A proteomics approach to understanding nicotine-dependent intracellular signaling

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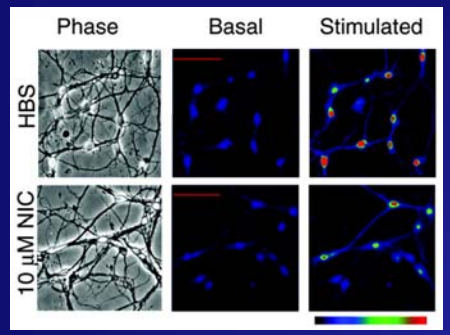
Questions to be answered:

How does repeated nicotine exposure result in long-term changes in behavior?

- What signaling pathways are involved?
- What are the downstream molecular consequences of repeated nicotine exposure?

Nicotine exposure decreases glutamate-mediated Ca2+ influx



WT nicotine

WT vehicle

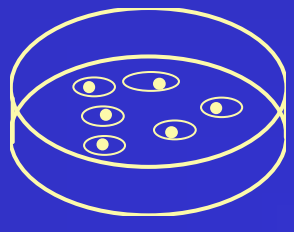
Δ f/f

10
9
8
7
6
5
4
3
2
1
0

0 5 10 15 20 25 30 35 40 45 50 55

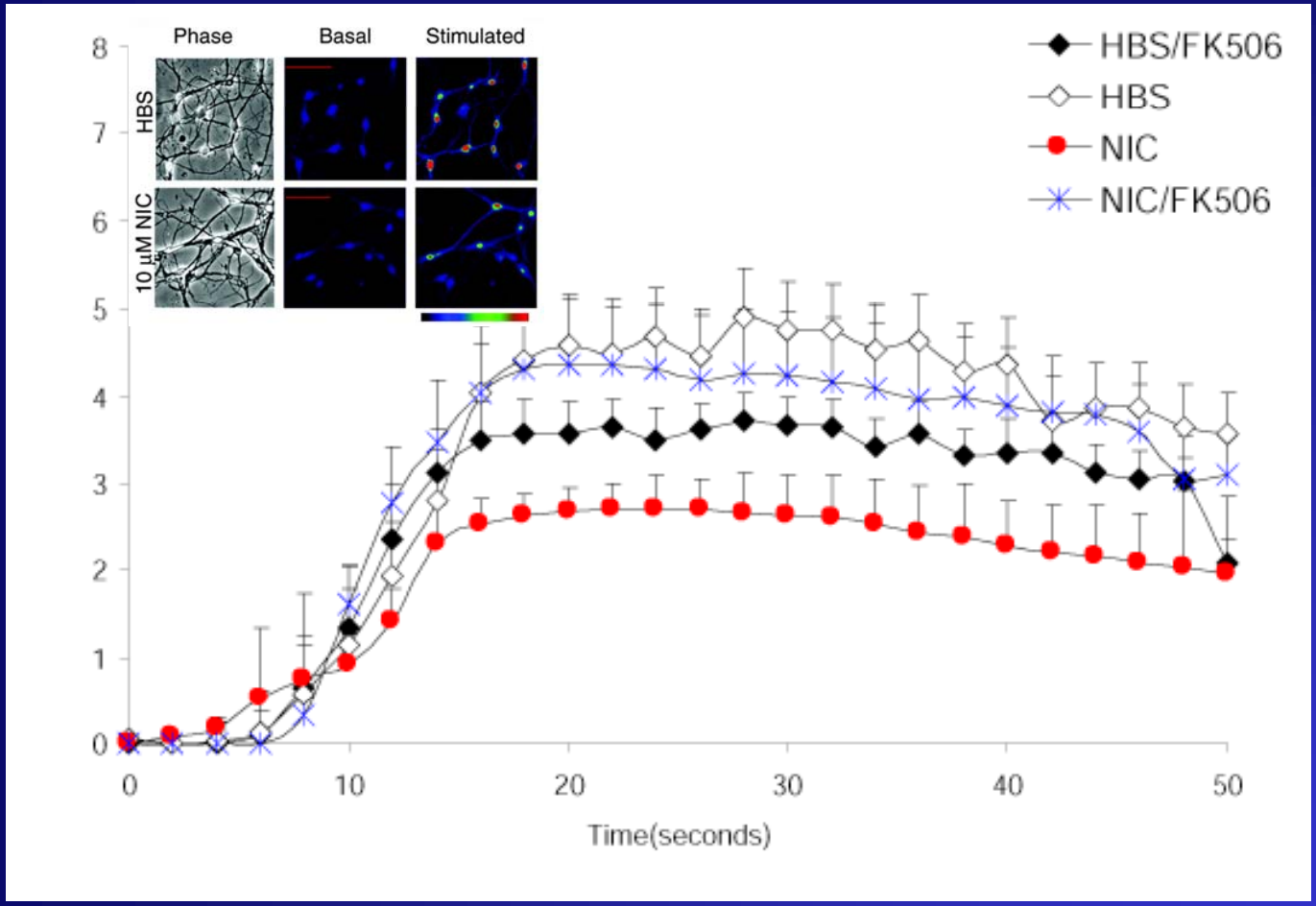
100uM Glutamate

Time (seconds)



Stevens et al, J Neurosci, 2003

Nicotine decreases glutamate-mediated Ca2+ influx via calcineurin activation



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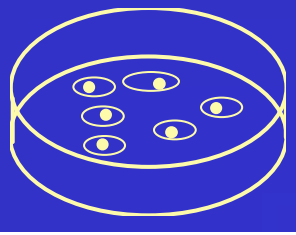
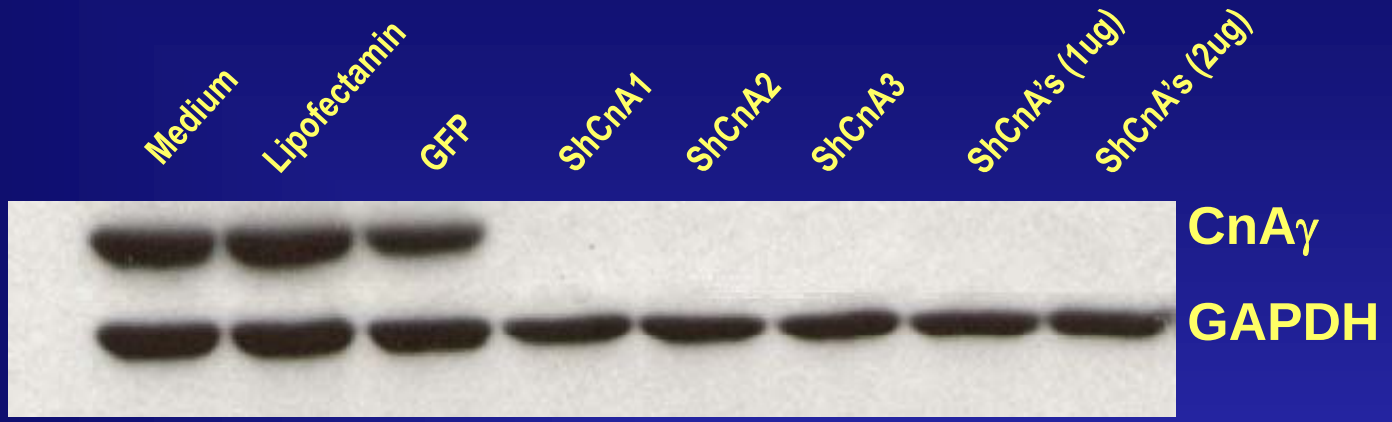
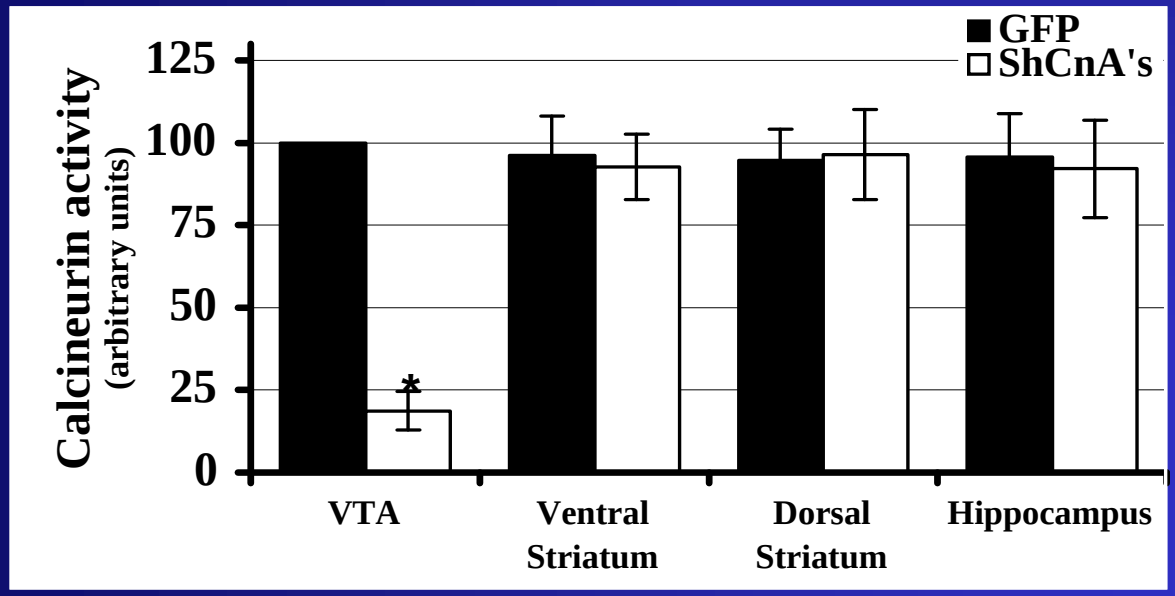


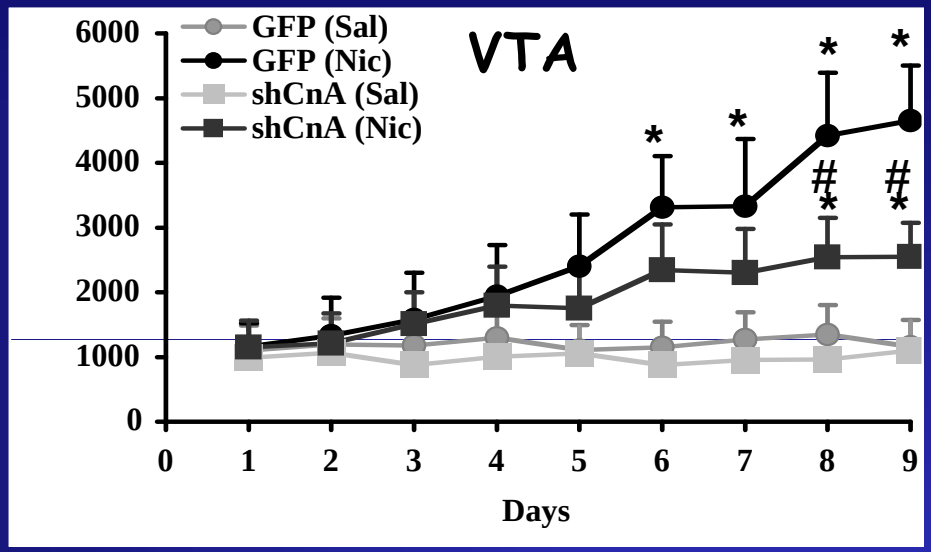
Calcineurin protein and activity is decreased by AAV-shRNAs

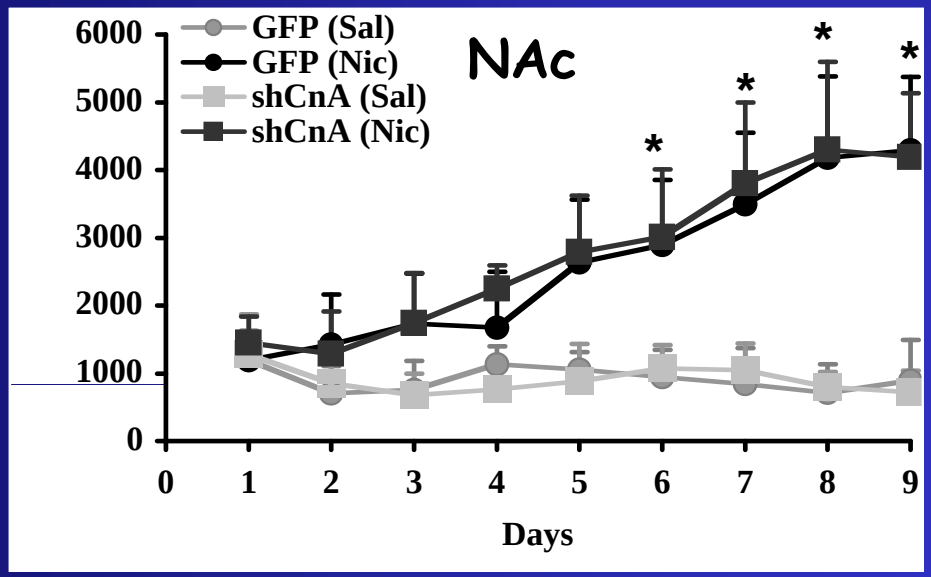




Amine Bahi

Nicotine locomotor sensitization is attenuated by AAV-shRNA in VTA but not NAc





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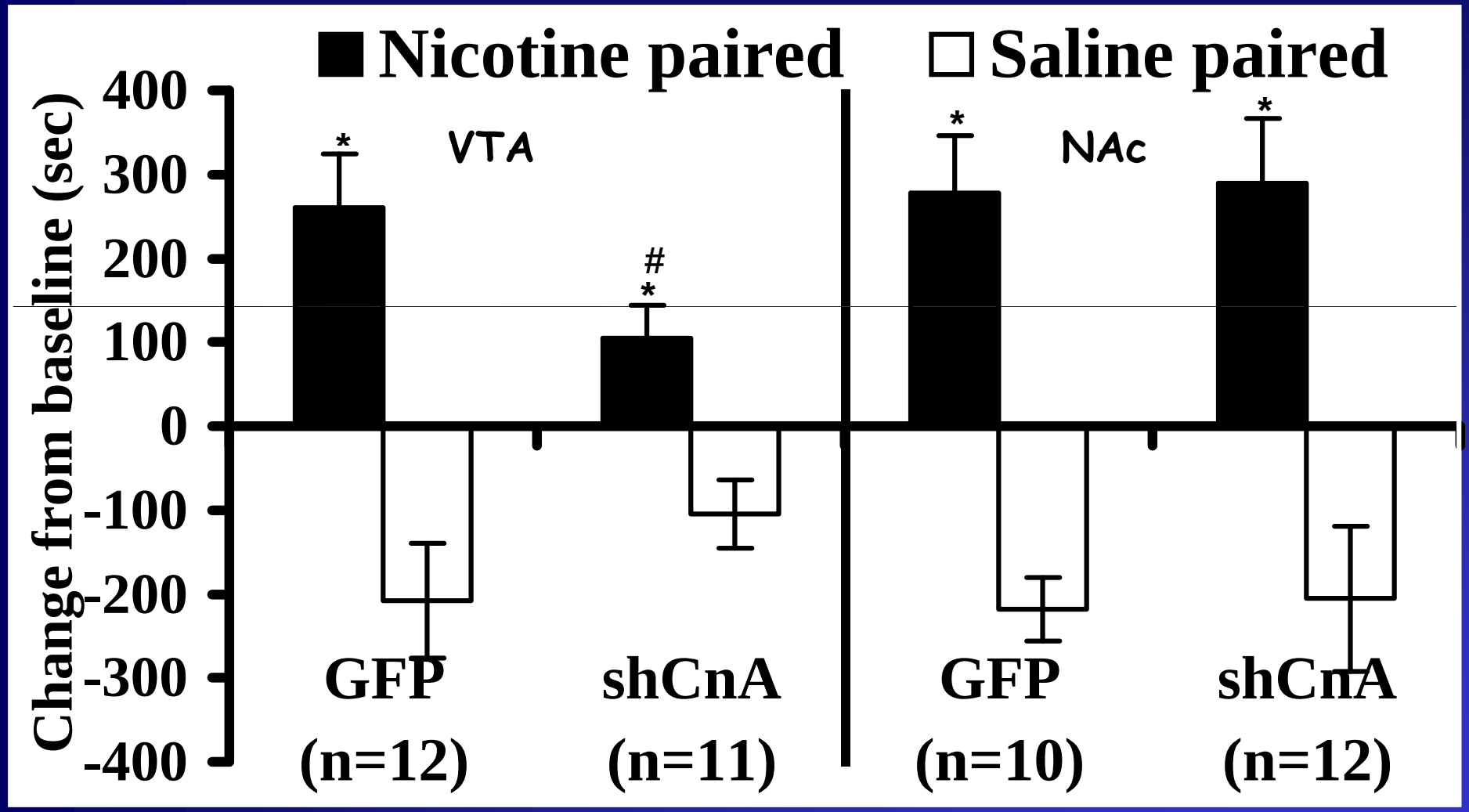
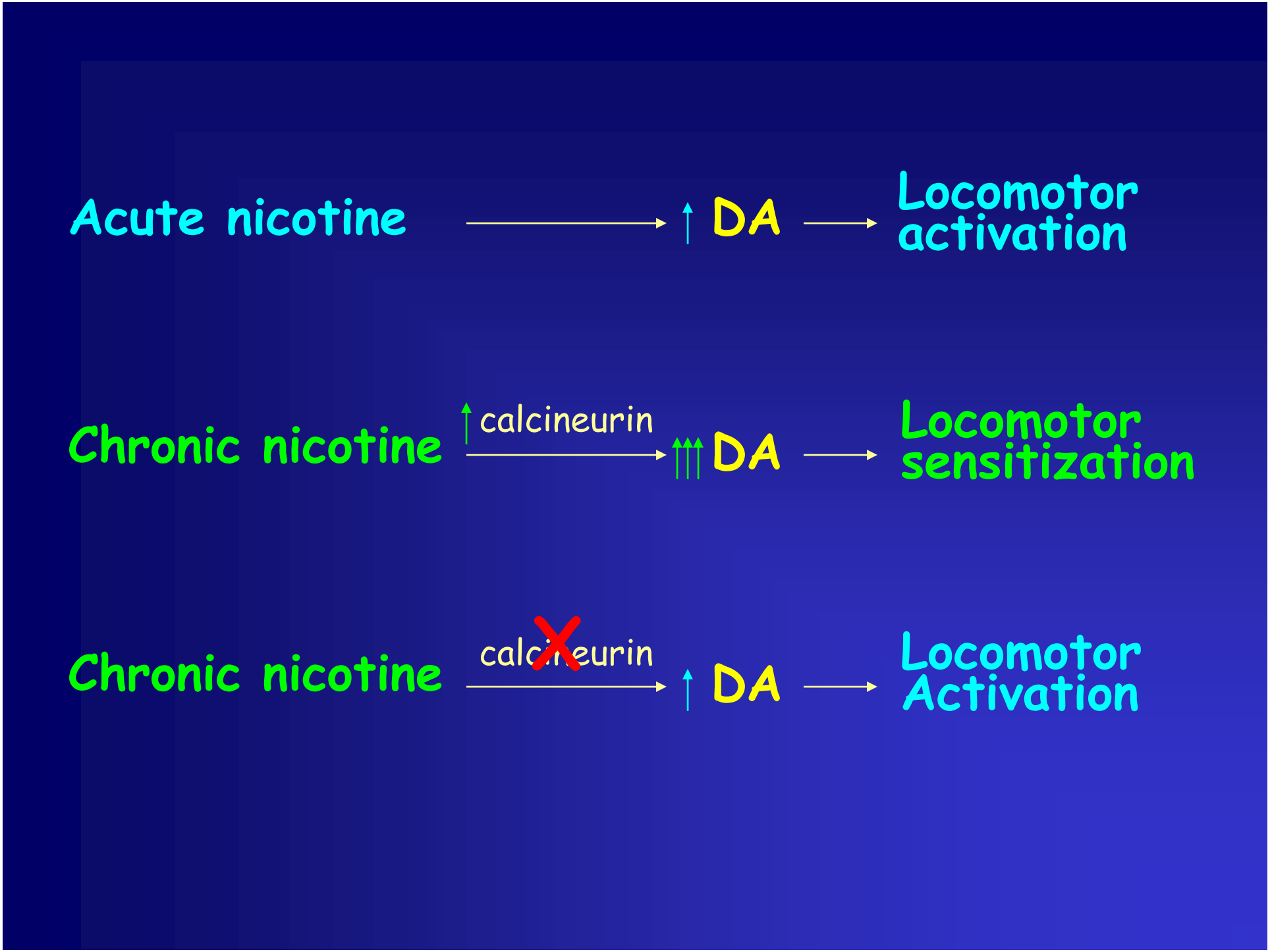
Nicotine CPP is attenuated by AAV-shRNA in VTA but not NAc



Amine Bahi

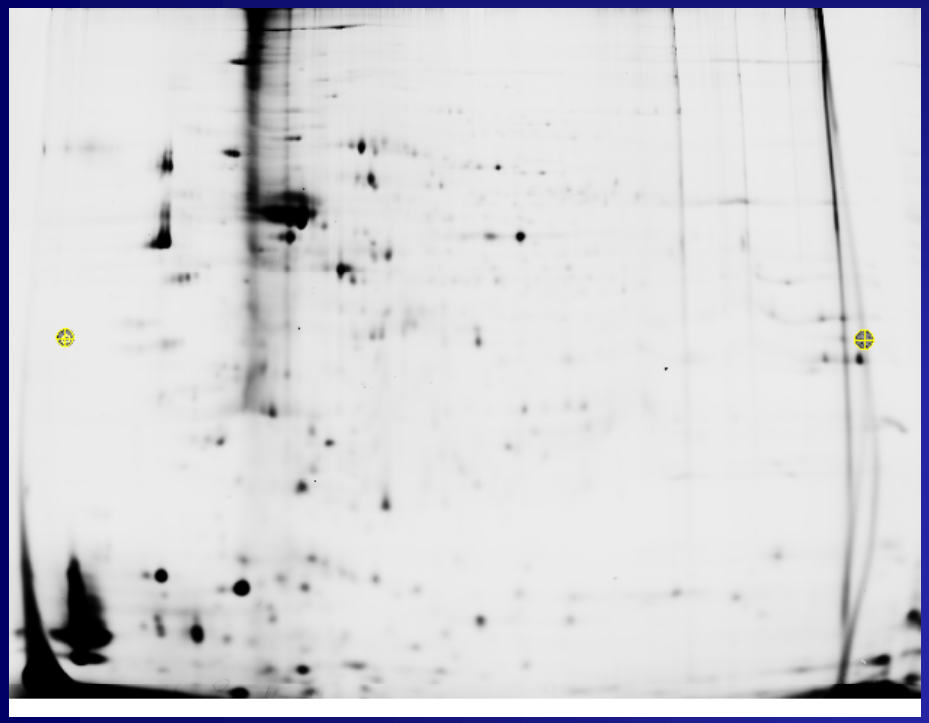

Acute nicotine
↑ DA
Locomotor activation
Chronic nicotine
↑ calcineurin
↑↑↑ DA
Locomotor sensitization
Chronic nicotine
calcineurin
↑ DA
Locomotor Activation

Previous work with DIGE: Chronic Nicotine - NAc

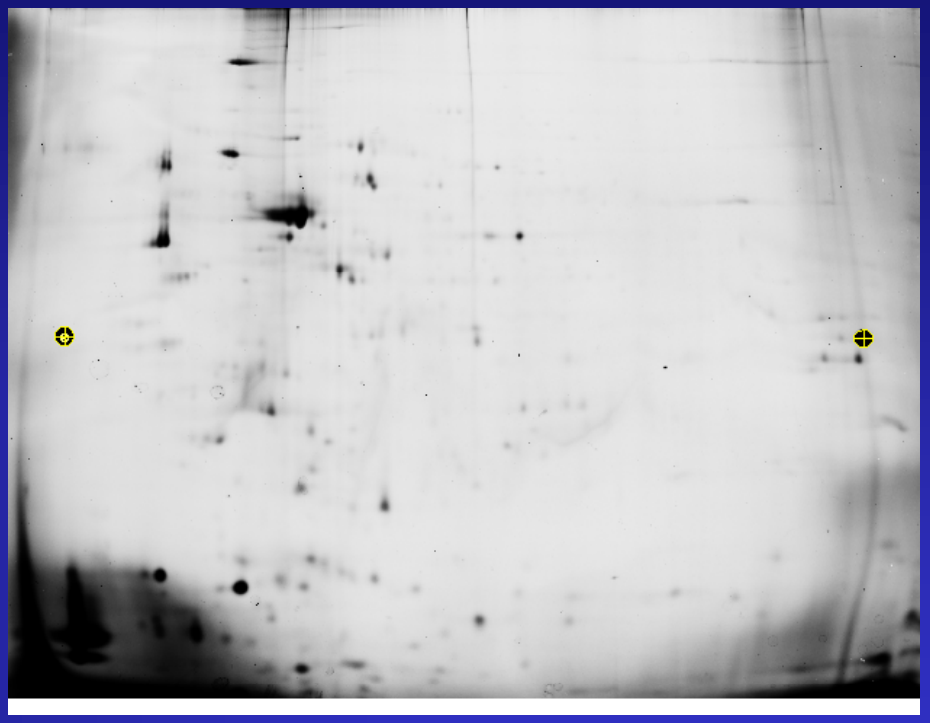
- 4 groups of mice were generated: WT-sac, WT-nic, KO-sac, KO-nic.
- Each group received the treatment in the drinking water for 28 days, a regimen of nicotine we have shown regulates CREB activity in WT mice.
- The NAc shell was dissected out for proteomic analysis.

Question:
What are the protein changes in the VTA and/or NAc that are responsible for nicotine-dependent plasticity?

Previous work with DIGE: Chronic Nicotine - NAc



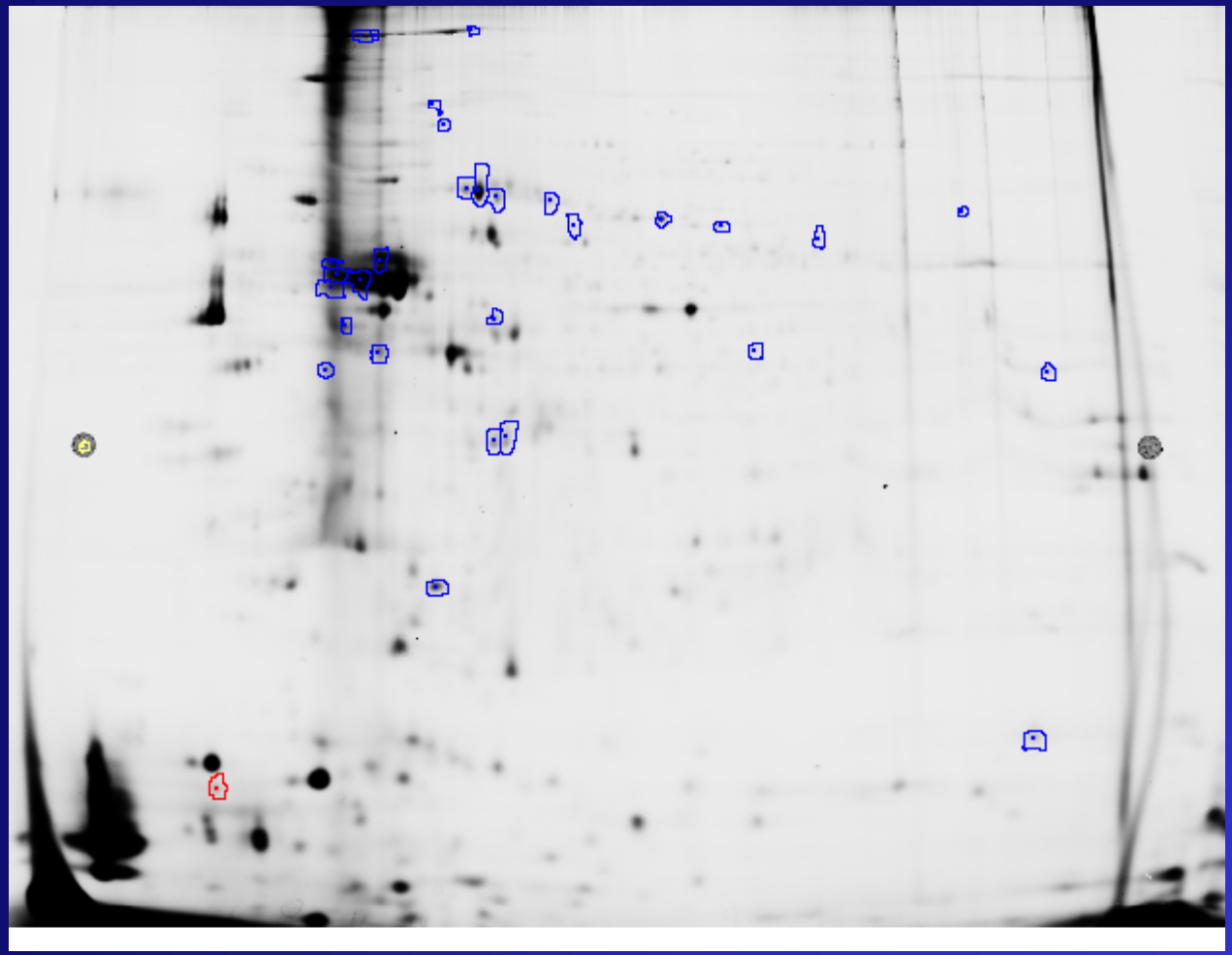
Wildtype
Chronic Nicotine



Beta-2 KO
Chronic Nicotine

Rebecca Steiner

Spot Differences



Up in Nic-WT
Up in Nic-KO

Rebecca Steiner

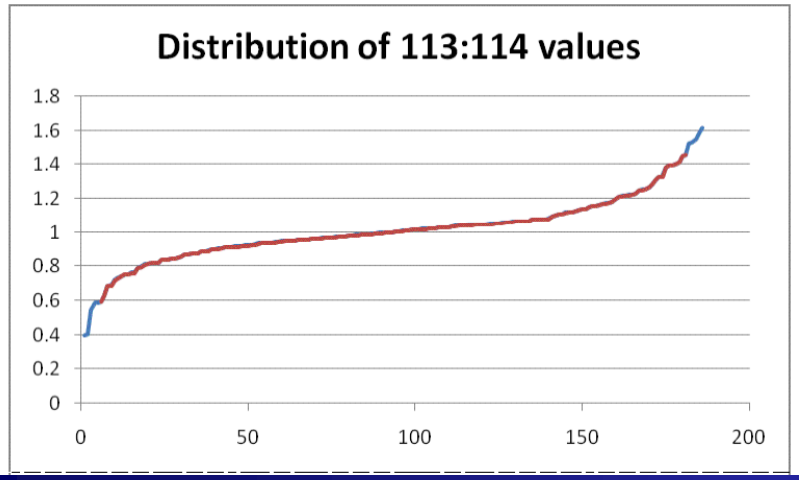
IDs for Chronic Nicotine - NAc

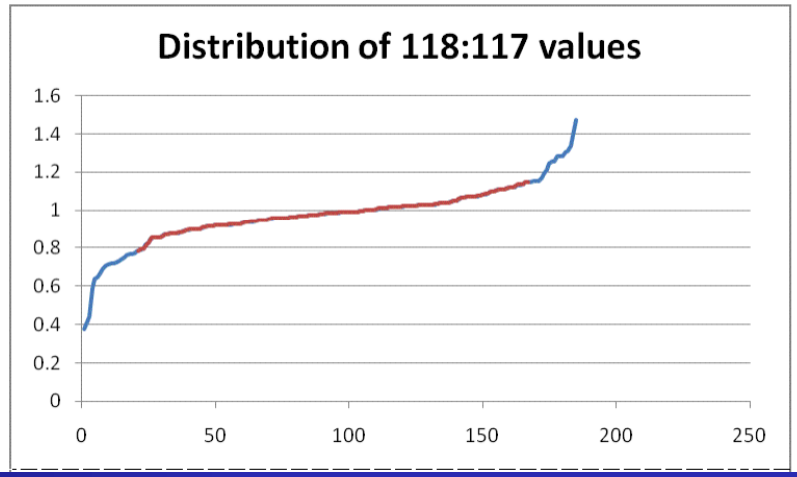
Cy5/Cy3	Identification	Function
1.56	BASP-1	Cytoskeleton?
1.58	Enolase-1 α	Energy metabolism
1.71	Neurofilament 3	Cytoskeleton
1.77	β-actin	Cytoskeleton
1.78	Enolase-2 γ	Energy metabolism
2.08	hsp70	Energy metabolism
2.46	ATP synthase cat sub A	Energy metabolism
2.55	Tubulin-β2	Cytoskeleton
2.56	MARCKS	Cytoskeleton

Unable to confirm by western blotting

Rebecca Steiner

iTraq results for VTA and NAc (analysis by Can Bruce)





VTA NAc

For the 186 proteins detected peptides in VTA, the 80th percentile boundaries are at 0.81 and 1.22. The p-value of values outside these boundaries is 0.04. Proteins outside this boundary whose identification depended on more than a single distinct peptide and whose fold change was +/- 1.33 fold were considered to be significantly different.

Amine Bahi

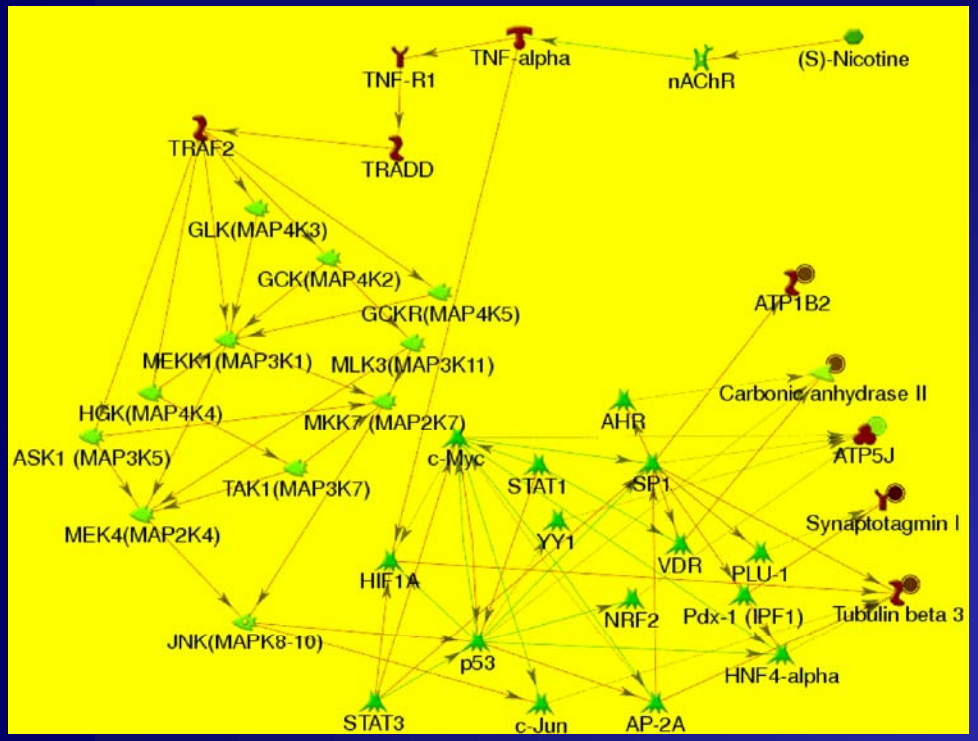
VTA

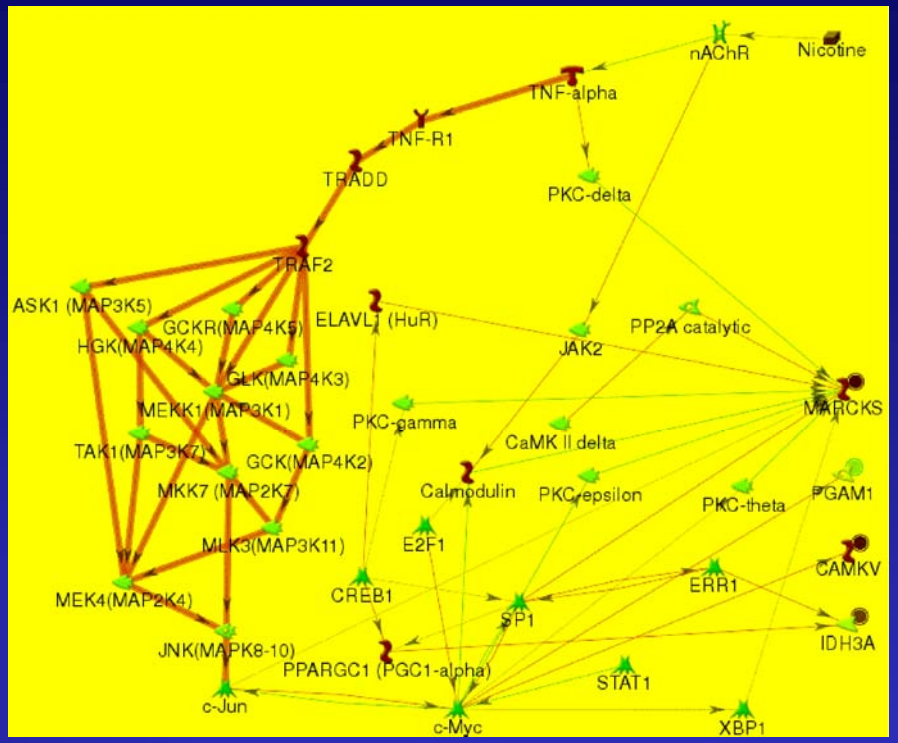
Protein name	Gene name	ratio	Fold change
synaptotagmin 1	Syt1	0.45	-2.24
Tubulin beta-3	Tubb3	0.61	-1.65
Sodium/potassium-transporting ATPase subunit beta-2	Atp1b2	0.71	-1.41
Carbonic anhydrase 2	Car2	0.72	-1.38
ATP synthase coupling factor VI	Atp5j	1.60	1.60

NAc

Myristoylated alanine-rich C-kinase substrate	Marcks	0.49	-2.06
CaM kinase-like vesicle-associated protein	Camkv	0.51	-1.95
Isoform 1 of Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial precursor	Idh3a	0.74	-1.35
Phosphoglycerate mutase 1	Pgam1	1.39	1.39

Pathway analysis (C. Bruce)



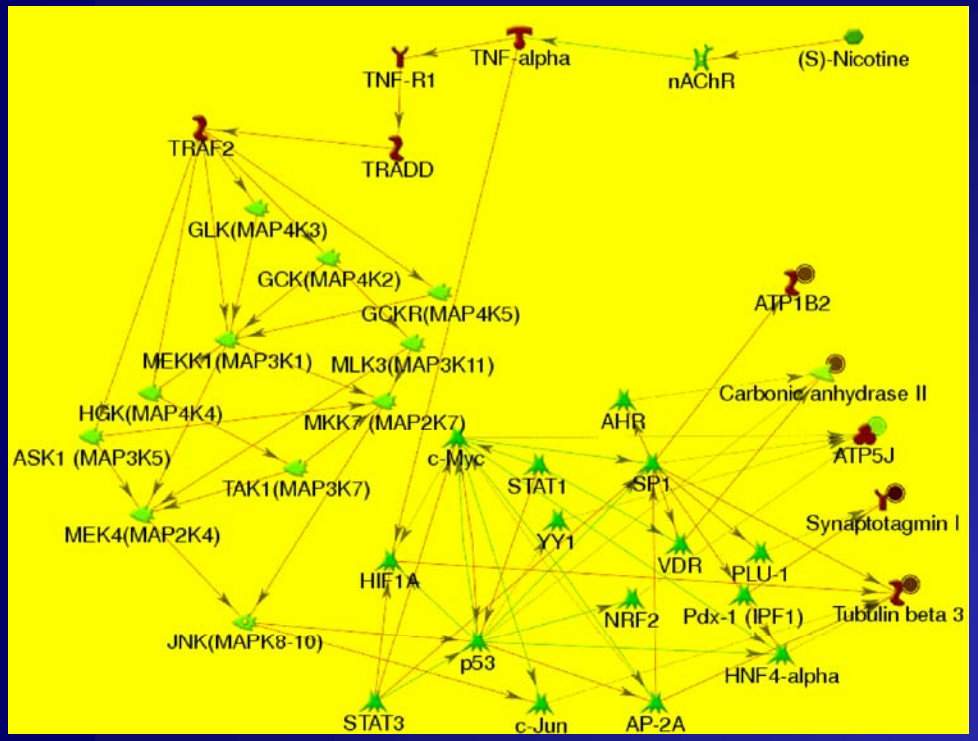


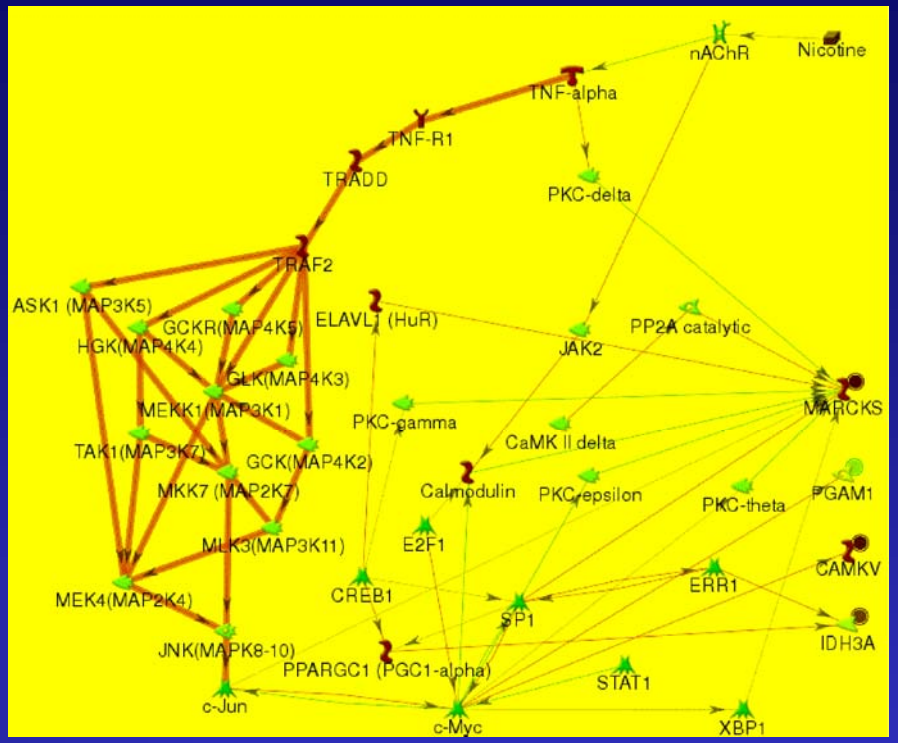
VTA NAc

Three out of five of the differentially regulated proteins in VTA and 3 out of 4 in the NAc are transcriptionally regulated by SP1. The probability of this enrichment is 0.003, suggesting that SP1's involvement with these proteins is unlikely to be due to chance.

Can Bruce

Pathway analysis (C. Bruce)





VTA

NAc

Calcineurin can regulate the activity of the SP1 complex by dephosphorylating cJun and modulating the association between SP1 and cJun.

Can Bruce

Phosphoproteins in VTA and NAc

Blue shading:

Total levels unchanged but phosphorylated peptide significantly changed.

VTA		Nic /Sal	P *
Ina Alpha-internexin	STEAIRASREEIHEYRRQLQAR	**1.87**	**0.043**
	QLQARTIEI	**1.08**	0.648
	RLPASDGLDLSQAAAR	**0.84**	0.535
	18 Unphos. peptides	**1.10**	0.350
Hspd1 Isoform 1 of 60 kDa heat shock protein, mitochondrial precursor	QMRPVSR	**1.34**	0.006
	2 Unphos peptides	**1.19**	0.341
Atp2b1 plasma membrane calcium ATPase 1	ISTIPTSRLK	**1.37**	**0.0001**
	3 unphos. peptides	**1.22**	0.270
Aldh5a1 Succinate-semialdehyde dehydrogenase, mitochondrial precursor	MATCFLLRSFWAAR	**1.46**	**0.011**
	1 Unphosphorylated peptide	**1.02**	0.896
spectrin beta 3	AASAGVPYHGEVPVSLAR	**2.07**	0.095
	GLTRAMTMPPVSQPEGSIVLR	**1.14**	0.789
	QTLPRGPAPSPMPQSR	**1.17**	0.561
	(no unphos peptides detected)	-	-
NAc			
Ina Alpha-internexin	RLPASDGLDLSQAAAR	**1.53**	0.031
	QLQARTIEI	**1.07**	0.591
	STEAIRASREEIHEYRRQLQAR	**1.57**	0.084
	18 Unphos. peptides	**0.88**	0.962
spectrin beta 3	AASAGVPYHGEVPVSLAR	**1.57**	-
	GLTRAMTMPPVSQPEGSIVLR	**0.88**	0.649
	QTLPRGPAPSPMPQSR	**1.00**	0.994
	(no unphos peptides detected)	-	-

Can Bruce

Questions and Future Directions:

1. Determine whether nicotine can regulate SP1.
2. Determine whether cJun and SP1 cooperate to regulate nicotine-dependent changes.
3. Identify a role for calcineurin in nicotine-dependent gene expression, potentially through regulation of the cJun-SP1 complex.

Ongoing projects for proteomics:

1) Calcineurin attenuates nicotine-induced cellular and behavioral plasticity: we want to find proteins that are dephosphorylated in VTA in response to nicotine sensitization or CPP.

2) CREB activity in NAc shell is necessary for nicotine reward: we want to look for proteins that are regulated in the NAc shell in response to nicotine CPP in WT but not beta 2 KO mice.

3) Developmental nicotine exposure or beta 2 KO result in hypersensitive passive avoidance learning due to effects on corticothalamic neurons. We have microarray data showing that the largest group of overlapping changes in expression with the hypersensitive PA is in cortex: we want to identify proteins in cortex and thalamus that are responsible for the permanent behavioral change resulting from developmental nicotine exposure.