Examining the Role of CaMKIIα in α4β2* Nicotinic Receptor Function

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Background

- Tobacco use is the leading cause of preventable death worldwide
- Nicotine binds to and activates nicotinic acetylcholine receptors (nAChRs)
- Primary reinforcing properties of nicotine are mediated by α4β2* nAChRs
- Molecular mechanisms underlying regulation and downstream signaling of α4β2* nAChRs are not completely understood
- CaMKIIα, a highly expressed brain kinase with well-established roles in synaptic plasticity, was recently identified as a protein interactor of α4β2* nAChRs in mouse and human brain
- In this study, we aimed to elucidate the role of CaMKIIα in nicotinic receptor function, focusing on receptor phosphorylation and localization

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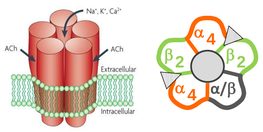
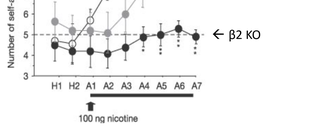
α4β2* Nicotinic Acetylcholine Receptors (nAChRs)



- Pentameric, ligand-gated ion channels
- High-affinity for nicotine and acetylcholine
- Highly expressed in the brain
- *Required for reinforcing properties of nicotine*
  - Nicotine-mediated increases in DA firing/ release (Picciotto et al, Nature 1998)
  - Expression in VTA is necessary and sufficient for nicotine self-administration and CPP (Maskos et al. Nature 2005)
- **Receptor regulation and downstream signaling are not well understood**

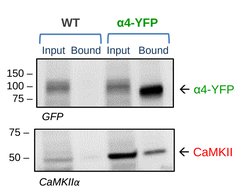


The α4β2* nAChR Protein Interactome

Table 3 Proteins with their abundances significantly positively correlated with that of β2 nAChR subunit across genotypes

Correlation coefficient	N	F score	P value	Protein	UniProtKB accession No.	Cellular compartment	Previously identified?	Molecular function
0.748	12	20.268	0	Glial fibrillary acidic protein	P03995	Cytoplasm	No	Protein binding, structural molecule
0.858	18	44.542	0	nAChR subunit α4	O70174	Cell junction	No	Neuro-transmitter receptor
1	18	∞	0	nAChR subunit β2	Q9ERK7	Cell junction	No	Neuro-transmitter receptor
0.652	18	11.844	0.003	Neurofilament light poly peptide	P08551	Growth cone	No	Protein binding, structural molecule
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 α	P11798	Cytoplasm	No	Transferase, nucleotide binding, protein binding
0.917	18	21.235	0.01	Calcium/calmodulin-dependent protein kinase type II subunit γ	Q923T9	Sarcoplasmic reticulum membrane	No	Transferase, nucleotide binding, protein binding
0.57	18	7.681	0.014	F-actin-capping protein subunit α2	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-α	P42669	Nucleus	No	Nucleic acid binding, translation regulator, 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 β 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
0.506	18	5.496	0.032	Myelin proteolipid protein	P60202	Cell membrane	No	Structural molecular, protein binding
0.502	18	5.378	0.034	Spectrin α chain, brain	P16546	Cytoplasm	Yes	Hydrolase, protein binding, nucleotide binding
0.493	18	5.149	0.037	Tubulin β-3 chain	Q9ERD7	Cytoplasm	No	Hydrolase, nucleotide binding, structural molecular, protein binding, peptide

McClure-Begley et al. GPB. 2013



- Immunoprecepitation from WT, β2 KO or heterozygous mouse brain
- Quantitative LC MS/MS for protein ID
- 17 proteins correlated significantly with β2 nAChR gene dose, including CaMKII isoforms
- CaMKII isoforms also found in nAChR interactome in human cortical tissue (*McClure-Begley, eNeuro 2016*)
- **CaMKIIα co-IP confirmed using lysates from α4-YFP transgenic mice**

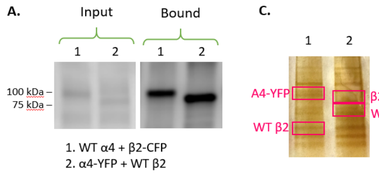
Aim 1: Characterize the interaction between CaMKIIα and α4β2* nAChRs

- Does CaMKIIα phosphorylate α4β2 nAChRs?
- Does CaMKIIα affect localization of α4β2 nAChRs?

Aim 2: Evaluate relevance of the CaMKII-α4β2* interaction to nicotine-mediated physiology and behavior

- Where is CaMKIIα expressed in the VTA?

In vitro phosphorylation of α4β2* nAChRs with CaMKIIα

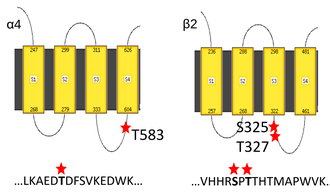


B.

Group	Treatment
A. Baseline	Extract with phosphatase inhibitors; no treatment
B. Dephosphorylated	Treat with Lambda Phosphatase
C. CaMKIIα	treat with Lambda phosphatase; stop; treat with activated CaMKIIα

D. nAChR Phosphorylation

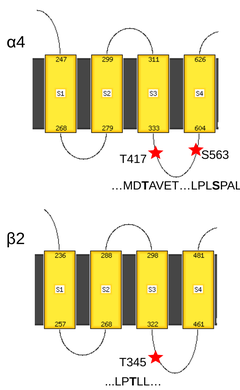
A		B		C	
Baseline		**Phosphatase only**		**CaMKIIα**	
α4	**β2**	**α4**	**β2**	**α4**	**β2**
S46	T79				
S69	S200				
S362			S325		**S325**
Y573	T327	Y573	T327		**T327**
T583	T328		T328	**T583**	



- nAChR subunits were expressed in HEK cells then isolated by immunoprecipitation on GFP resin
- Bound fractions were treated as indicated in panel B
- nAChR subunits were eluted, separated by SDS PAGE, silver stained then cut from the gel and analyzed by LC MS/MS for phosphorylation status

Baseline Phosphorylation of α4β2* nAChRs in Mouse Forebrain

Subunit	% Coverage (total/loop)	site	Predicted CaMKII site?	Conserved in Human?
α4	30/45	T417	No	No
		S563	No	Yes
β2	21/30	T345	Yes	No



S365...KRPSVV... PKA site covered but not phosphorylated
S470 ...SLSVQ... predicted CaMKII site, observed PKA site; covered but not phosphorylated
S489 ...SRSIQ... observed PKA site, not covered
T583 ...EDTDF... site IDed in alpha4 from HEK lysates; covered but not phosphorylated here

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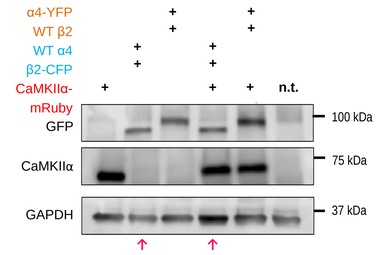
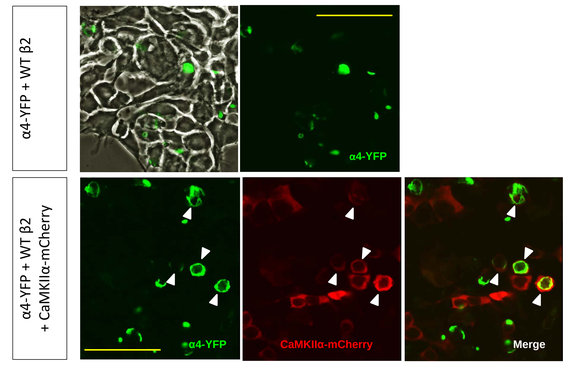
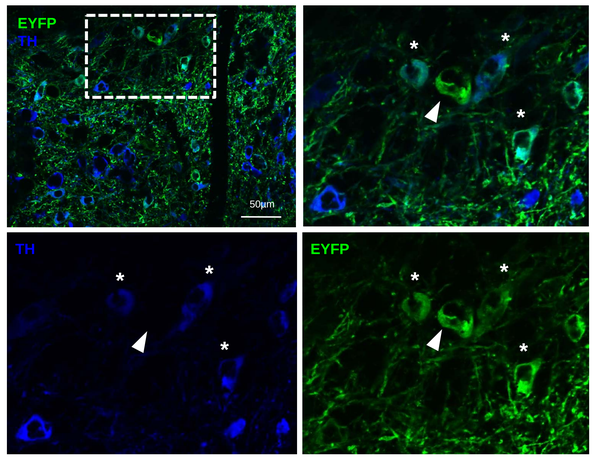
CaMKIIα may promote surface localization of α4β2 nAChRs

- HEK cells: Transiently transfected with nAChR subunits with or without CaMKIIα-mRuby
- Cells were fixed 24hrs after transfection and imaged for changes in receptor localization
- **α4-YFP* nAChRs display increased surface localization when co-expressed with CaMKIIα-mRuby**





Evaluating CaMKIIα expression in the VTA



- CaMKIIα-Cre mice injected with floxed hChR2-EYFP (AAV) into the VTA
- Immunostained for Tyrosine Hydroxylase (TH, dopamine neurons) and GFP (CaMKII+ cells)
- Imaged using Olympus FluoView confocal microscope and 60X objective
- **Both DA (asterisks) and non-DA (arrow heads) cells in the mouse VTA express CaMKIIα**

Conclusions

- CaMKIIα co-IPs with α4β2* receptors from mouse brain
- CaMKIIα is capable of phosphorylating α4 and α4-YFP at T583 *in vitro*
- α4β2* receptors are differentially phosphorylated in the mouse brain at baseline
- CaMKIIα-mRuby promotes surface expression of α4β2 nAChRs in HEK cells
- CaMKIIα is expressed in the VTA, where it is present in both DA and non-DA cells

Future Directions

- Evaluate α4β2* receptor phosphorylation in mouse brain after nicotine exposure and/or withdrawal
- Evaluate the role of the CaMKIIα-α4β2* interaction on nicotine-mediated physiology and behavior