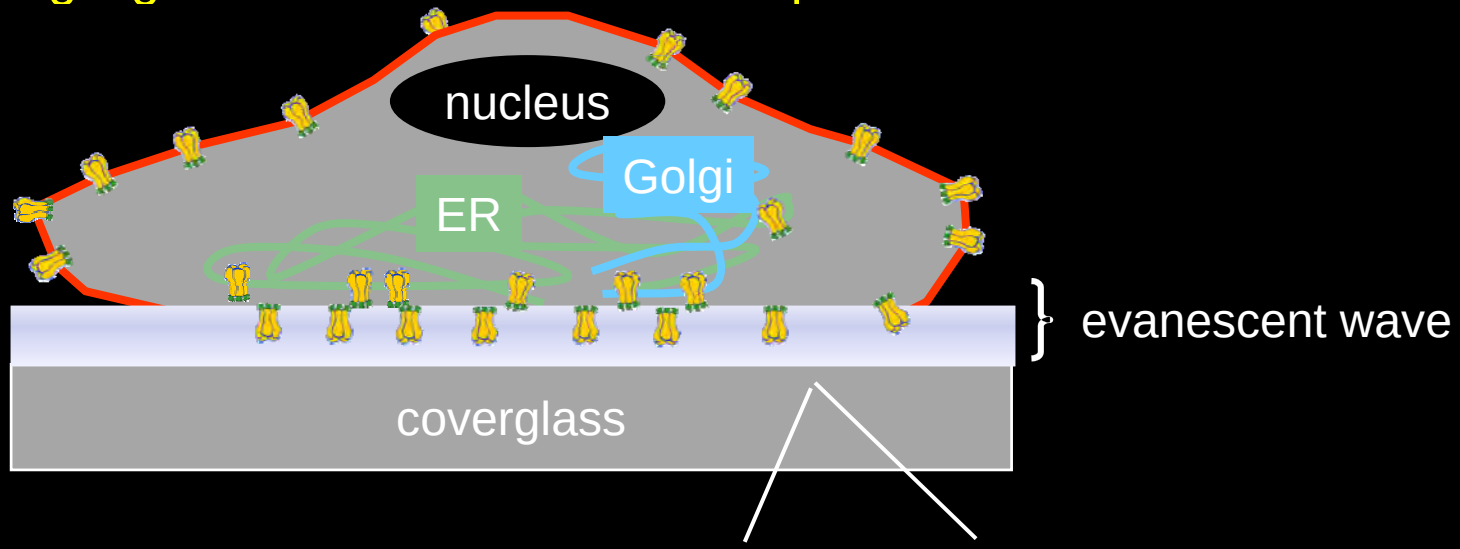


. . . and the change occurs upstream of the Golgi (presumably in the ER)

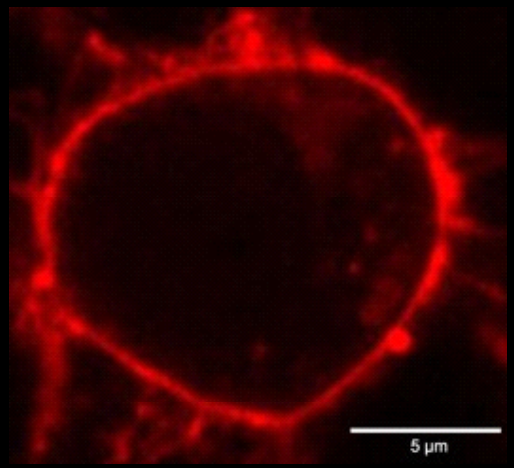


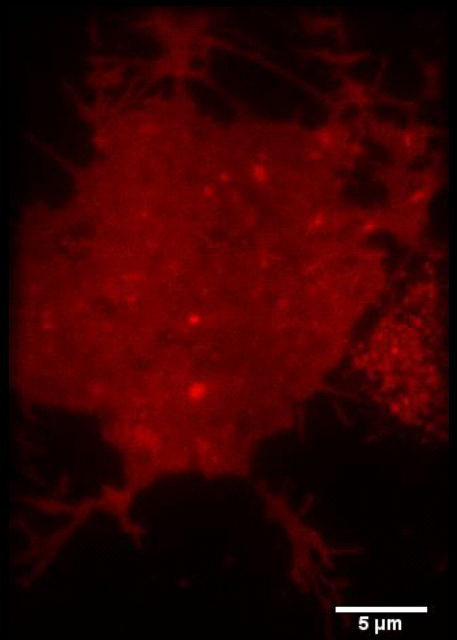


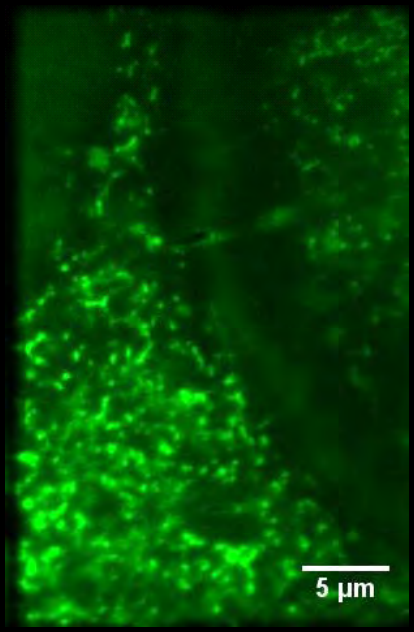
Total internal reflection fluorescence (TIRF) microscopy highlights events at and near the plasma membrane



Confocal







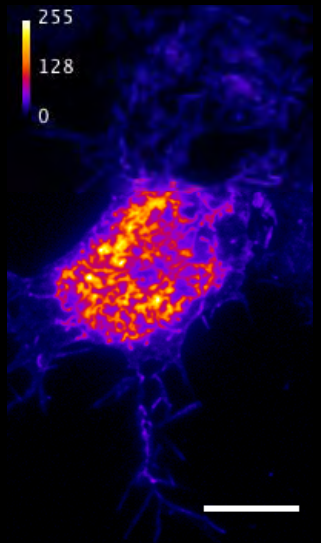
N2a cell pCS2-mcherry (plasma membrane label)

(ER) Tracker green

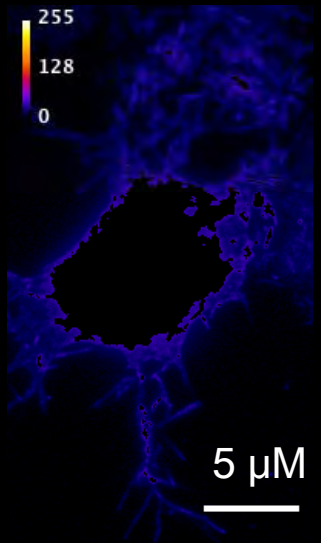
TIRF measurements recapitulate α4β2 nAChR upregulation at the plasma membrane

α4-eGFP β2

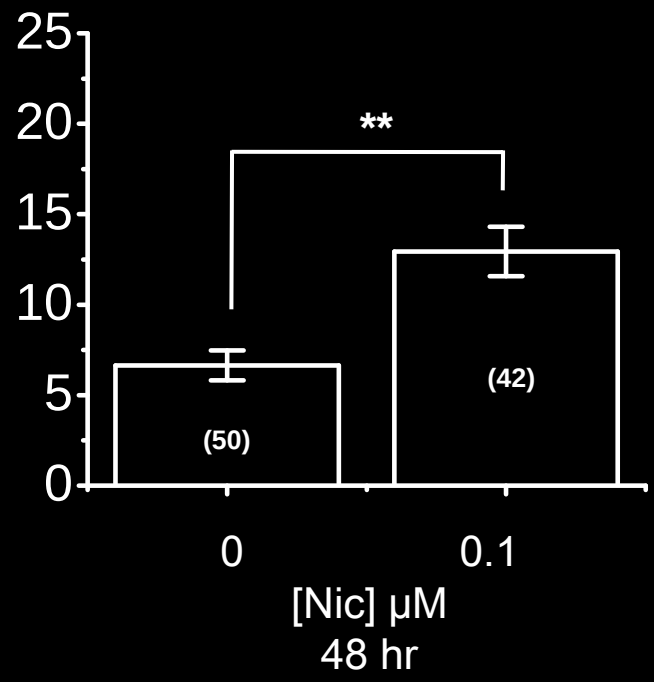
ER + PM



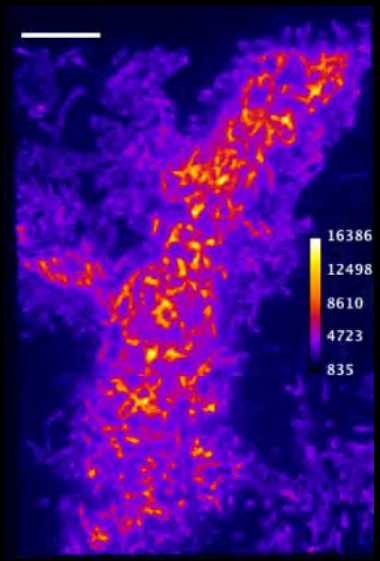
ER subtracted



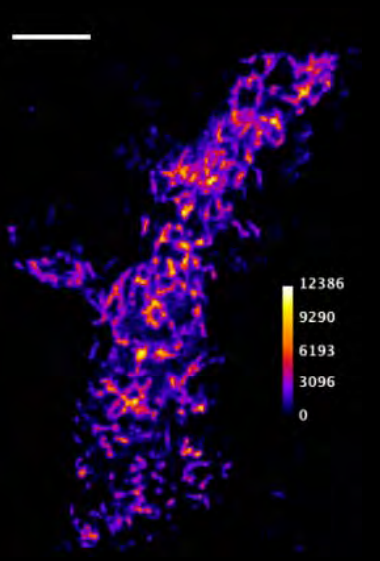
PM integrated intensity:
Thousands
of
arbitrary units



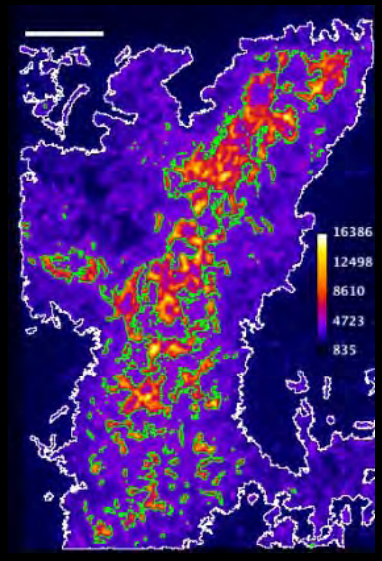
ER + PM



PM subtracted

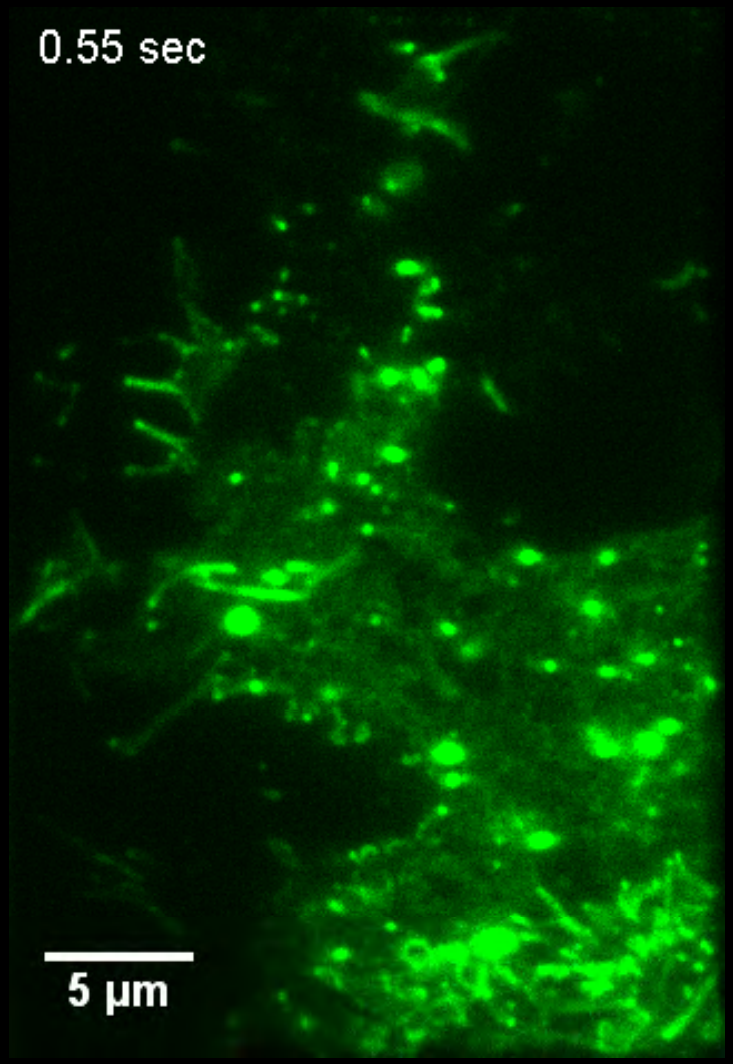


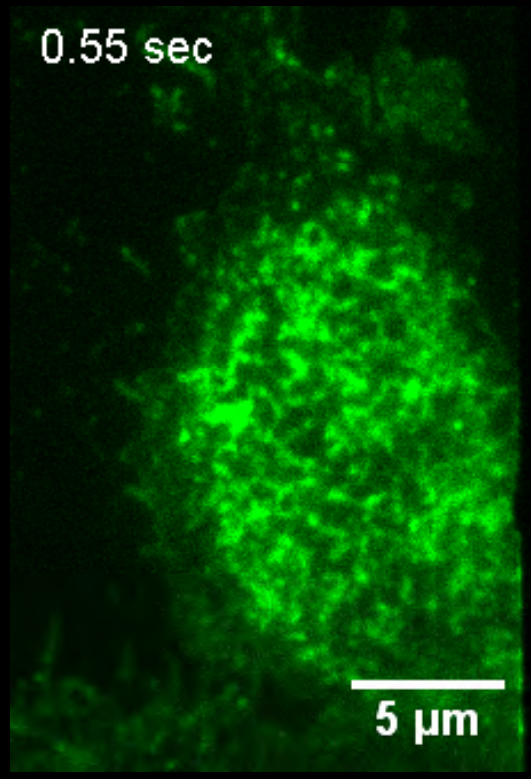
ER + PM (ROIs)



Sequences in the β subunit M3-M4 loop mediate ER retention of α4β nAChRs

α4eGFPβ4





α4eGFPβ2

Mouse

putative COP 1 retrieval motif

β2 **VHHRSPTTHTMAPWVKVVFLEKLPTLLFLQQPRHRCARQRLRL**RRRQR**EREGAGTLFFREG**

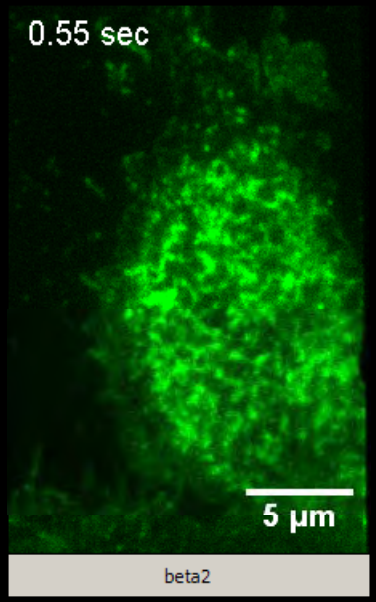
β4 **VHHRSPSTHTMASWVKECFLHKLPTF**LFM**KRPGLEVSPARVPHSSQLHLTTAEATSTSALG**

putative ER export motif

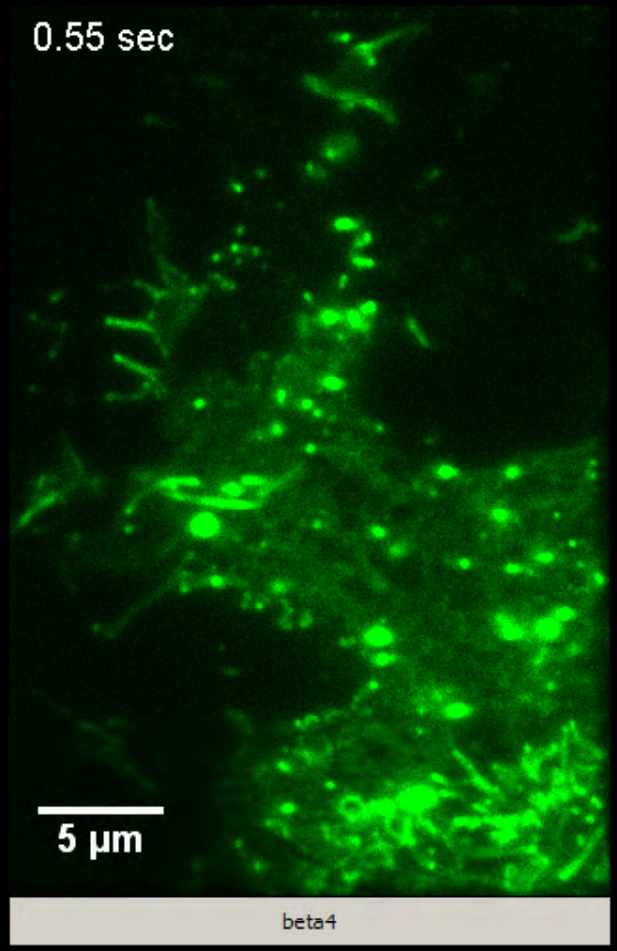
Rigo Pantoja, Rahul Srinivasan

The β2 nAChR subunit mediates ER retention of receptors

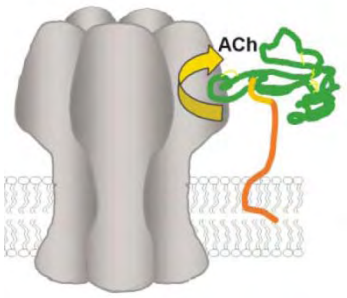
α4eGFPβ2



α4eGFPβ4

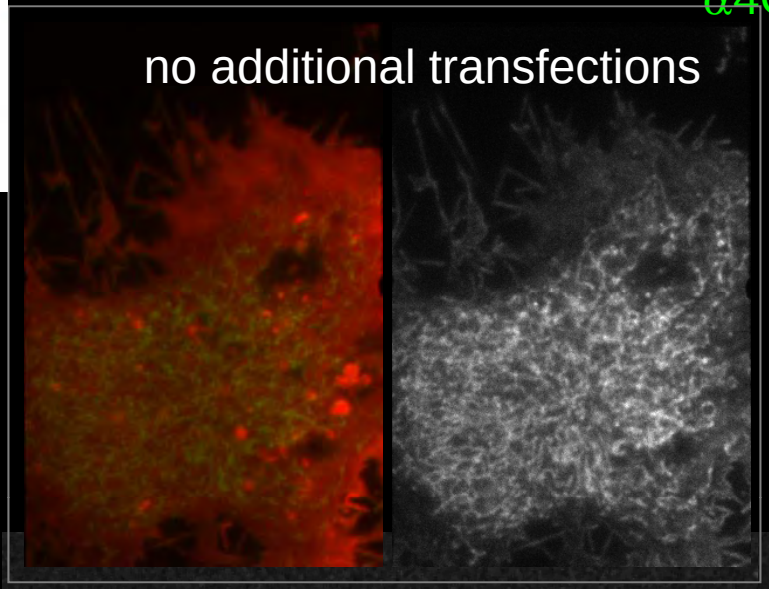


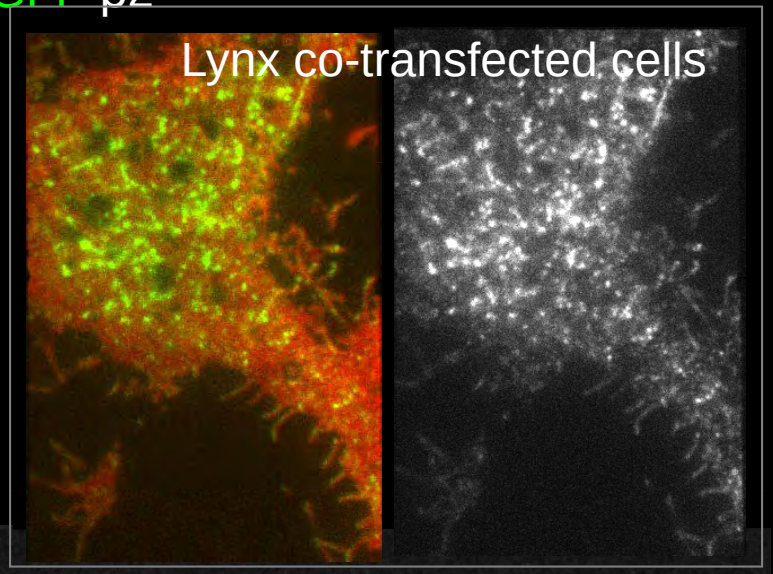
Rigo Pantoja
Rahul Srinivasan



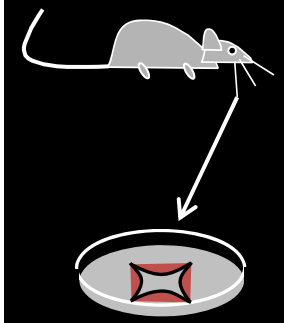
Lynx proteins: endogenous chaperones for α4β2 nAChRs?

α4GFP β2

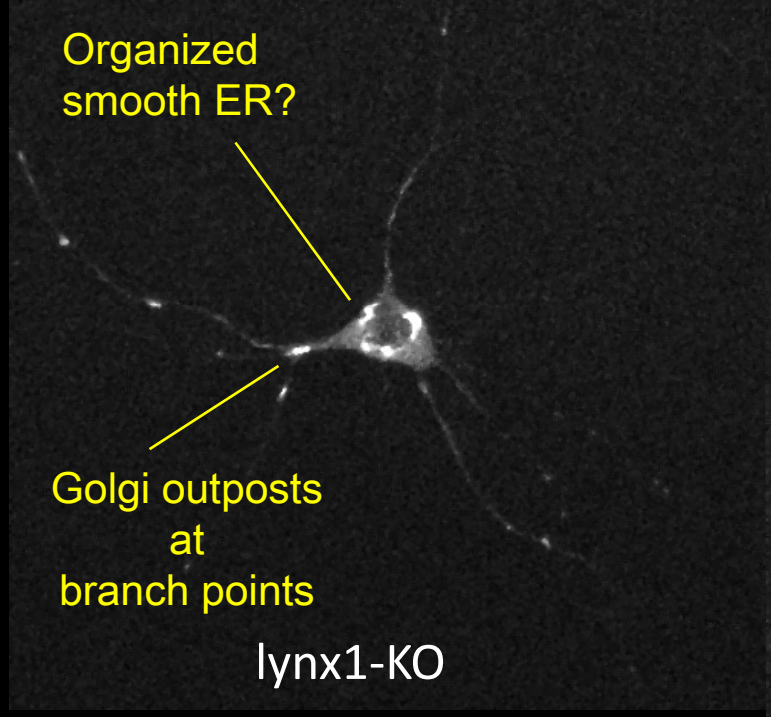


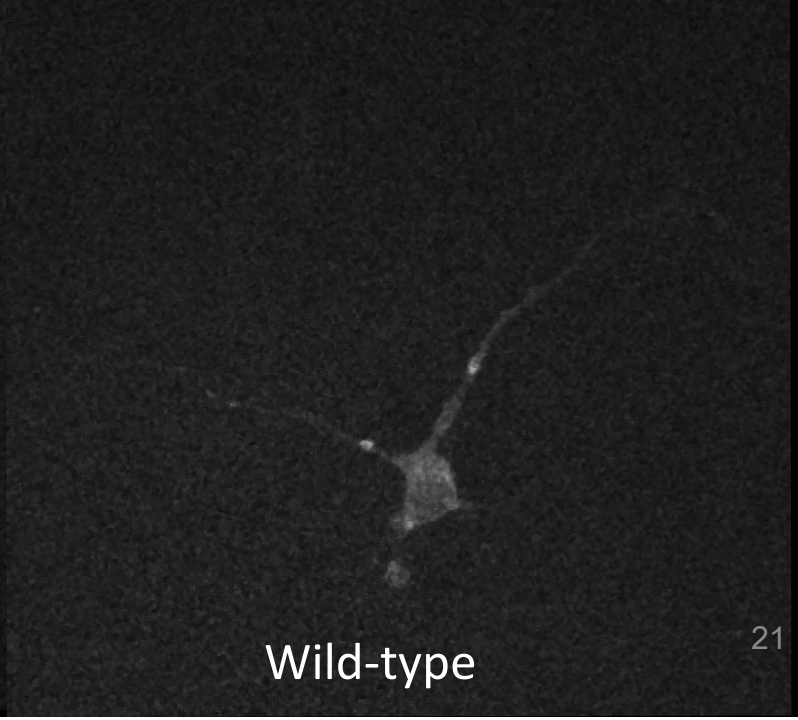


Neuro2a
(HEK)



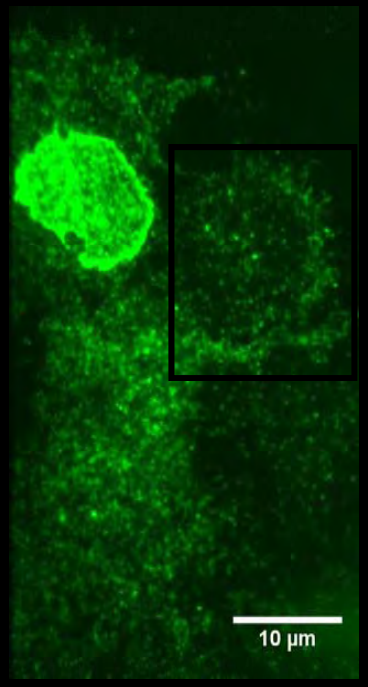
Julie Miwa



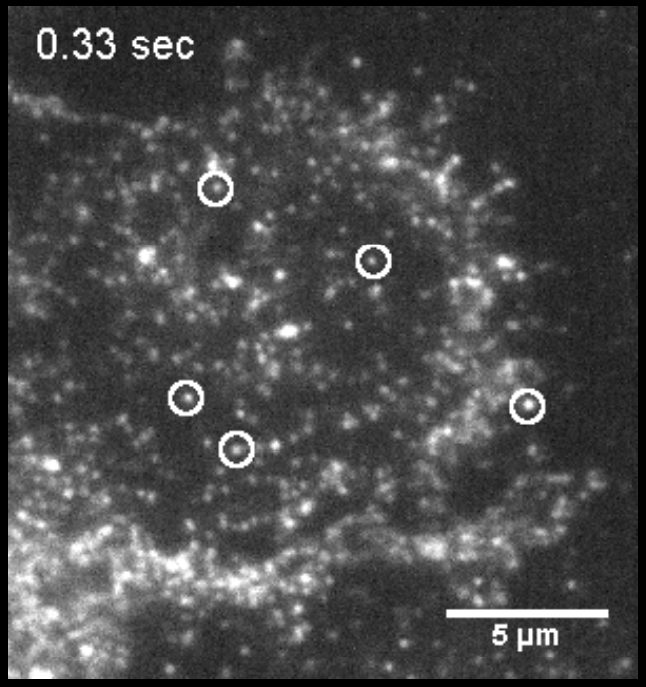


Toward single-molecule resolution of α4β2* nAChRs in mammalian cells: receptor clusters and subunit stoichiometry

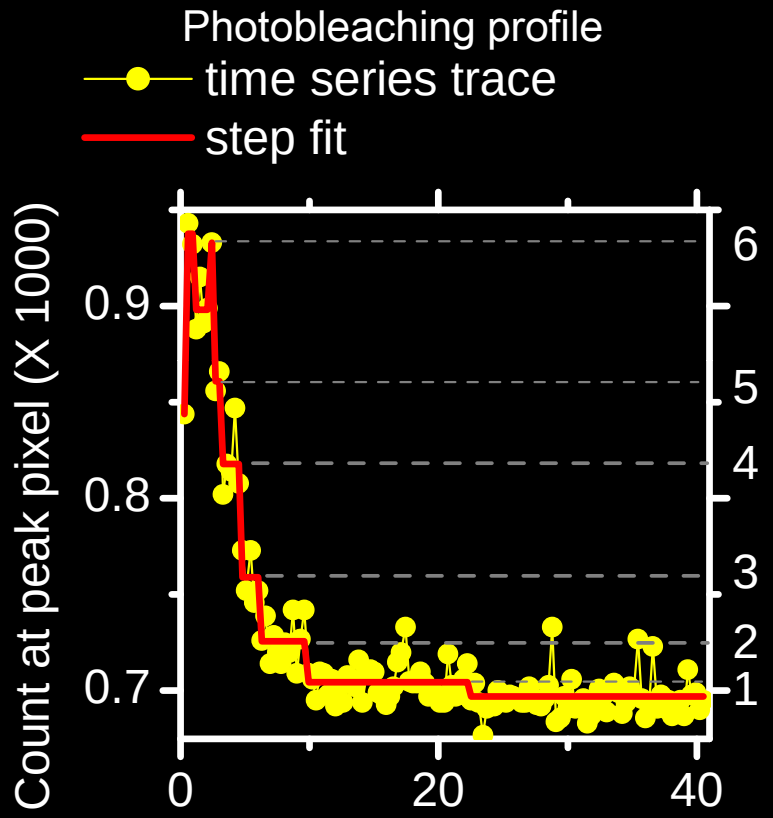
α4GFPβ2 (4:1)



Immobile diffraction-limited puncta



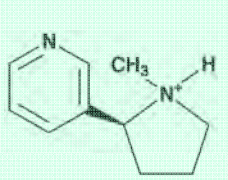
Photobleaching profile



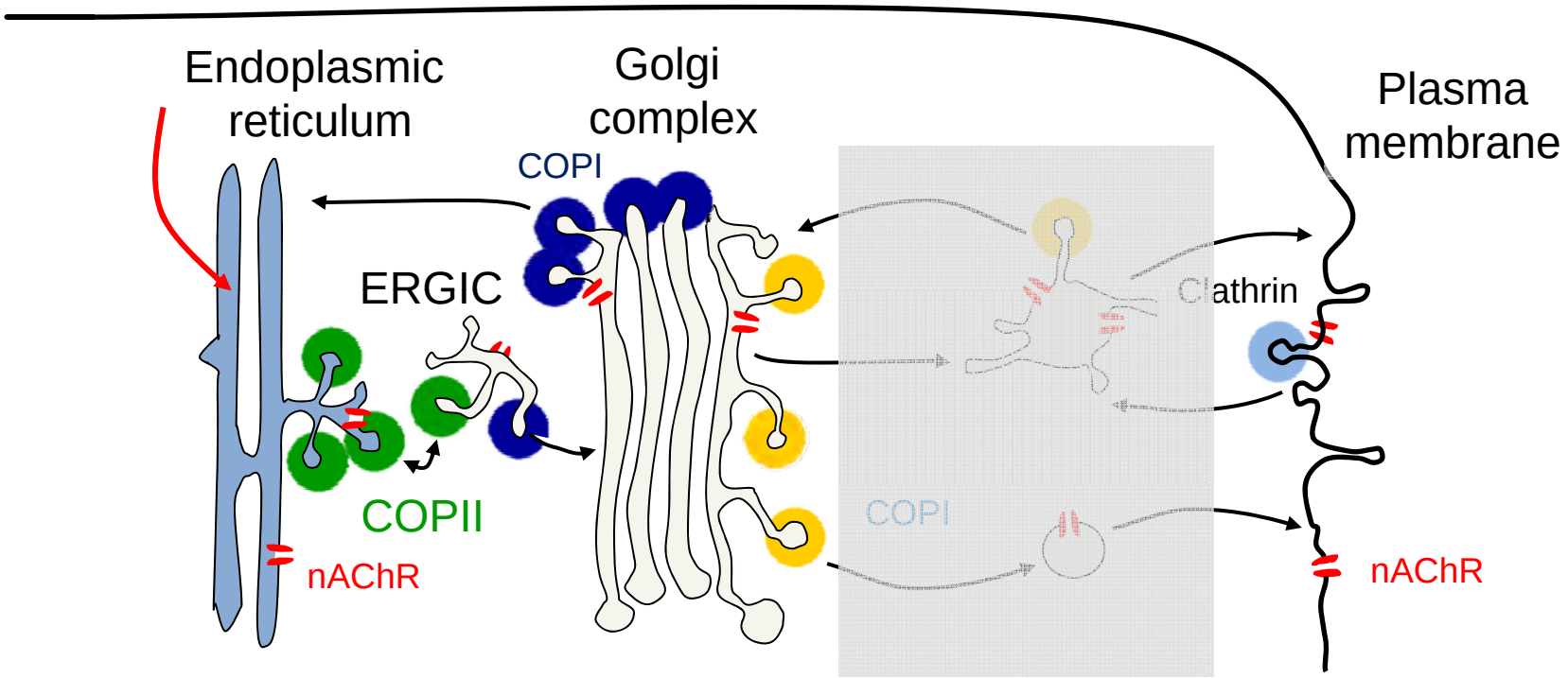
Criteria for plasma membrane inserted nAChRs

- Immobile
- Diffraction limited
- Discrete photobleaching steps

Rigo Pantoja, Chris Richards



How does nicotine upregulate
Wild type nAChRs on the plasma membrane?
Our best current hypotheses



≥ 3 β2 subunits

Co-express lynx1

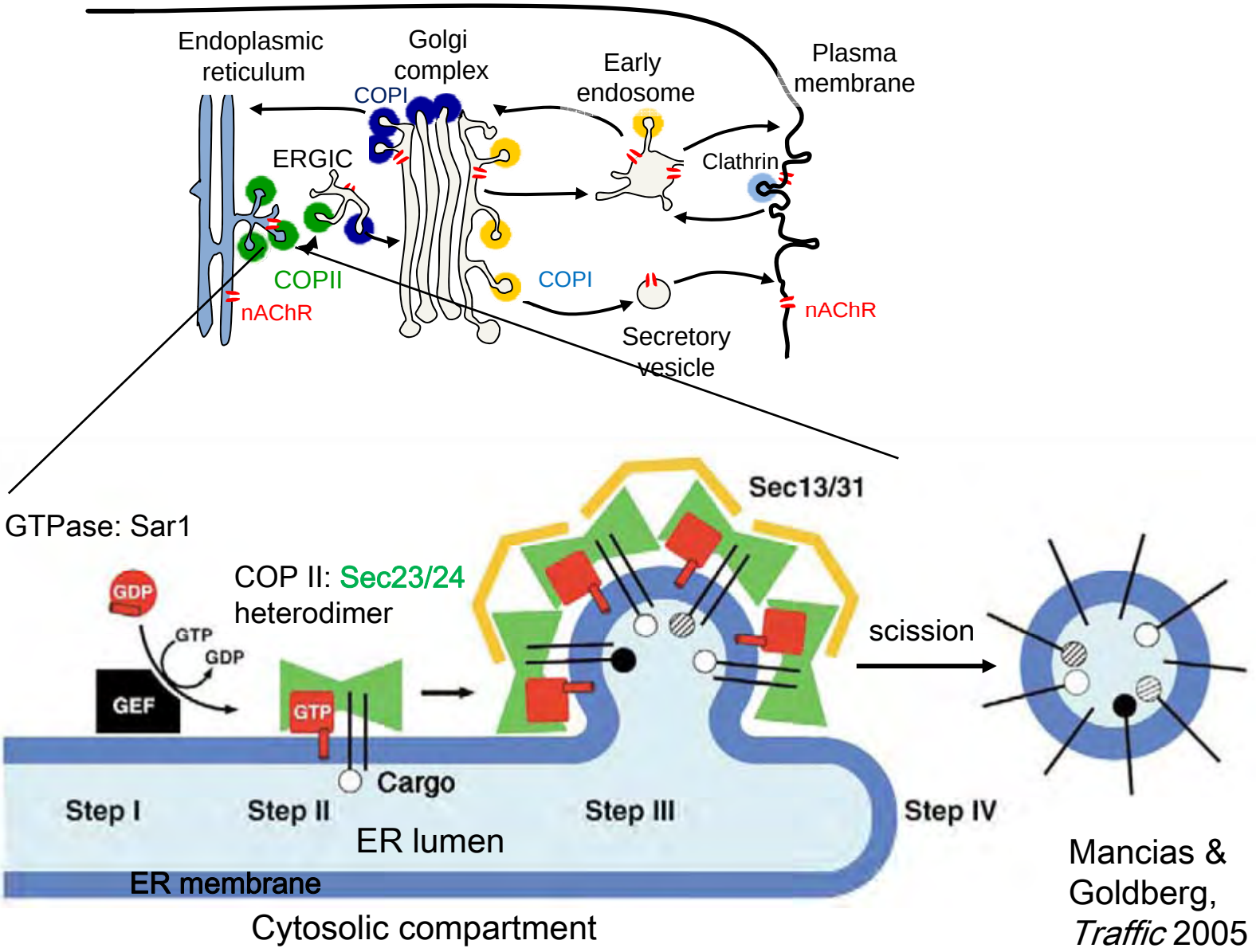
≤ 2 β2 subunits

Remove ER retention motifs

Insert ER exit motifs

β4 replaces β2 subunit

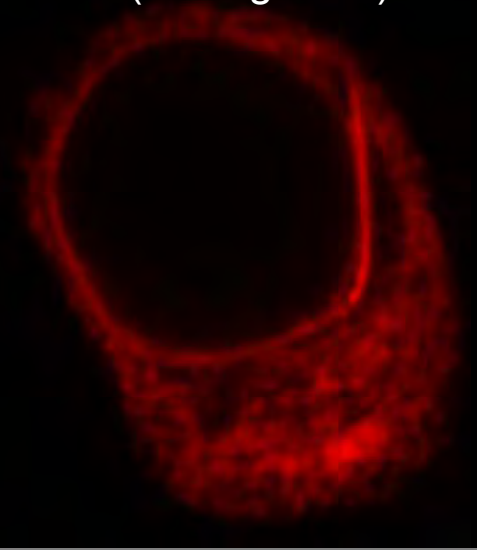
Most membrane proteins exit the ER in a COPII-dependent manner at ER exit sites (ERES)



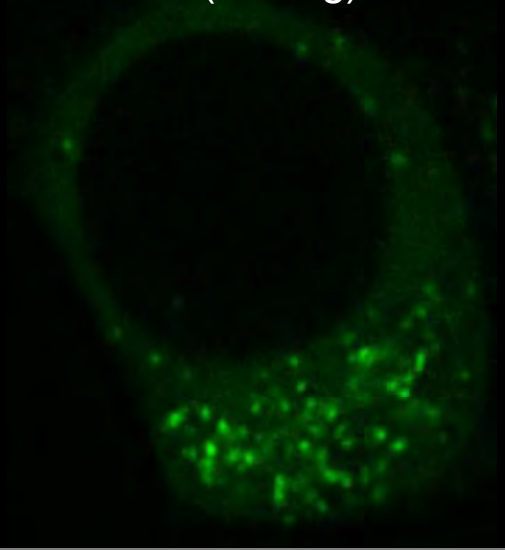
Mancias & Goldberg, *Traffic* 2005

ER exit sites and nAChRs

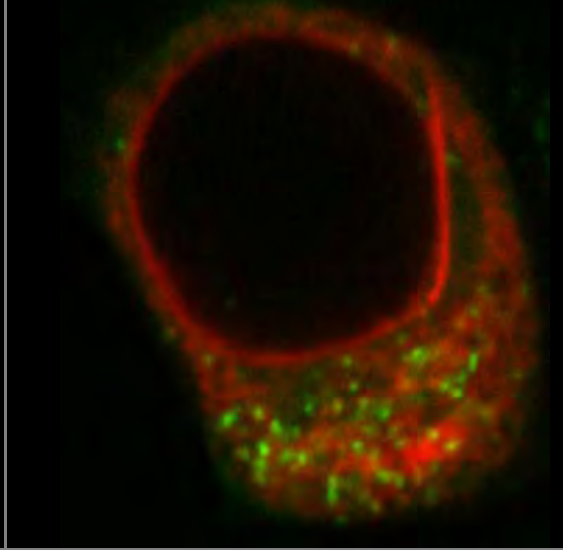
α4-mCherry + β2
(500 ng each)



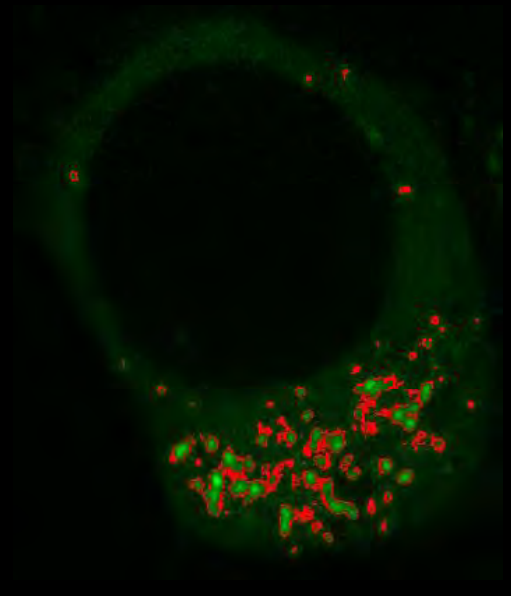
Sec24D-eGFP
(250 ng)



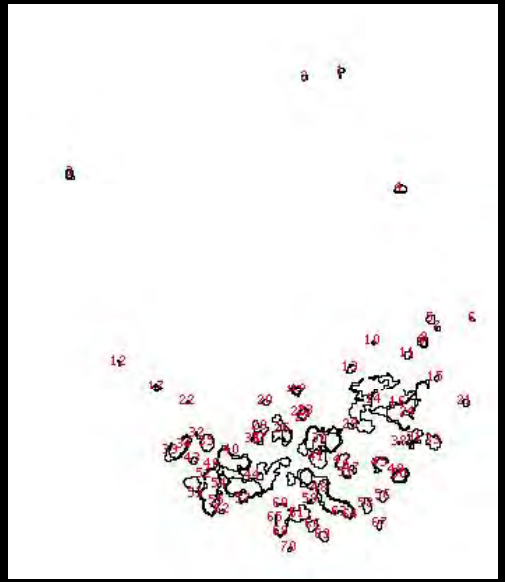
Merge



ERES selection



Outlines of selected ERES

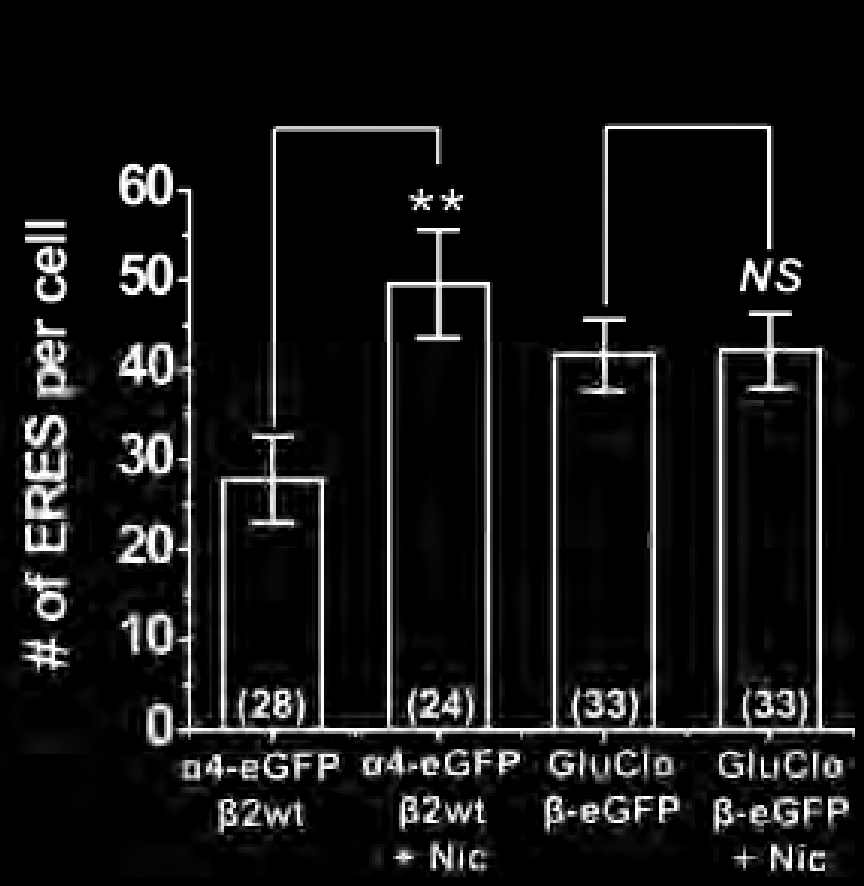


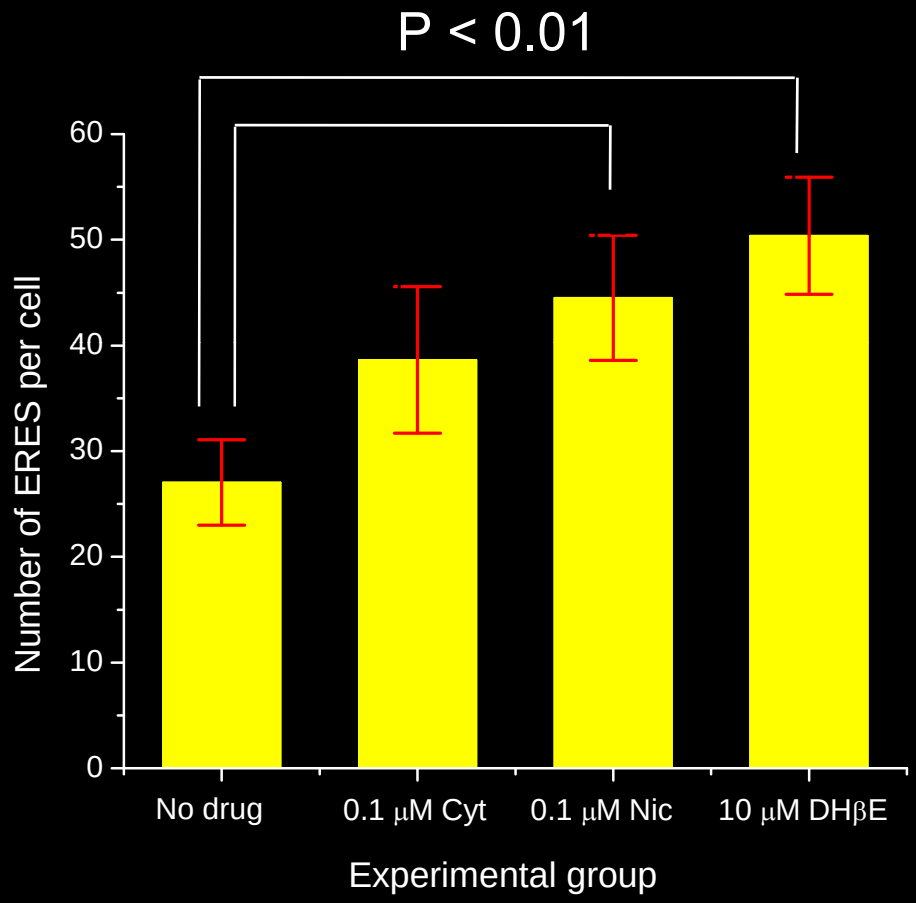
Methodology

- Transfect N2a cells with nAChRs + ERES marker
- Incubate with drug (48 h)
- ERES number (Confocal)
- ERES intensity (Confocal)
- ERES dynamics (TIRFM)

Rigo Pantoja
Rahul Srinivasan

48 h exposure to nicotine increases number of ER exit sites (ERES), and this depends on nAChR receptor expression

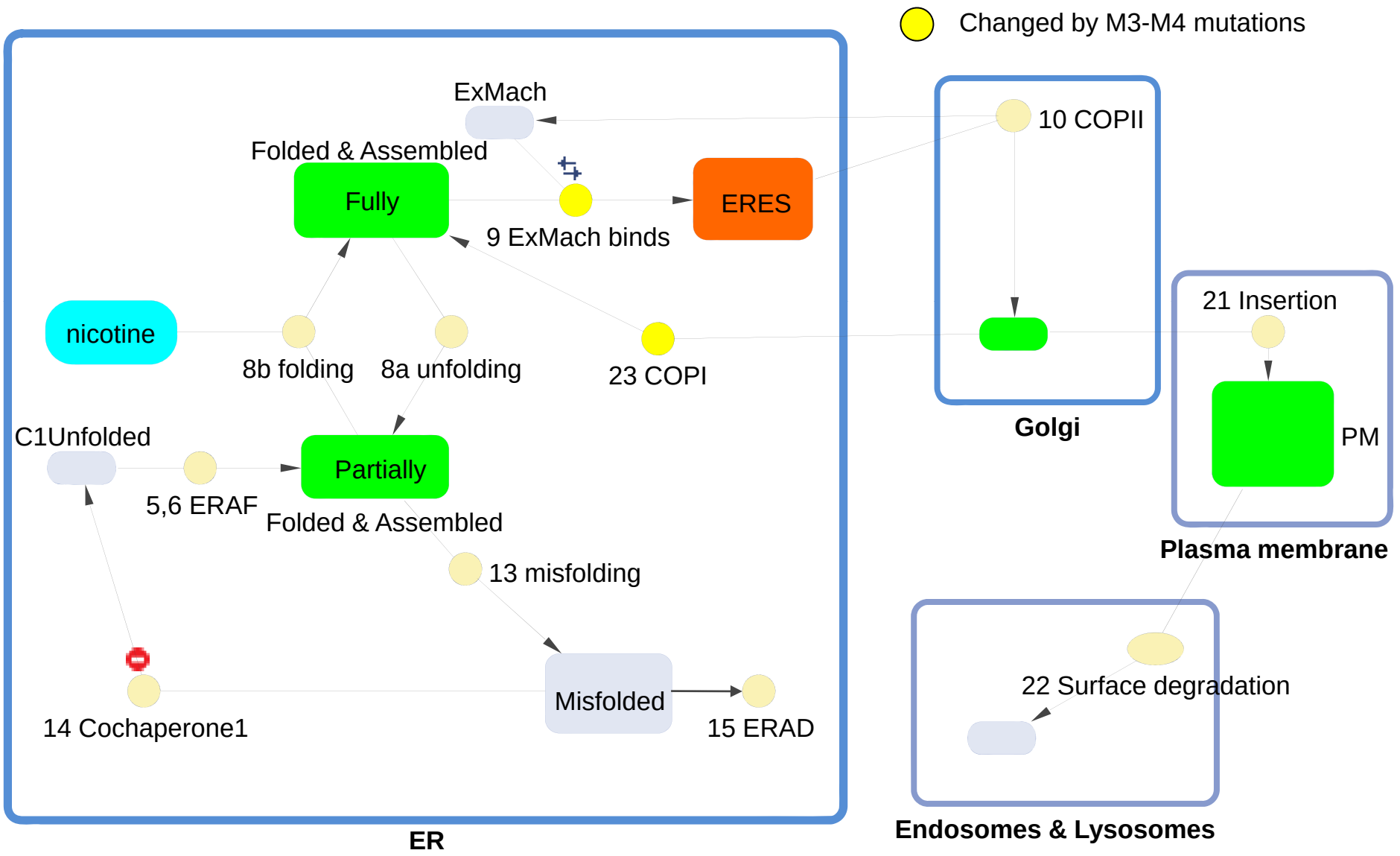




A cell-delimited phenomenon related to PD neuroprotection?

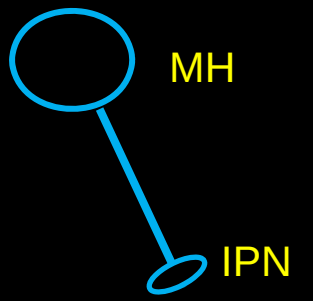
Rigo Pantoja, Rahul Srinivasan

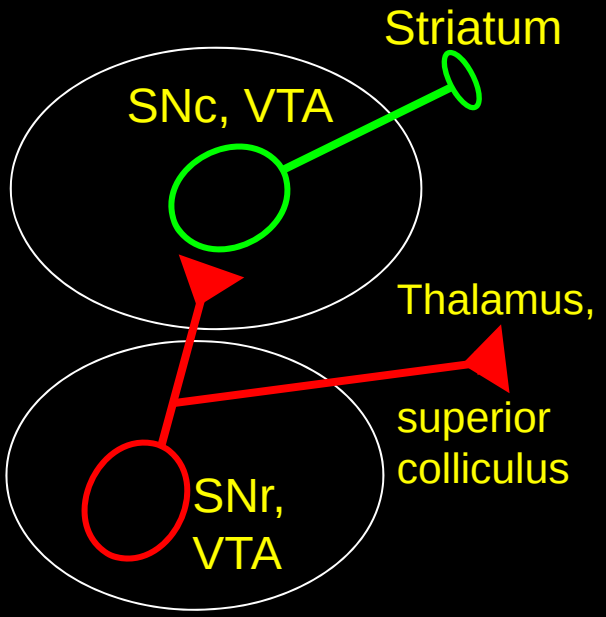
We're testing a formal model for SePhaChARNS by matching seven measured fluorescence ratios: nicotine/control, mutant/WT

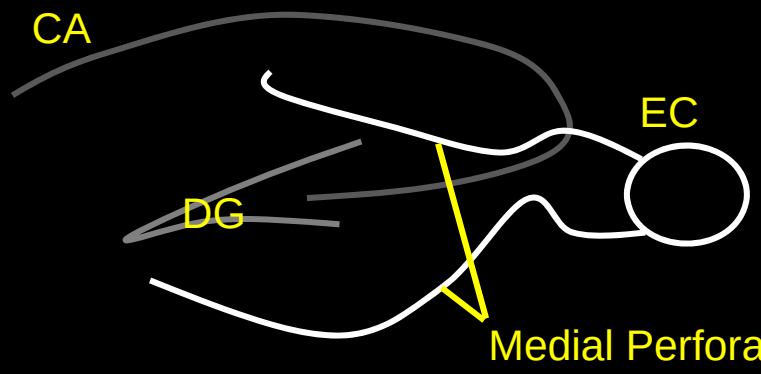


Based on FoldEx Wiseman, Powers, Buxbaum, Kelly & Balch *Cell* 2007

Cellular and subcellular specificity of SePhaChARNS: α4* nAChRs



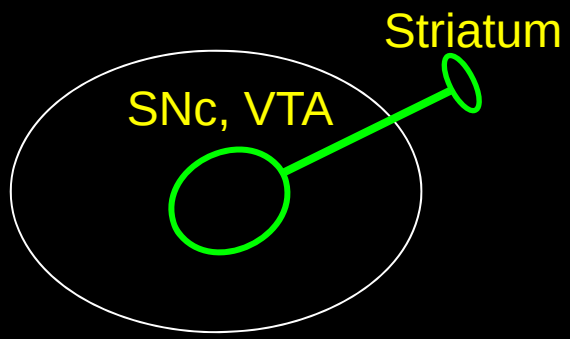




	Upregulation?		
Transmitter	Soma	Term.	Region / projection
Glu	??	Yes	Entorhinal cortex → dentate gyrus
ACh	No	No	Medial habenula → Interpeduncular nucleus
DA	No	Yes	Ventral tegmental area, substantia nigra pars compacta → Striatum
GABA	Yes	Yes	SN pars reticulata, VTA → SNC, VTA

Nashmi et al J Neurosci 2007; Xiao et al, J. Neurosci 2009

α6* nAChRs are much simpler



	Upregulation?		
Transmitter	Soma	Term.	Region / projection
Glu			No expression
ACh			No expression
DA	No	No	Ventral tegmental area, substantia nigra pars compacta → Striatum
GABA			No expression

Drenan et al, Neuron 2008; papers from several other labs

Strategy to evaluate the cellular and subcellular specificity of α4* upregulation

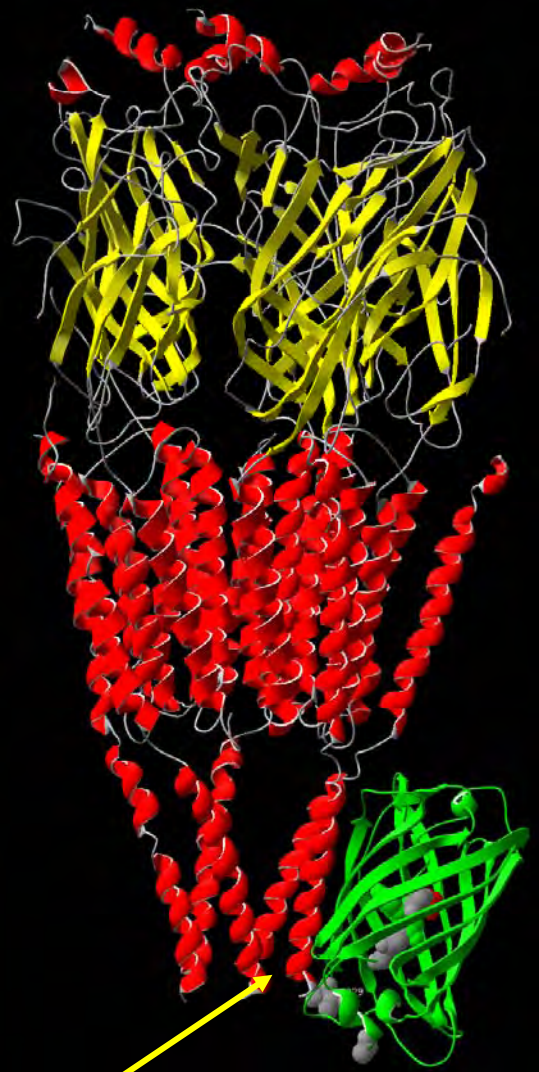
1. Generate knock-in mice with fully functional, fluorescent α4* receptors

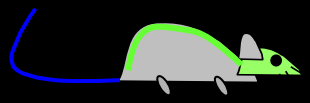
2. Expose the mice to chronic nicotine

3. Find the brain regions and cell types with changed receptor levels

4. Perform physiological experiments on these regions and cells to verify function

5. Model the cellular and circuit changes





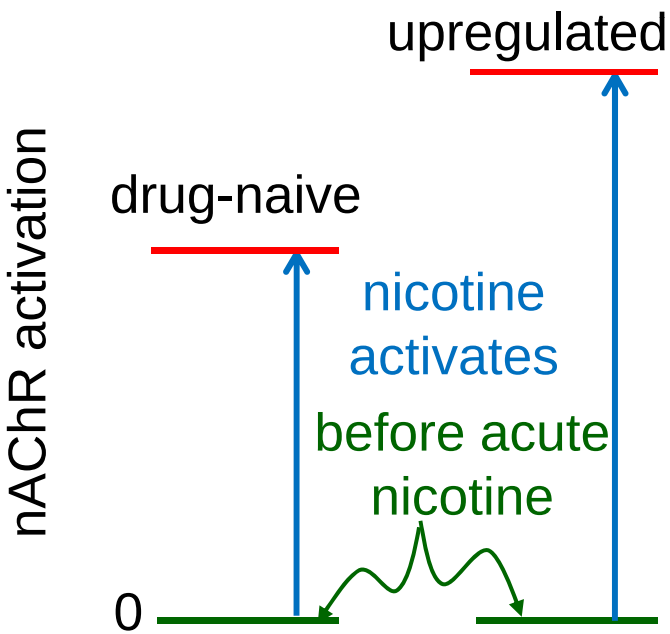
CFP

Leu9'Ala-YFP,

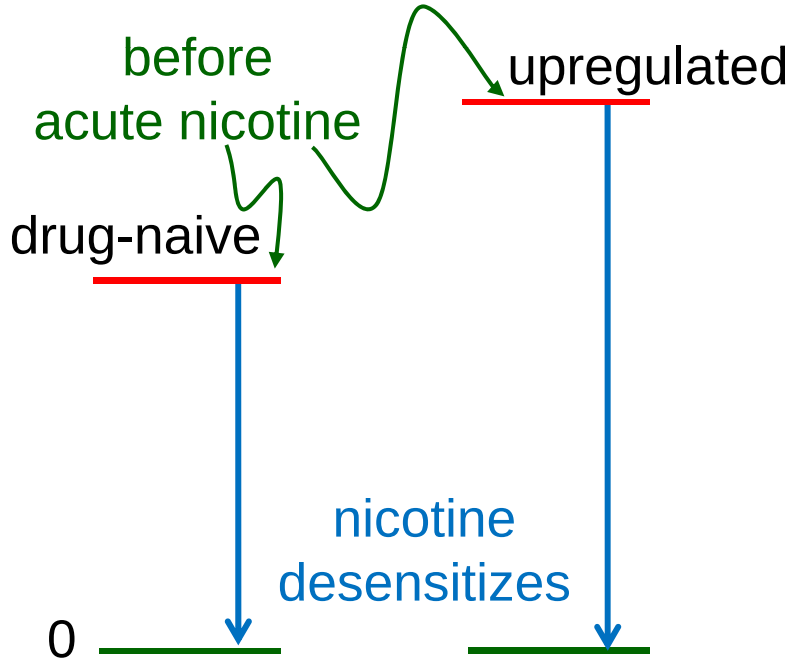
YFP,

Either activation and/or desensitization can be amplified by upregulation

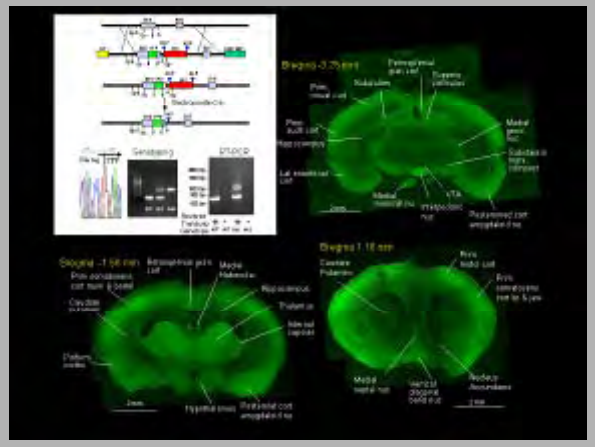
A. No endogenous activation; Nicotine activates nAChRs

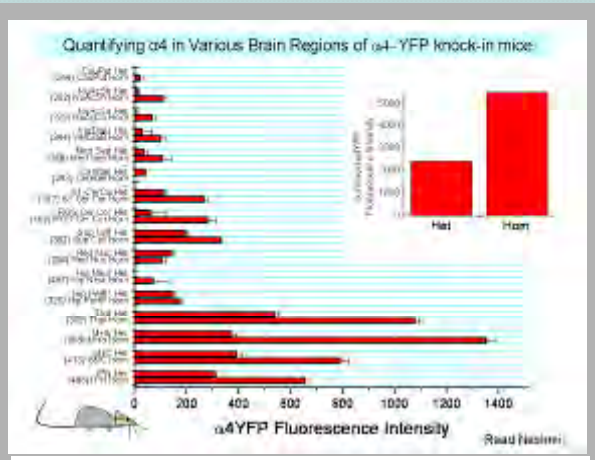


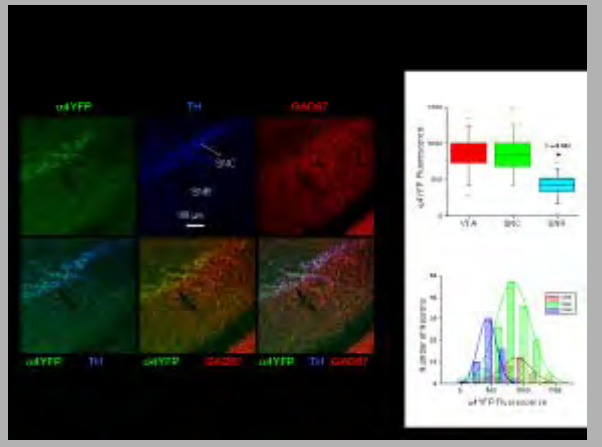
B. Activation by endogenous ACh; Nicotine desensitizes nAChRs

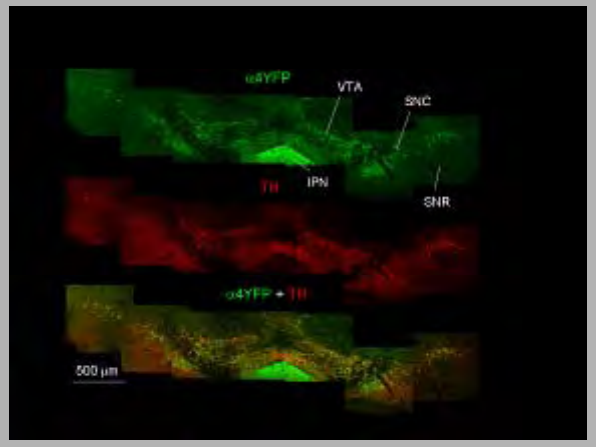


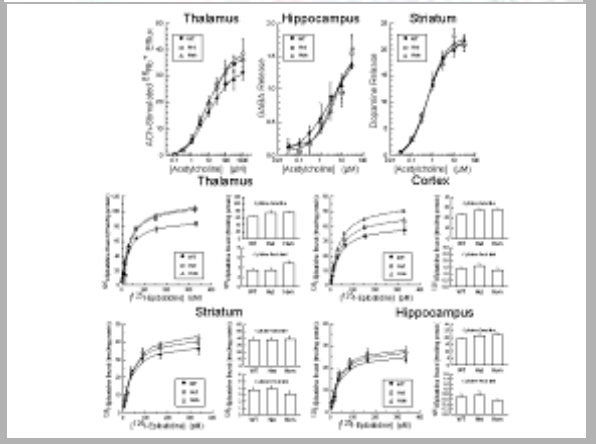
The Caltech α4 fluorescent mice . . . normal in all respects

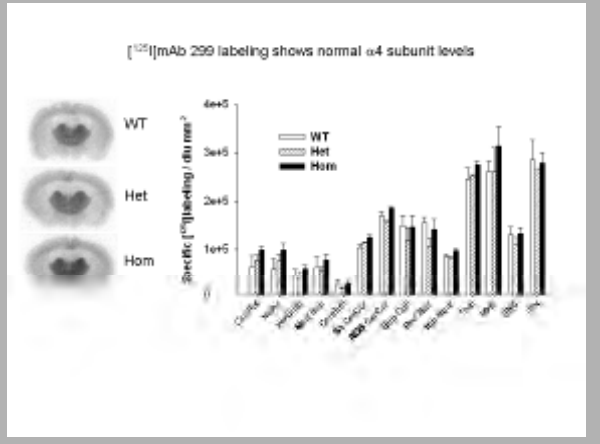


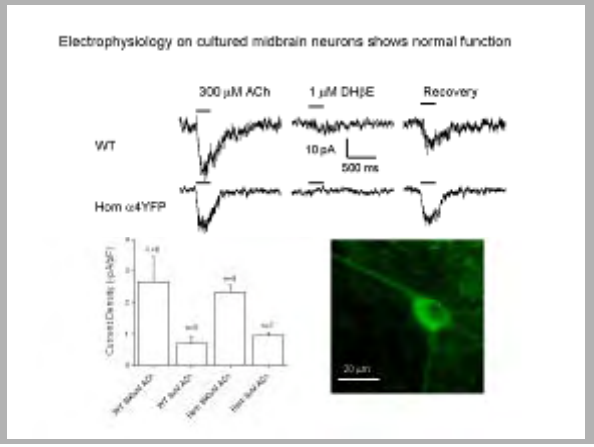


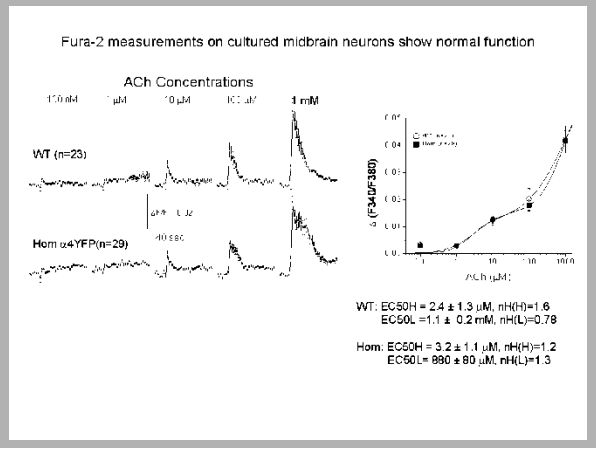


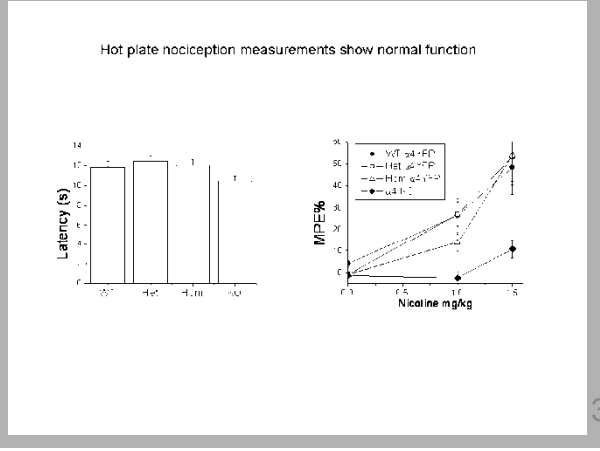




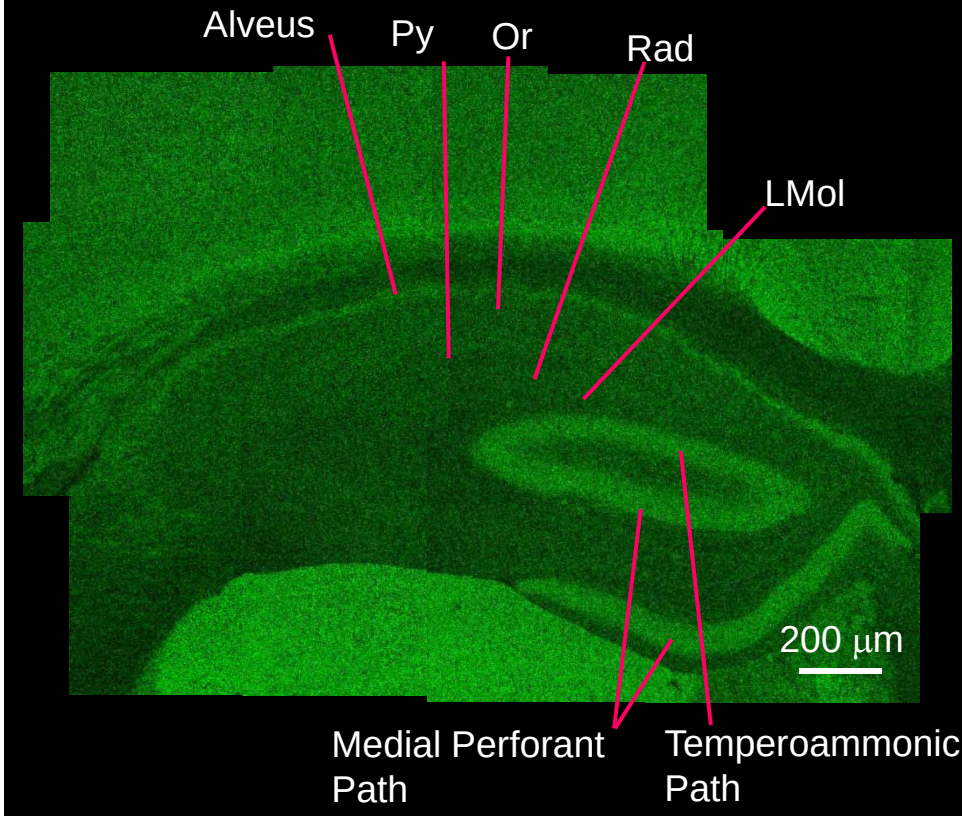


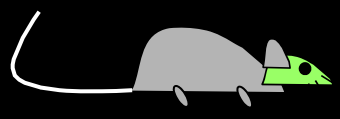






Chronic nicotine increases medial perforant path α4 fluorescence ~ 2-fold. Relevant to cognitive sensitization?



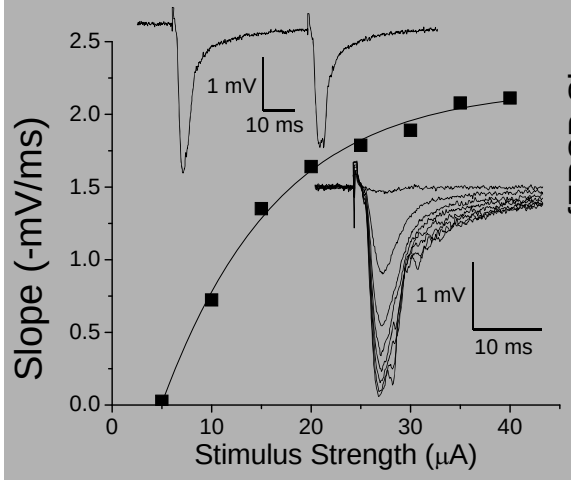


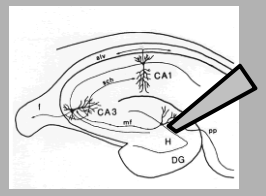
Humans:
Some smokers report that they think better when they smoke;
smokers who smoke nicotine cigarettes (but not nicotine-free cigarettes) display certain cognitive enhancements (Rusted and Warburton, 1992; Rusted et al., 1995).

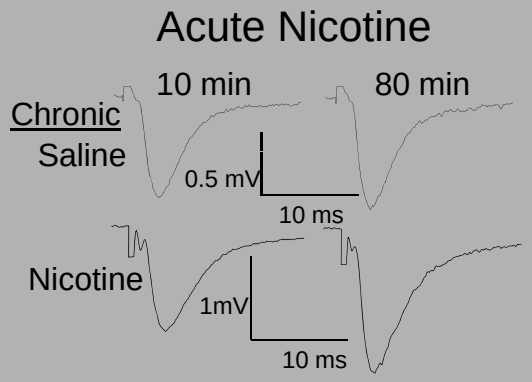
Rodents:
Mice show more contextual fear conditioning if, one day after withdrawal from chronic nicotine, they receive an acute nicotine dose (Davis et al., 2005); this is α4β2* dependent.
Also chronic nicotine produces better spatial working memory performance in the radial arm maze (Levin et al., 1990; Levin et al., 1996).

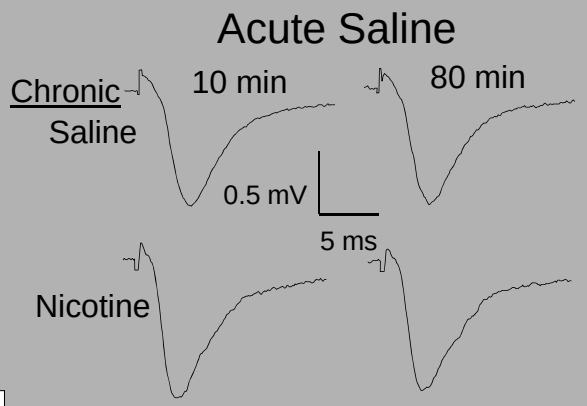
Simple model for cognitive sensitization:

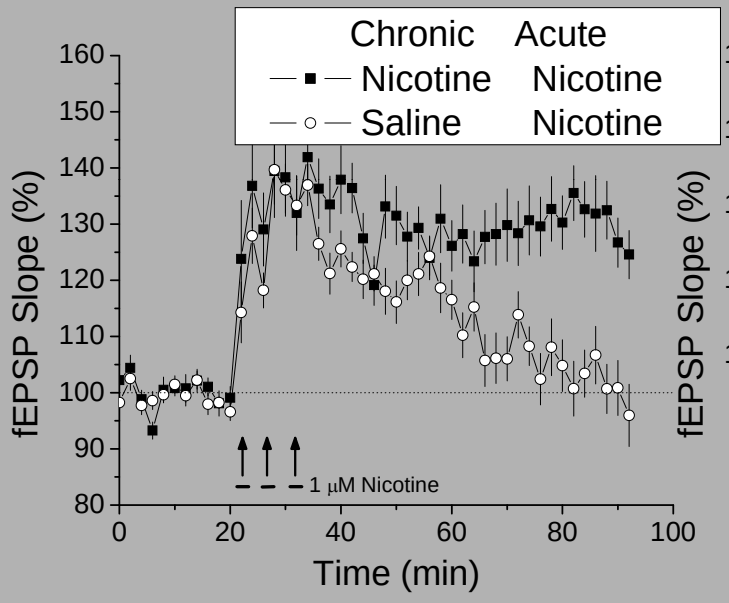
chronic nicotine
+
acute nicotine
lowers the threshold for perforant pathway LTP

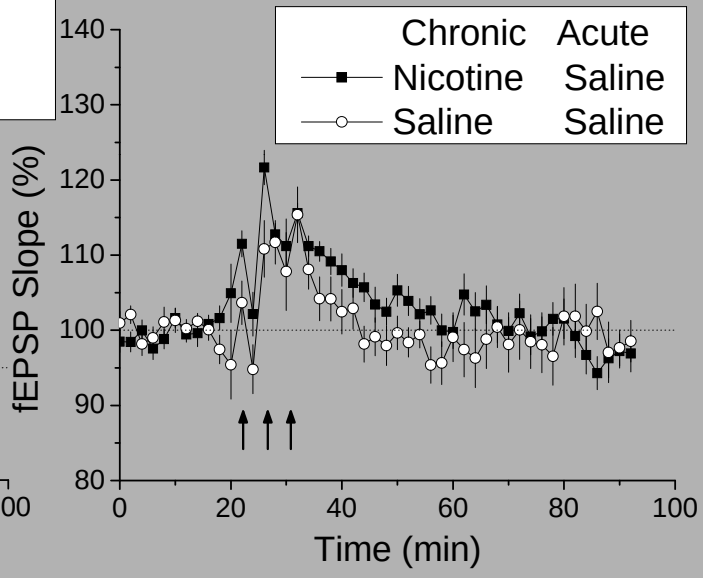


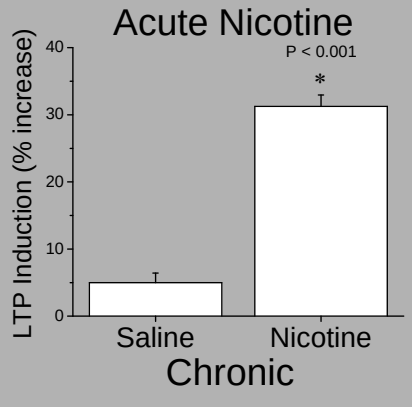


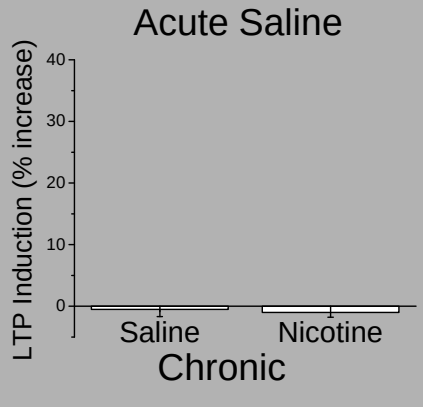


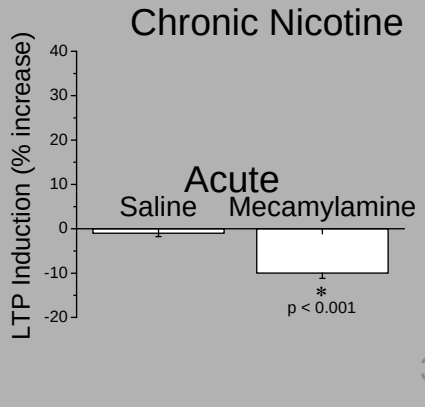




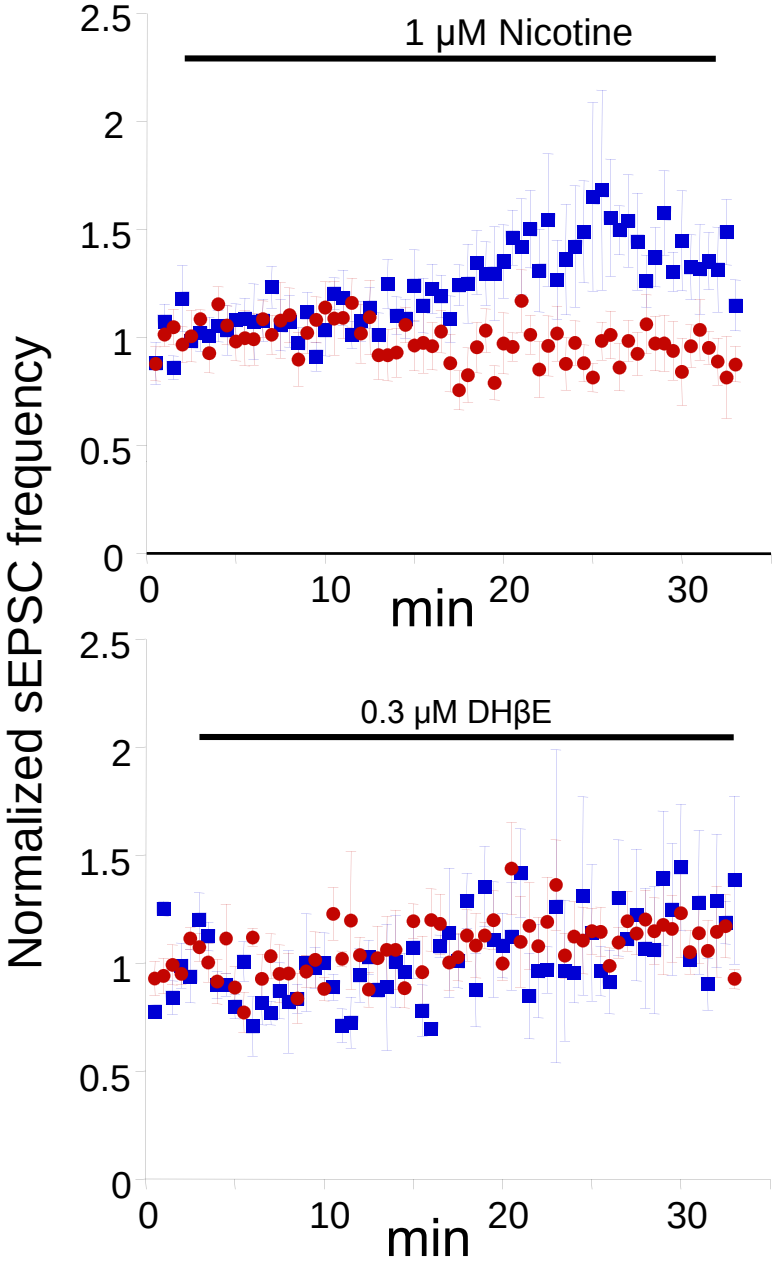


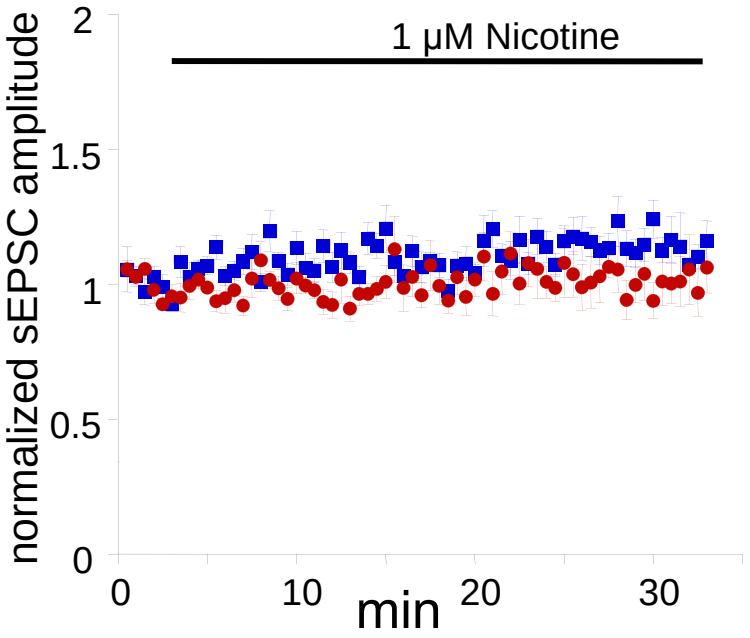






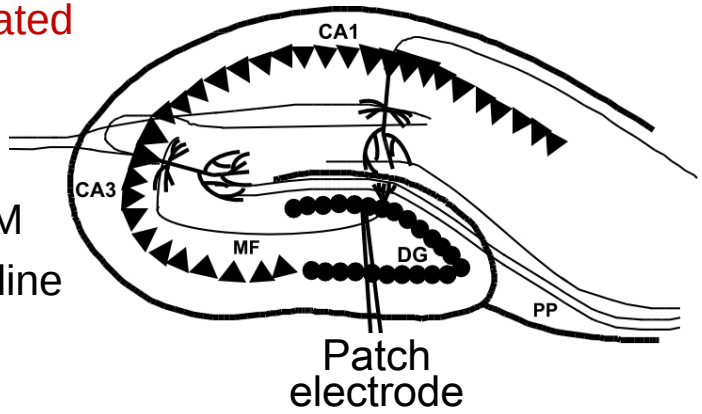
Acute nicotine increases sEPSC frequency in 1-day nicotine-treated animals





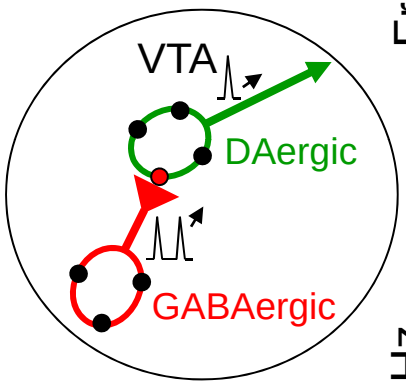
Nicotine-treated
Saline-treated

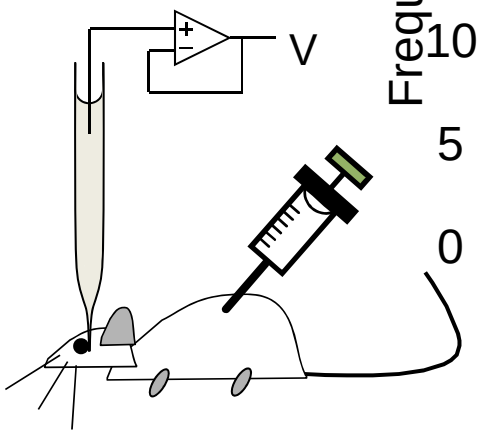
10 μM bicuculline

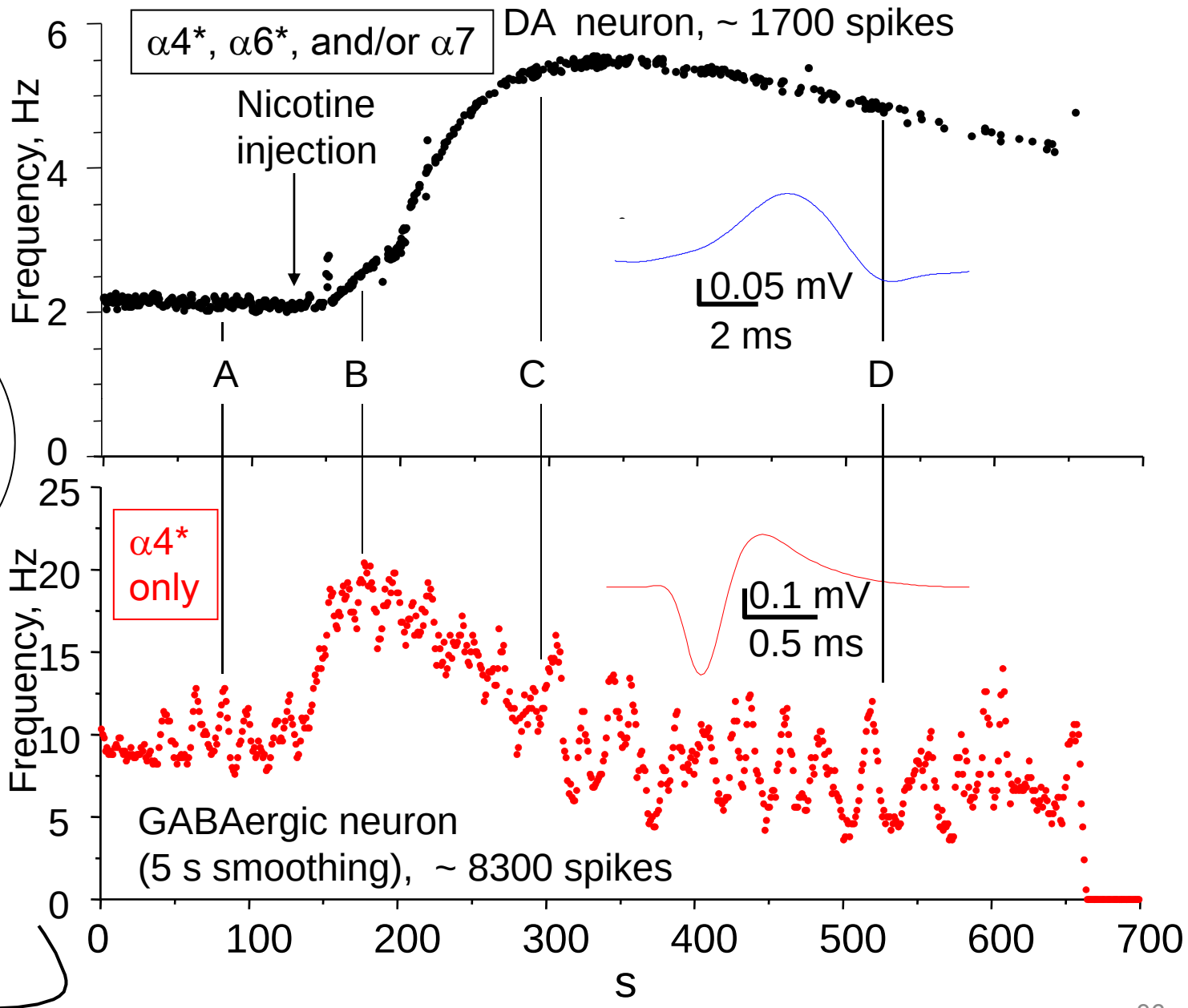


VTA GABAergic and DA neurons have contrasting responses to nicotine *in vivo*

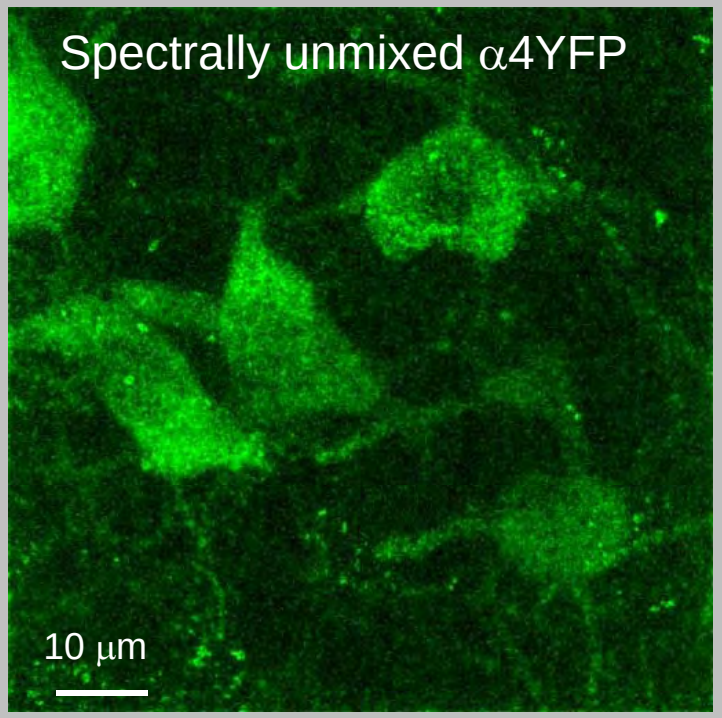
WT mouse

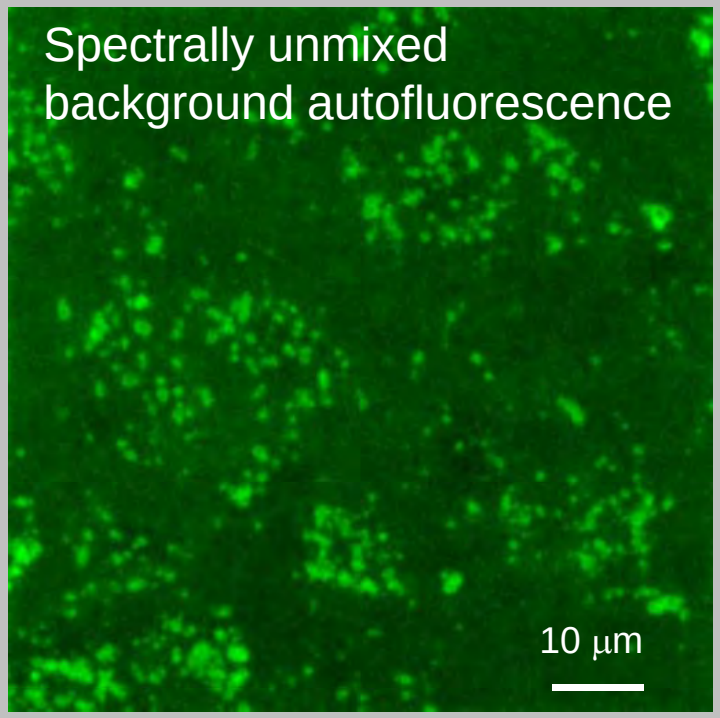


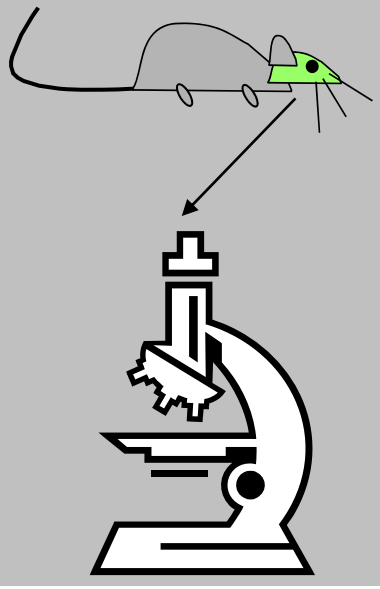


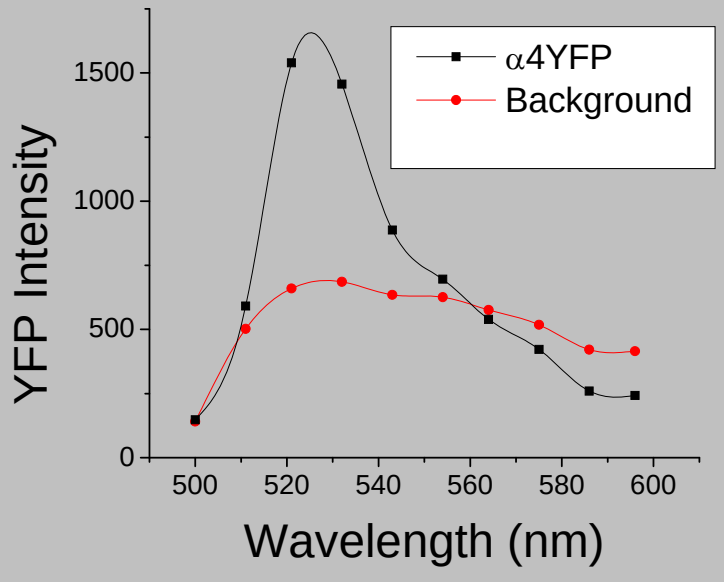


α4-YFP knock-in: substantia nigra pars compacta neurons







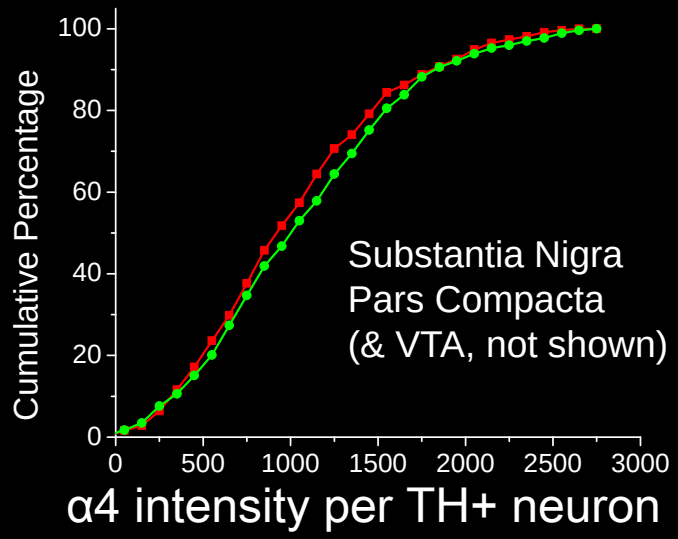


Shortcut to Projections of 32-32-LS5unmix.avi.lnk

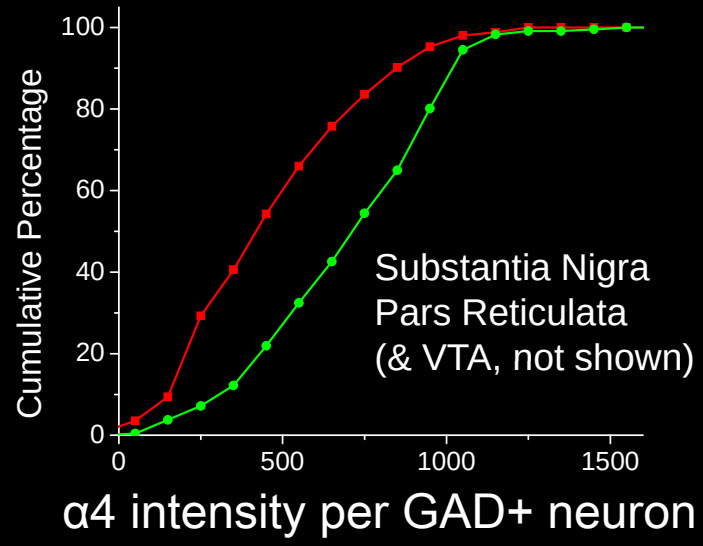
Raad Nashmi

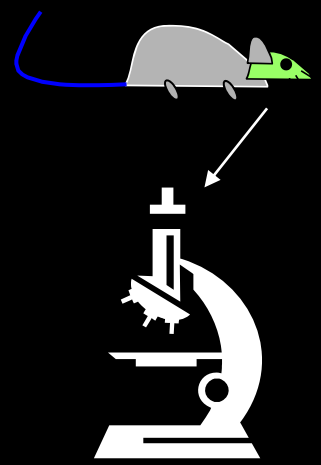
Midbrain data show cell specificity of SePhaChARNS

Chronic nicotine does not change
α4 levels
in dopaminergic neuron somata . . .



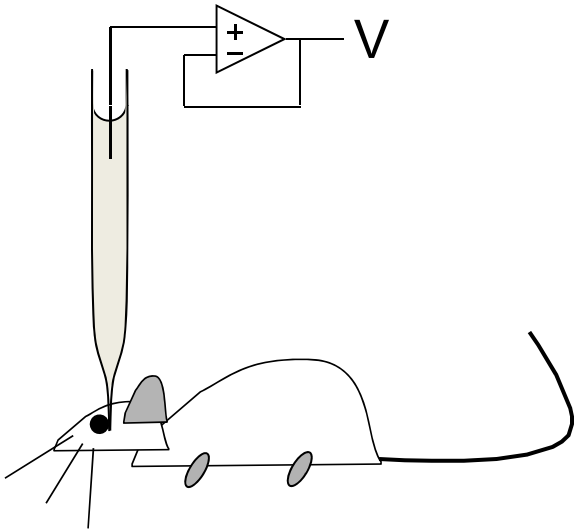
. . . but does upregulate
α4 levels
in GABAergic inhibitory
neuron somata.



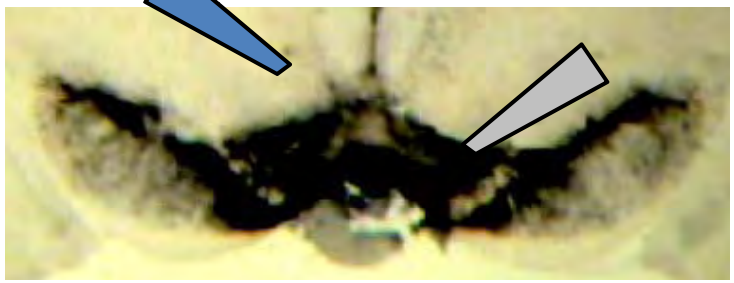


Test for functional α4* upregulation:
Electrophysiology in slices
and
intact anesthetized mice

Including studies with α4 knockout (KO) mice



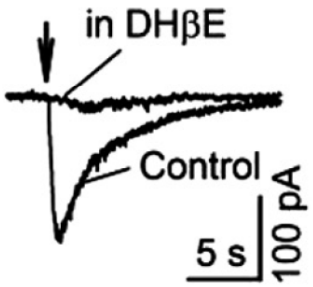
ACh, nicotine puffs

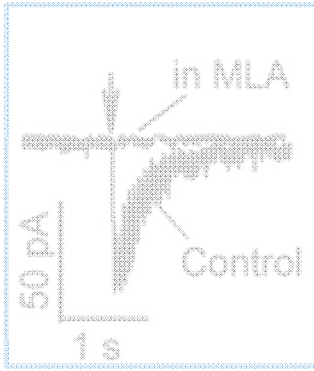


(Tyrosine hydroxylase immunostain)

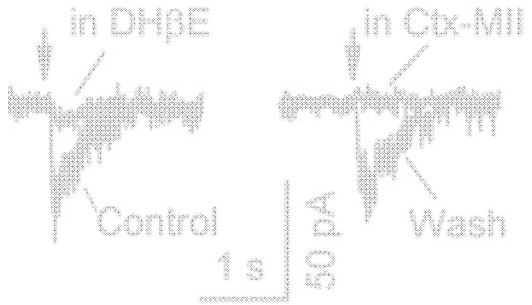
Chronic nicotine modifies α4* currents in substantia nigra neurons

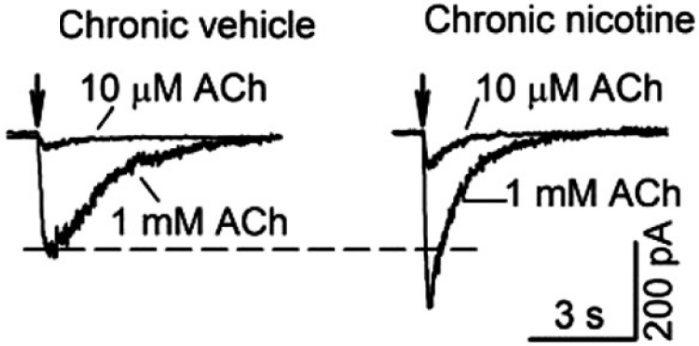
SN pars reticulata GABAergic somata

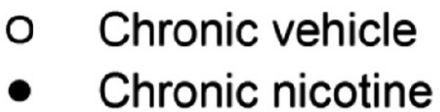


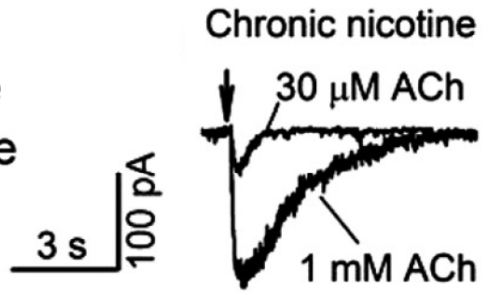


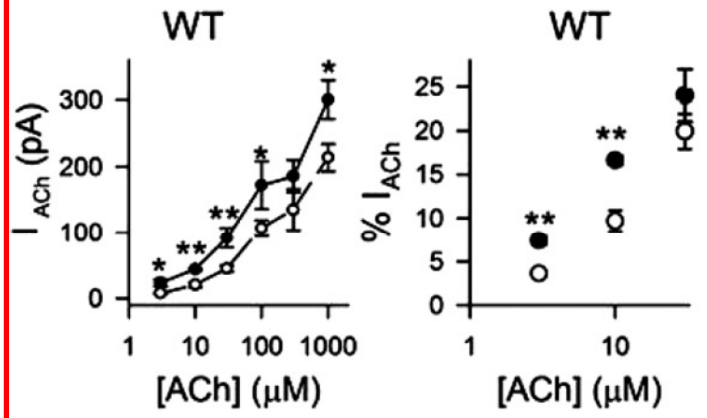
α4 KO



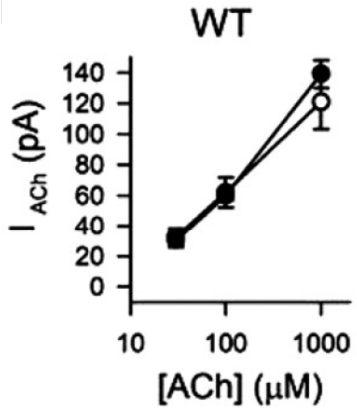




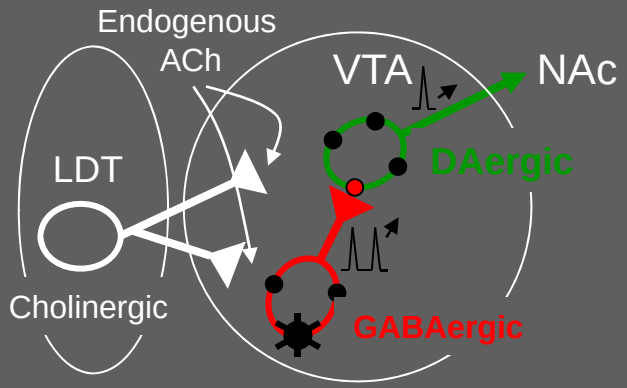


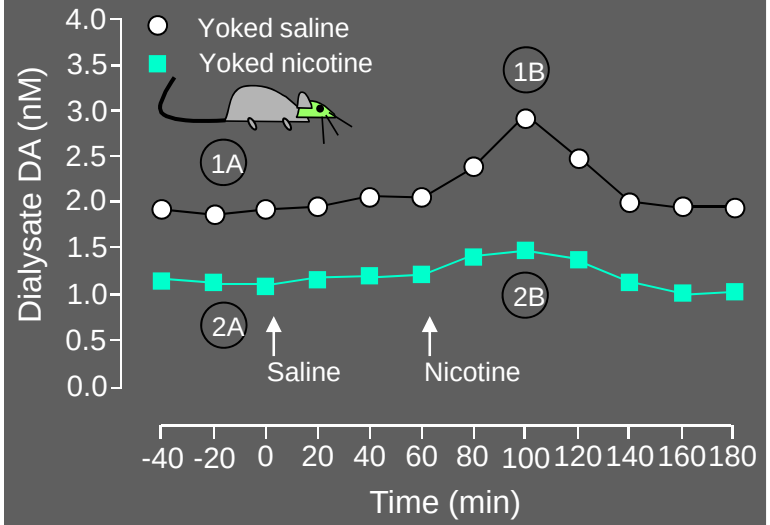


SN pars compacta DA somata



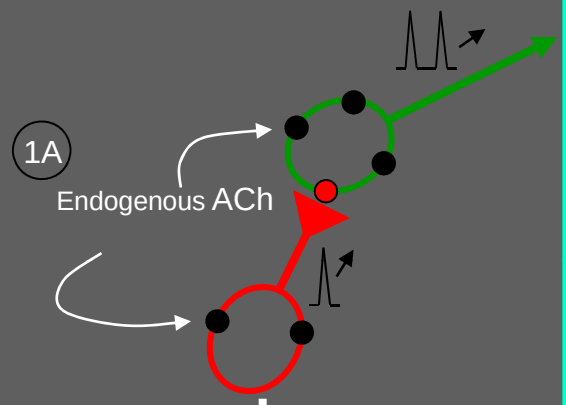
Chronic nicotine cell-specifically up-regulates functional α4* receptors: Hyothesis for circuit-based tolerance in midbrain (Nashmi et al, 2007)





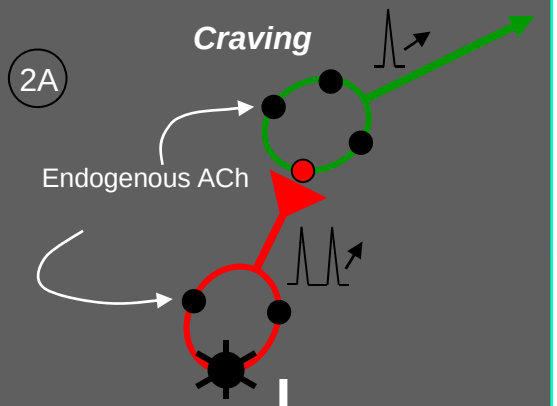
Rahman et al, 2004

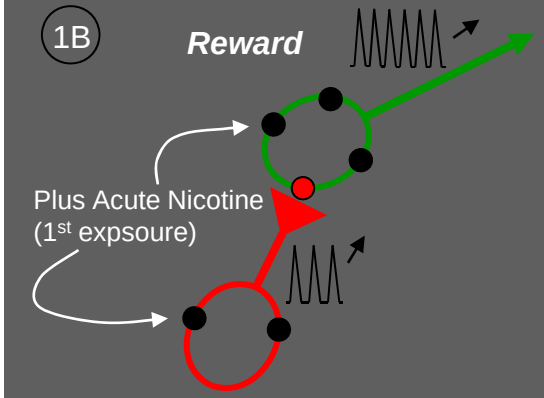
Chronic Saline

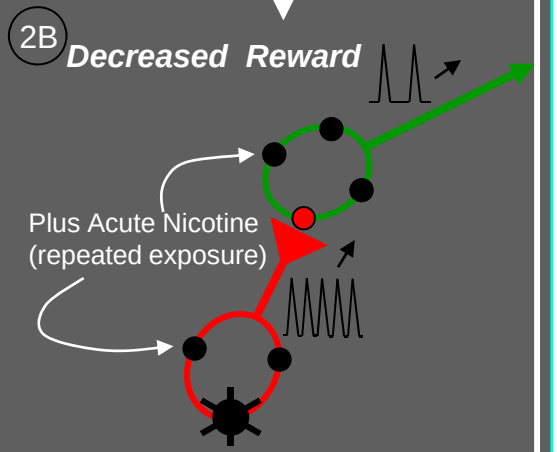


Chronic Nicotine →Tolerance

Upregulated α4* nAChRs



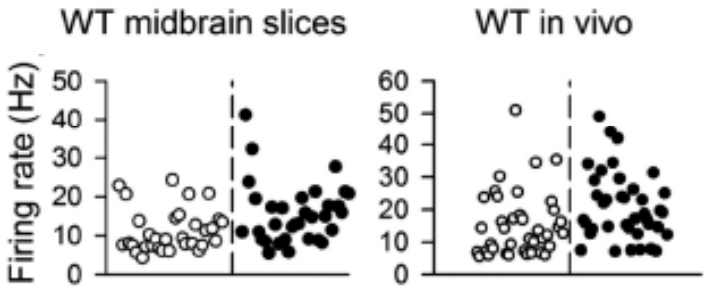




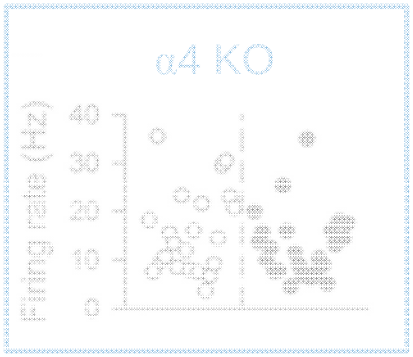
+ acute nicotine

Chronic nicotine modifies firing rates in substantia nigra neurons: the role of α4* nAChRs on GABAergic neurons

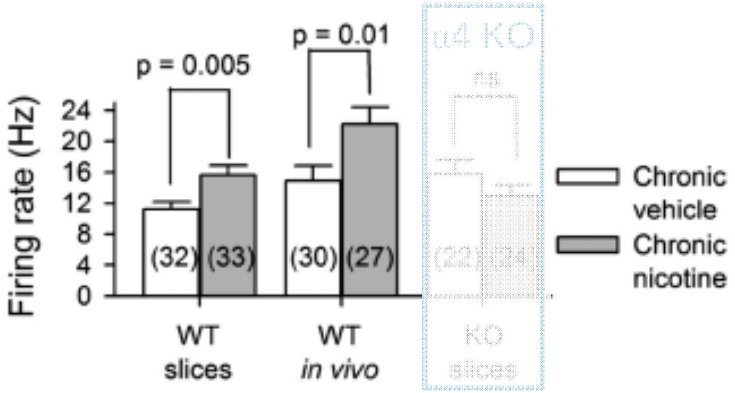
SN pars reticulata GABAergic neurons



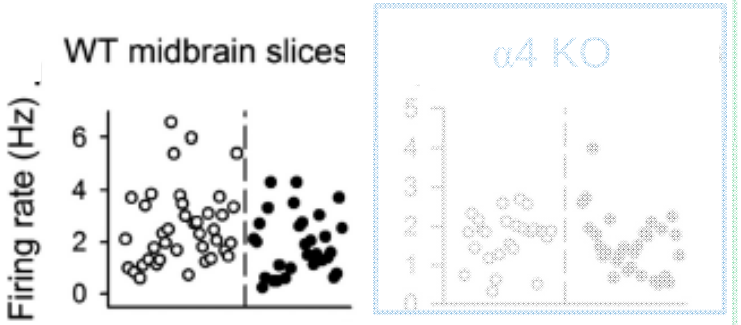
Also Tan . . . Laviolette
Neuropharm 2009

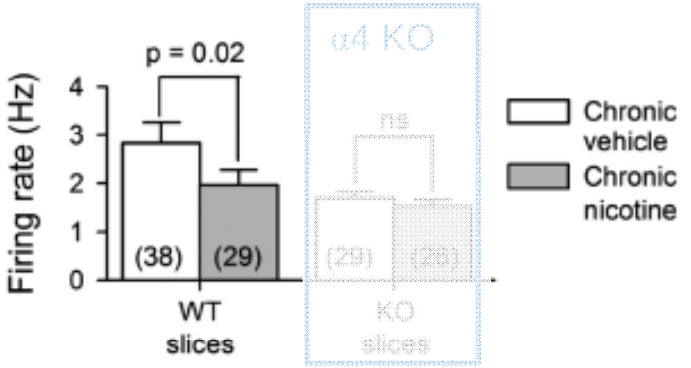


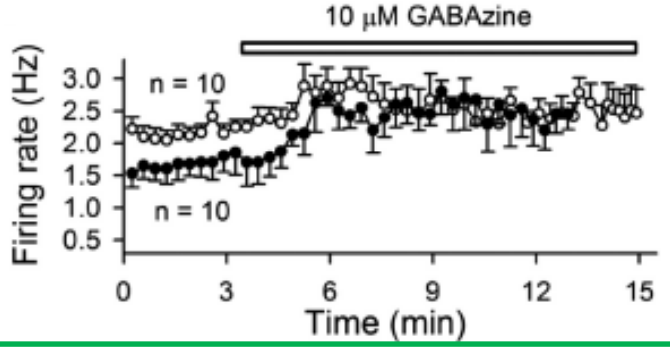
Chronic vehicle
Chronic nicotine



Firing of SNc DA neurons

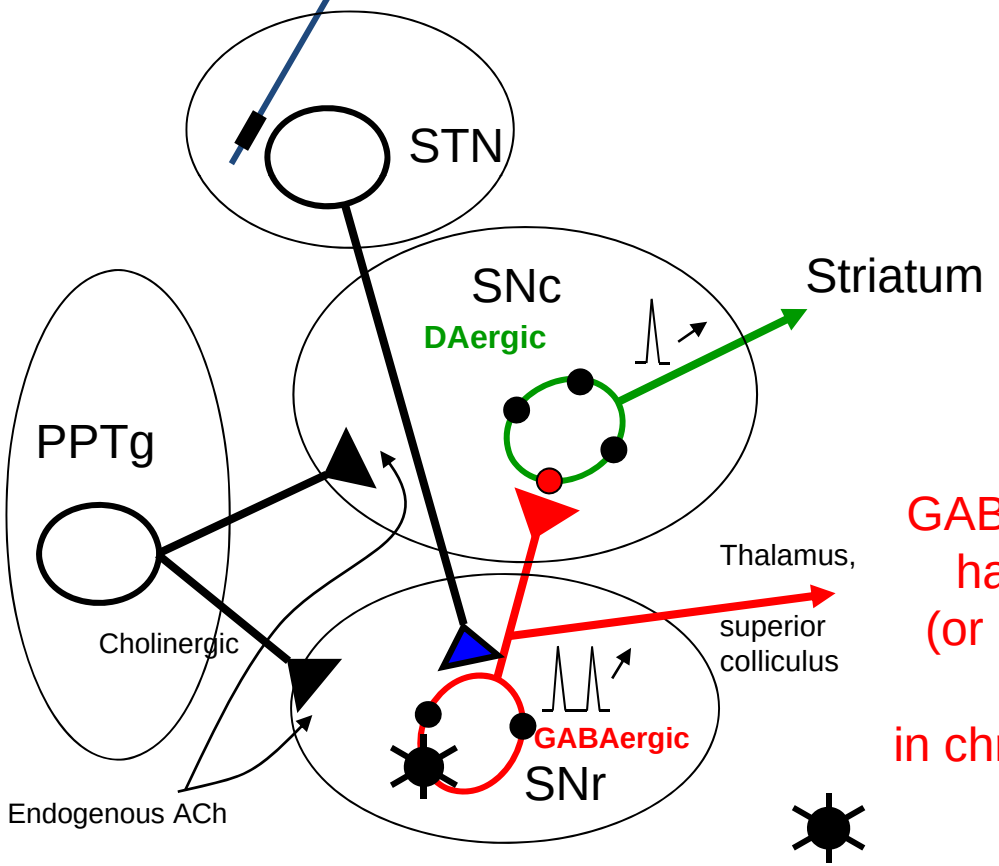






2nd Hypothesis for PD neuroprotection by Chronic nicotine: Circuit-based mechanism in substantia nigra via Cholinergic, Dopaminergic, and GABAergic neurons in Hindbrain & Midbrain

. . . Analogous to
"deep brain stimulation" in subthalamic nucleus?



GABAergic neurons have increased (or more regular?) firing in chronic nicotine. . .

Upregulated a4* nAChRs

Regional specificity of α4* nAChR upregulation by chronic nicotine

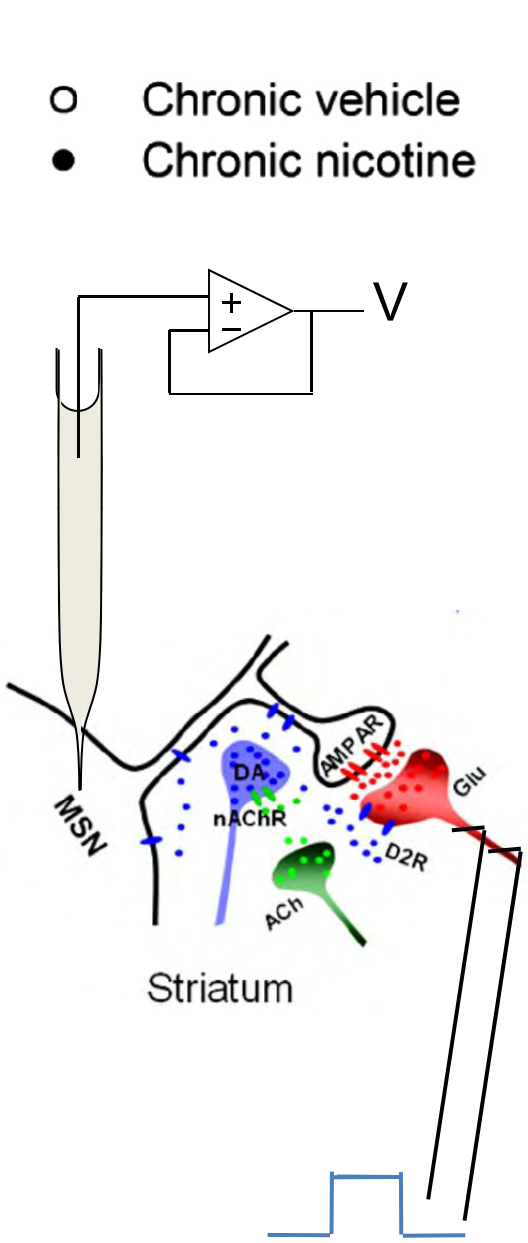
	Saline	Nicotine	Nicotine, % of saline
Superior colliculus	195 ± 20	262 ± 21	134 ± 12
Medial habenula	669 ± 55	749 ± 24	112 ± 4
Fasciculus retroflexus	599 ± 86	673 ± 41	112 ± 7
Interpeduncular nucleus	1216 ± 71	984 ± 38	81 ± 3
Perforant path	287 ± 8	555 ± 7	194 ± 2
Nucleus accumbens	318 ± 25	357 ± 18	112 ± 6
Caudate putamen	353 ± 11	417 ± 15	118 ± 4
Cerebral cortex	273 ± 18	236 ± 16	86 ± 6
Anterior cingulate cortex	299 ± 21	407 ± 23	136 ± 8
Thalamic DLG nucleus	840 ± 71	928 ± 38	111 ± 4

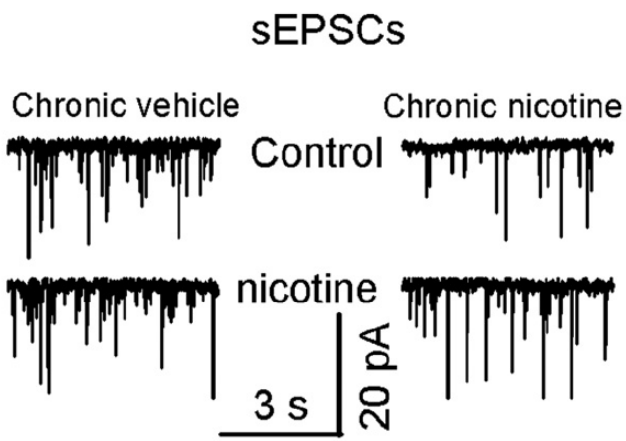
We sought α4* nAChRs in striatal neurons, using fluorescence and electrophysiology.

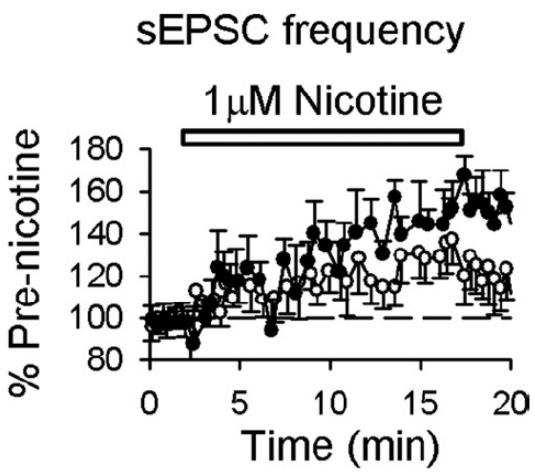
We found none. Therefore,

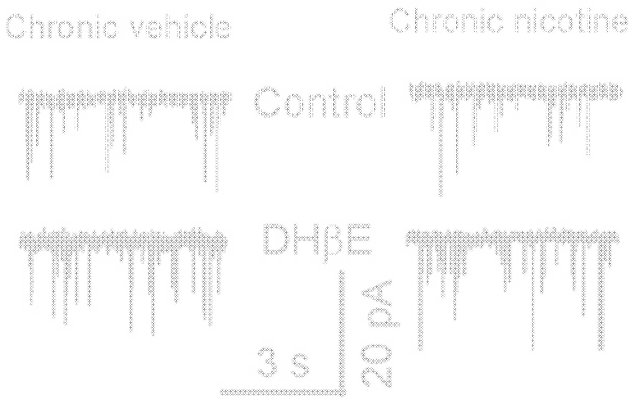
we developed assays for the α4* nAChRs on dopaminergic nerve terminals in striatum . . .

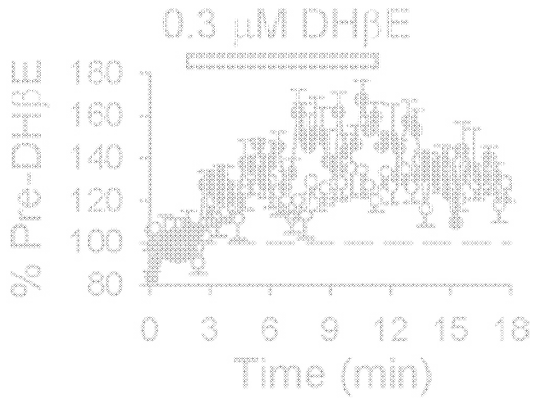
Chronic nicotine augments nicotinic modulation of sEPSCs in MSNs

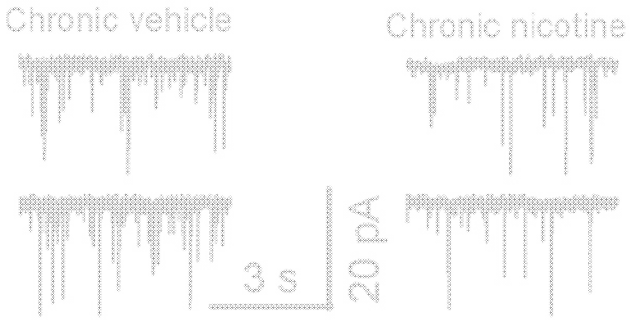


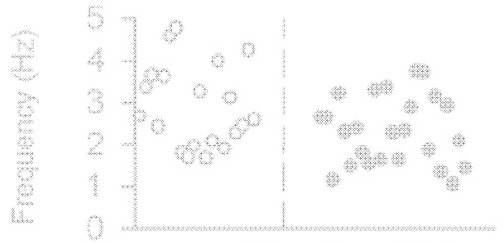




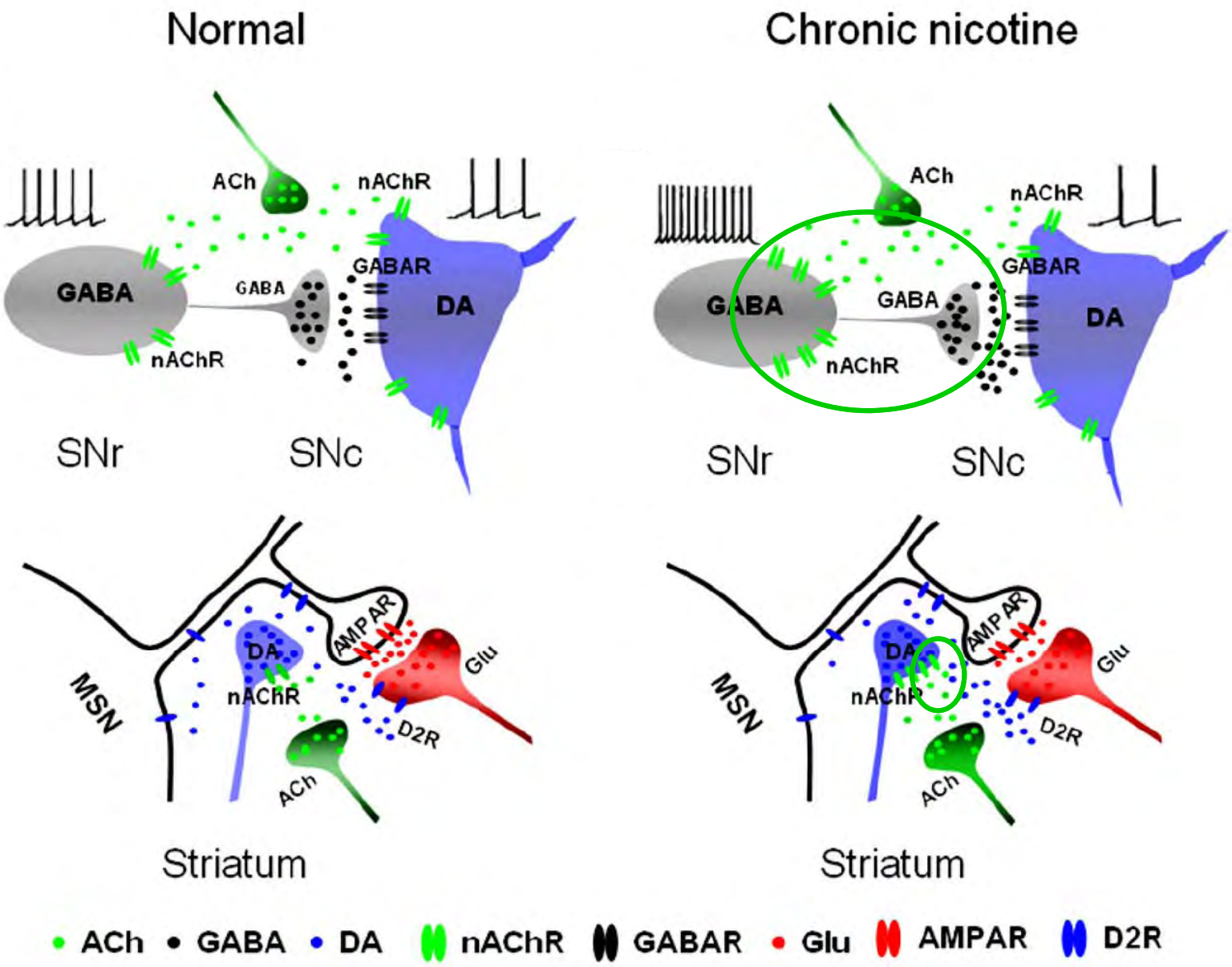






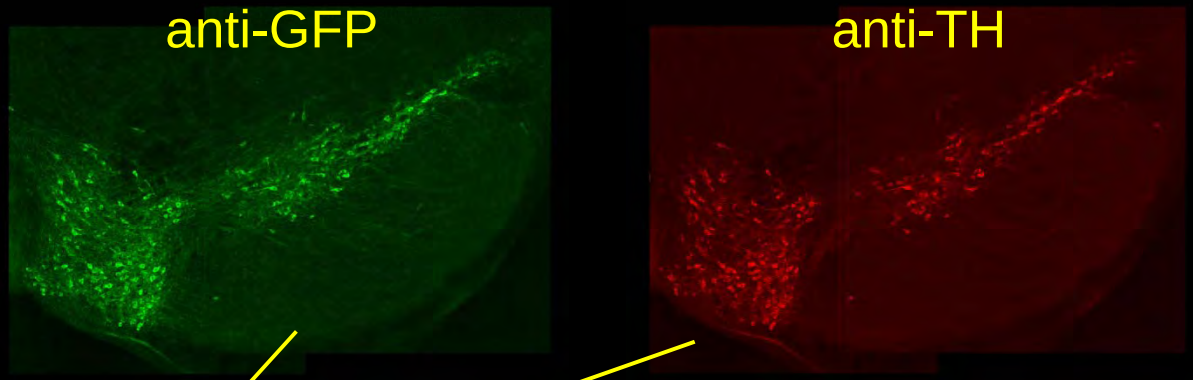


Chronic nicotine regulates the nigrostriatal pathway via α4β2* nAChR upregulation, with cellular and subcellular selectivity

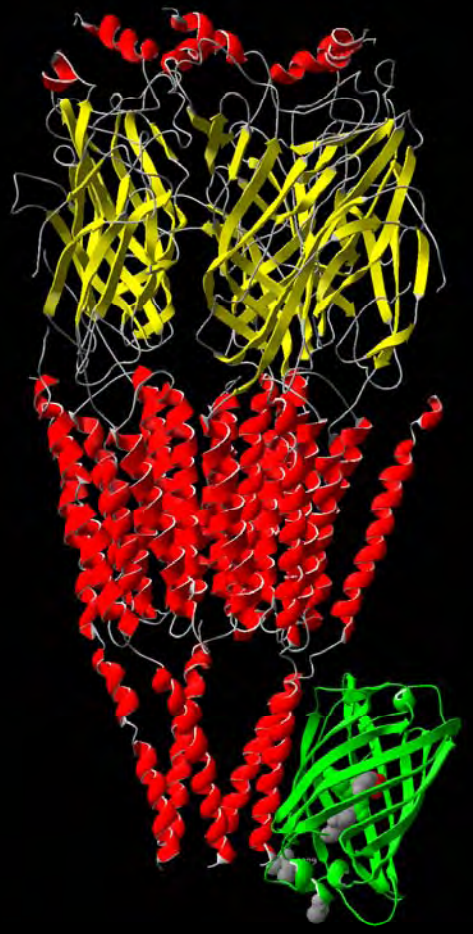


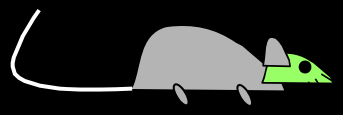
In planning & construction phases

Knock-in and BAC Transgenic mice with Monomeric GFP and Cherry nAChR subunits for studies on localization & assembly (FRET)

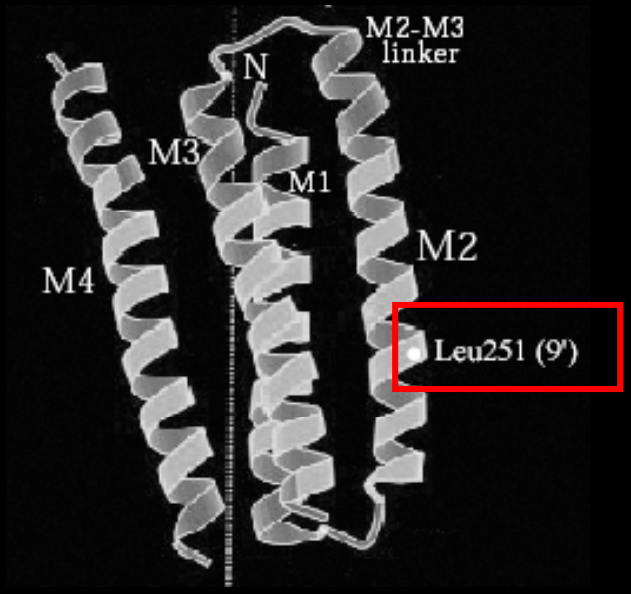


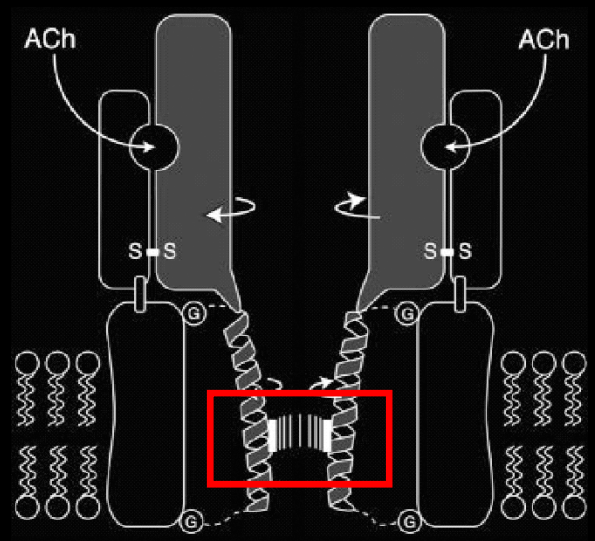
Subunit	*Known FRET partners*	*References*
α6	α4, α6, β2, β3	Drenan et al, 2008
β2	α4, α6, β2, β3	Nashmi et al, 2003 Drenan et al, 2008
β3	α4, a6, β2, not β3	Drenan et al, 2008
α7	α7	T. Murray, SFN
α5	α4	C. Dilworth, unpub





Engineering α-subunit nAChR hypersensitive mice: TM2 Pore-Lining Leu9' Residue Controls Receptor Sensitivity

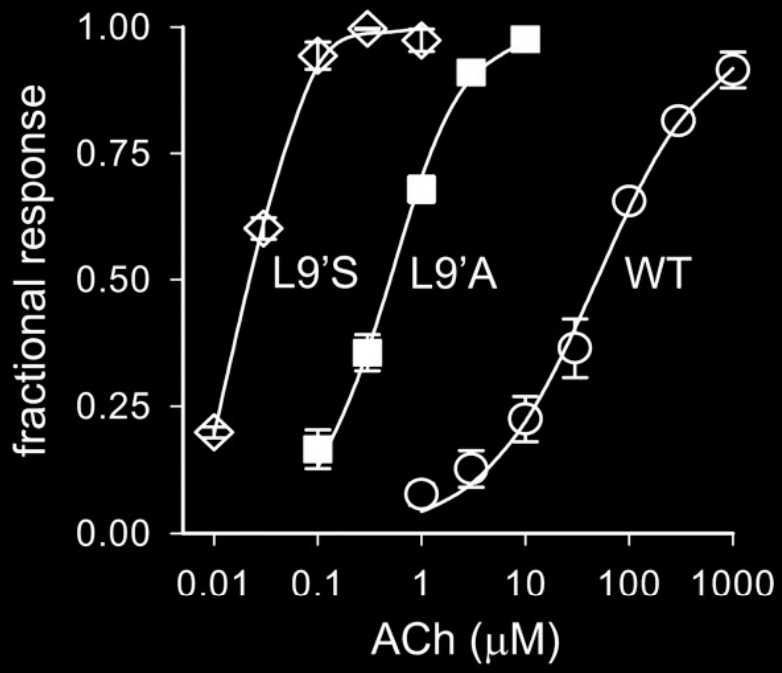




Miyazawa, Fujiyoshi, Unwin, *Nature* 2003

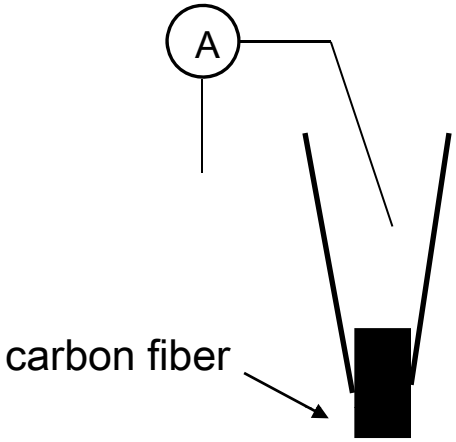
- Leu9' Lines the Ion Channel Pore
- Leu9' Mutations Shift Dose-Response Curve to Left
- Leu9' Mutations are Dominant & Gain of Function

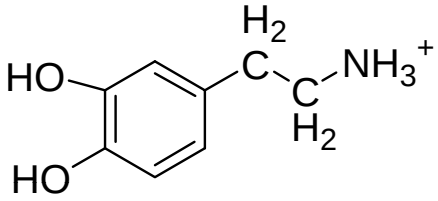
α4 data, not α6!

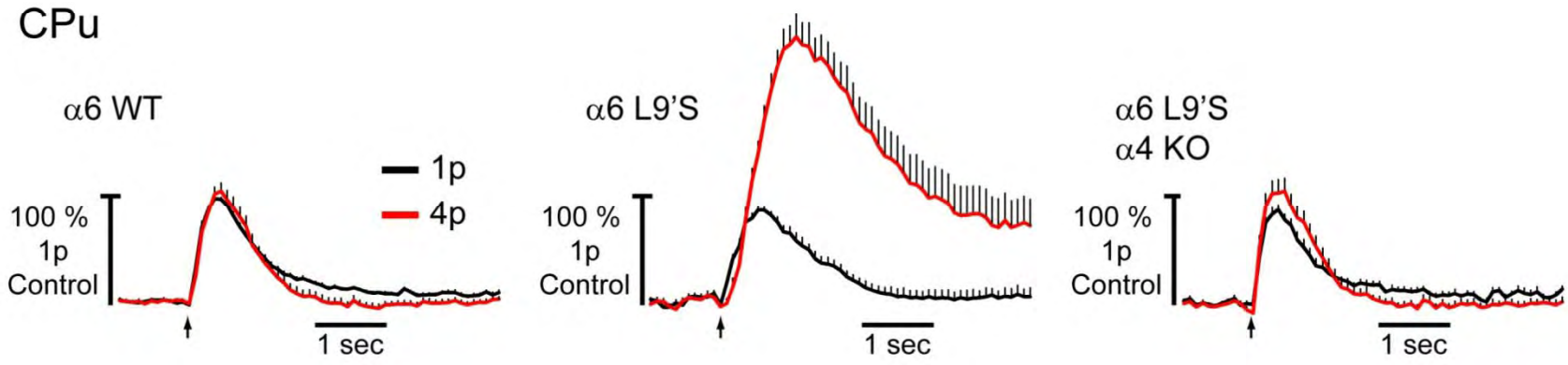


Fonck, et al. J. Neurosci. 2005

Carbon fiber electrochemistry shows that hypersensitive α6* nAChRs c can lead to burst-enhanced DA release

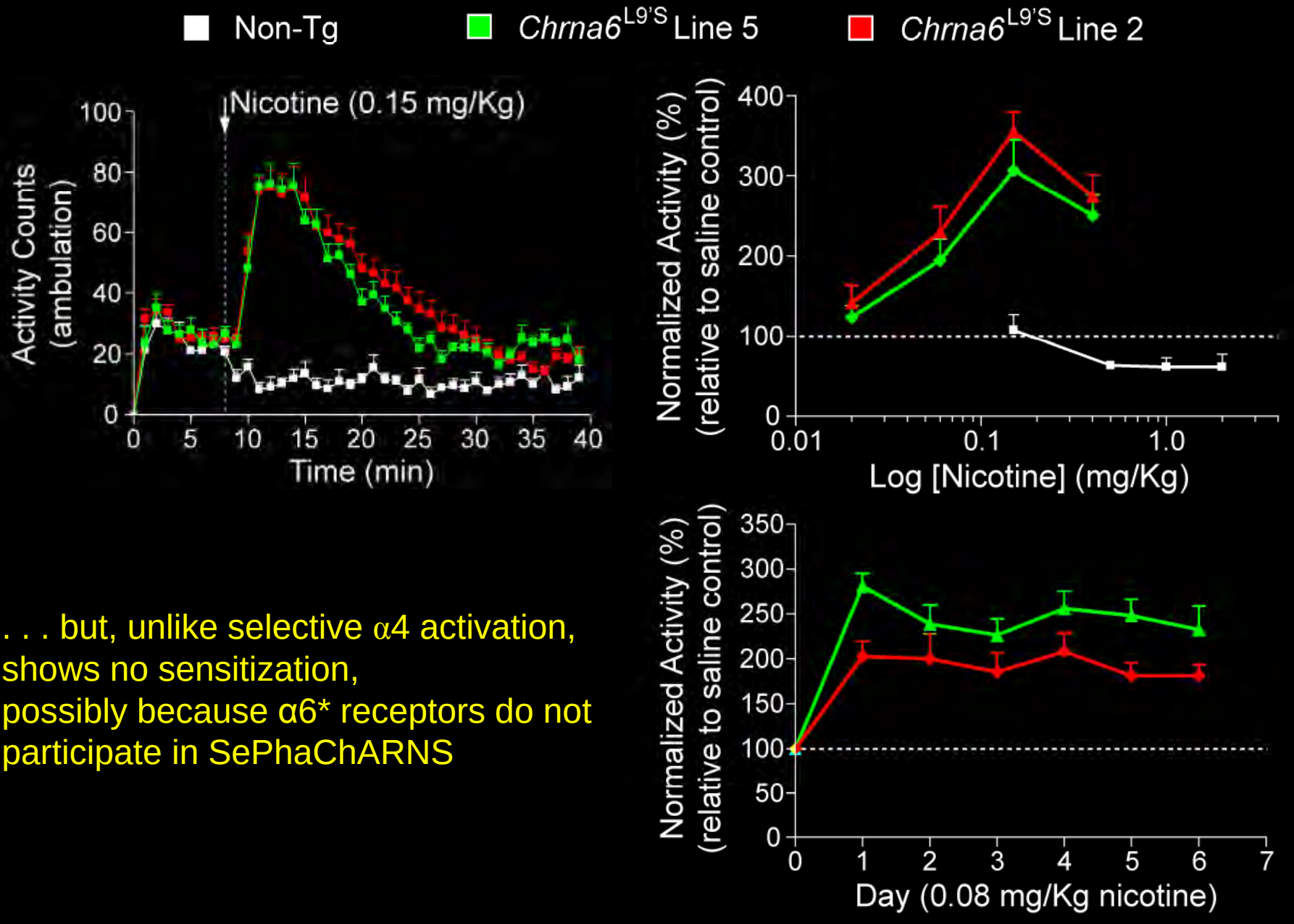






Ryan Denan

Selective Activation of DA Neurons via α6* nAChRs Stimulates Locomotor Activity . . .



. . . but, unlike selective α4 activation, shows no sensitization, possibly because α6* receptors do not participate in SePhaChARNS

SePhaChARNS participates in sequelae of chronic exposure to nicotine

1. Nicotine potently activates some neuronal nAChRs (because it participates in both cation-π and H-bond interactions within the conserved aromatic box).

2. This high affinity allows nicotine to act as a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry.

3. These processes lead to α4β2* upregulation, with cellular and subcellular specificity.

4. Chaperoning may underlie chronic nicotine's effects on suppression of ADNFLE seizures.

5. β2 vs β4 subunits are processed and trafficked differentially, iun part because of distinct trafficking motifs in the M3-M4 loops.

6. Lynx proteins may also function as chaperones.

7. These phenomena may soon be resolved at the single molecule level.

Behavior

Circuits

Synapses

Neurons

Intracell.

Binding

Nic vs ACh

Proteins

RNA

Genes

Selective nAChR upregulation during chronic exposure to nicotine

8. In the medial perforant path, α4* upregulation explains enhanced LTP, via a direct presynaptic mechanism. This is a simple model for cognitive sensitization.

Nicotine Addiction

Parkinson's Disease

ADNFLE

9. a. In midbrain, α4* upregulation in GABAergic neurons explains tolerance to chronic nicotine, via the GABAergic-DA circuit.

b. A similar circuit mechanism may protect DA neurons against harmful burst firing in PD.

10. In striatal DA terminals, α4* upregulation may increase the influence of cholinergic interneurons on DA release.

11. Repeated selective activation of DA neurons, via hypersensitive α6* receptors, produces neither locomotor tolerance nor sensitization.

Behavior

Circuits

Synapses

Neurons

Subcell.

Binding

Nic vs ACh

Proteins

RNA

Genes

We do not yet understand several processes, *e. g.*
somatic signs of withdrawal,
stress-induced nicotine use,
weight gain in people who stop smoking,
and ANFLE circuitry.

SEBASTIAN JUNGER *NY Times* April 21, 2010

Farewell to Korengal

For much of 2007 and 2008, I was an embedded reporter with a platoon of airborne infantry at a remote outpost called Restrepo . . .

The psychological pressure was enormous. One soldier told me,

"I've only been here for four months and I can't believe how messed up I am. I went to the counselor and he asked if I smoked cigarettes and I told him no and he said, 'Well, you may want to think about starting.'"

Caltech Prof. Dennis Dougherty, Nyssa Puskar, Jai Shanata, Joanne Xiu

Caltech Purnima Deshpande, Crystal Dilworth, Ryan Drenan, Elisha Mackey, Sheri McKinney, Julie Miwa, Raad Nashmi, Rigo Pantoja, Rachel Penton, Chris Richards, Johannes Schwarz, Rahul Srinivasan, Cagdas Son, Andrew Tapper, Ying Wang, Cheng Xiao

Univ of Colorado, Boulder Al Collins, Sharon Grady, Mike Marks, Erin Meyers, Tristan McClure-Begley, Charles Wageman, Paul Whiteaker

Univ. of Colorado, Denver Robert Freedman, Sherry Leonard

Univ. Utah J. Michael McIntosh

Univ. Michigan Dan Axelrod

Support: NIH (NIDA, NINDS, NIA), Targacept, CA Tobacco-Related Disease Research Program, MJ Fox Foundation Louis & Janet Fletcher