Neuronal Subtype-Specific Effects of the Transcription Factor ΔFosB on Synaptic Physiology

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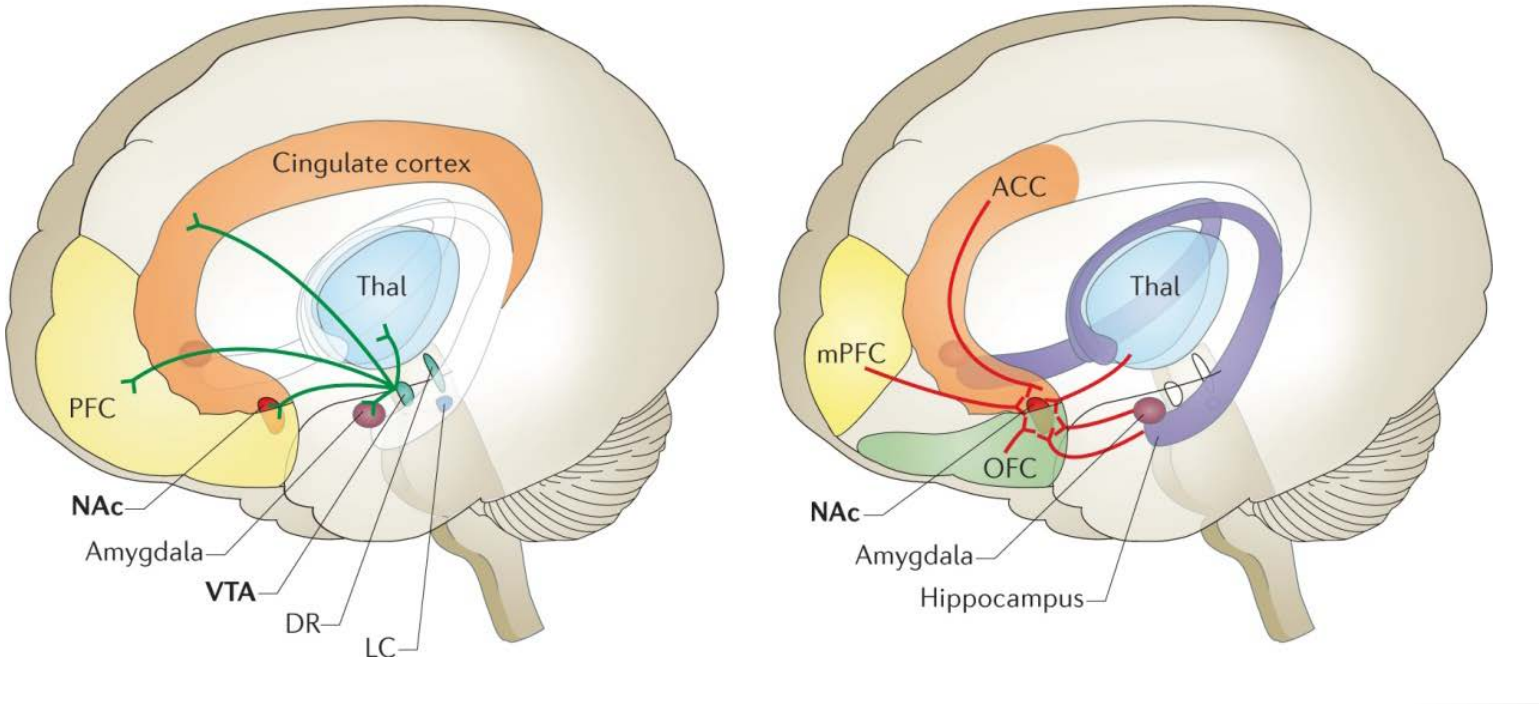
Drug Addiction

-Addiction can be best defined as the loss of control over drug use, or the compulsive seeking and taking of drugs despite adverse consequences.

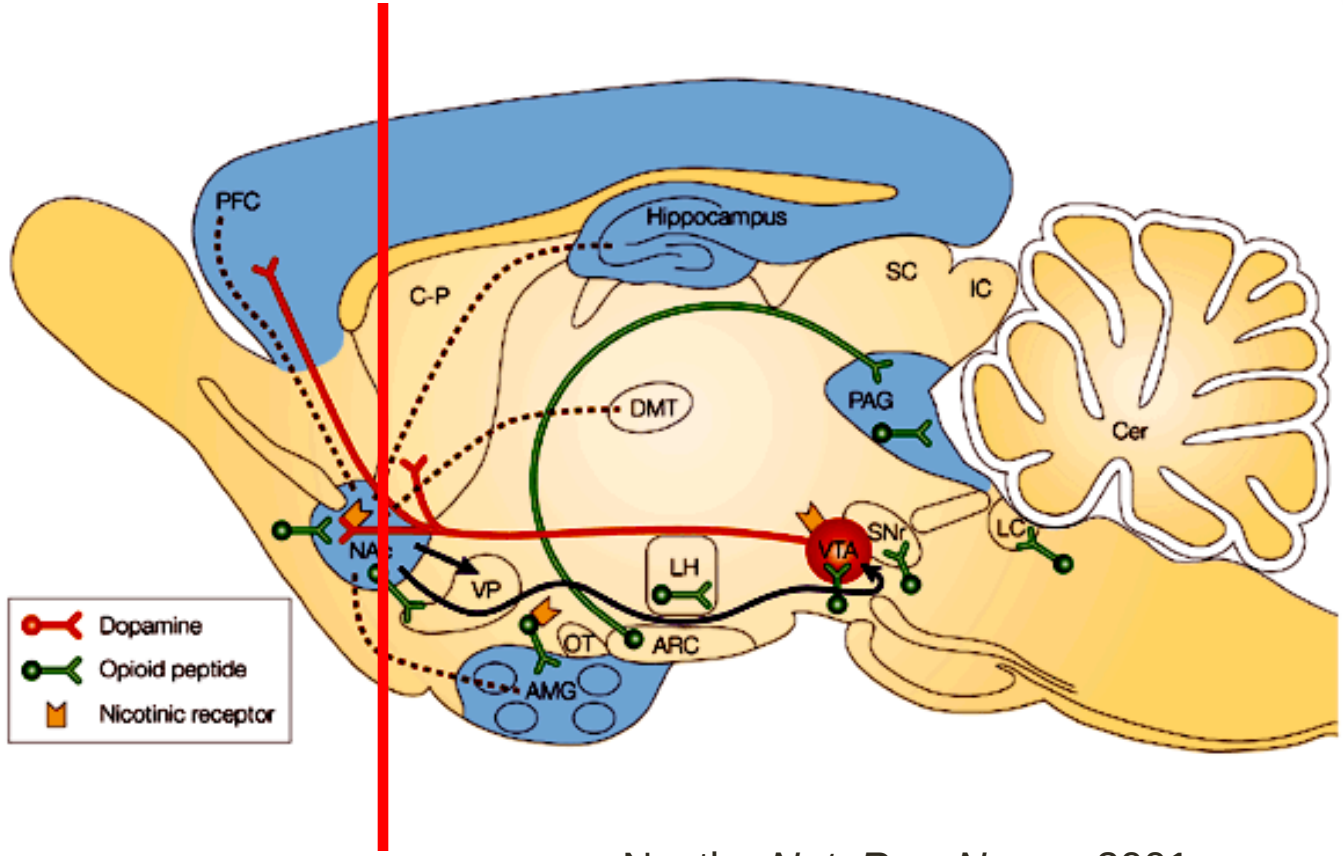
-Hypothesis: regulation of gene expression is one important mechanism by which chronic exposure to a drug of abuse causes long-lasting changes in the brain, which underlie the behavioral abnormalities that define a state of addiction.

Reward Circuitry



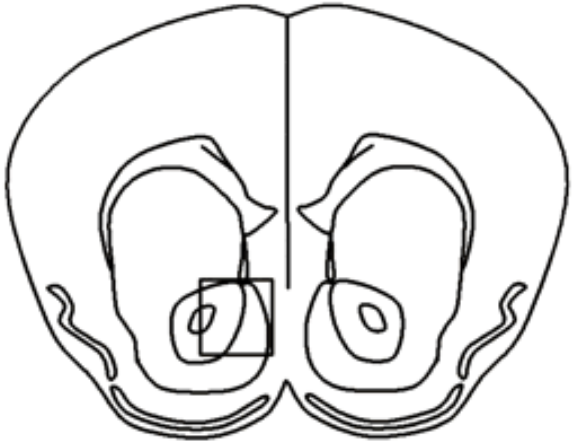
Robison & Nestler *Nat. Rev. Neuro.* 2011

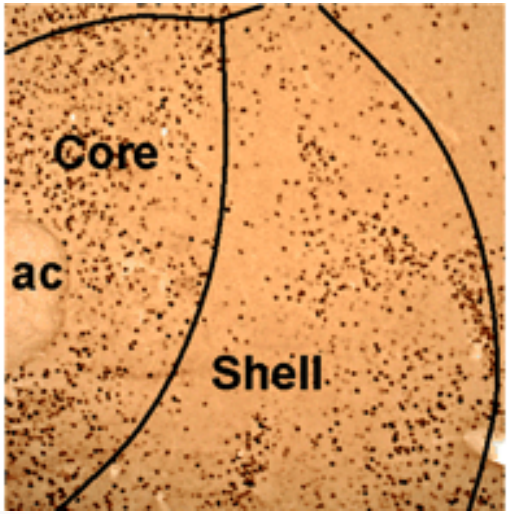
Reward Circuitry



Nestler *Nat. Rev. Neuro.* 2001

NAc Shell and Core





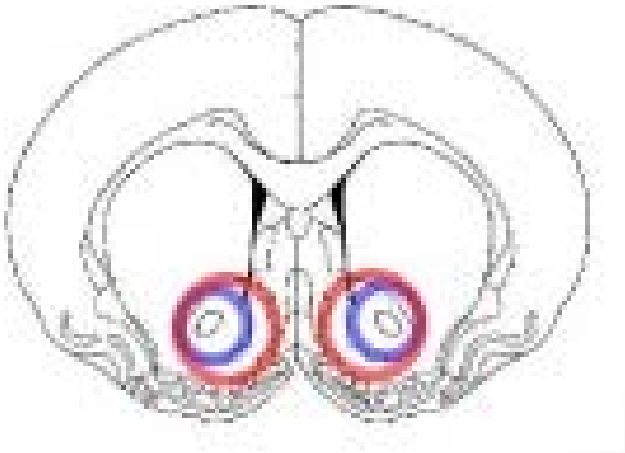
-Shell and core differ:

-Anatomically

-Biochemically

-Electrophysiologically

-Behaviorally



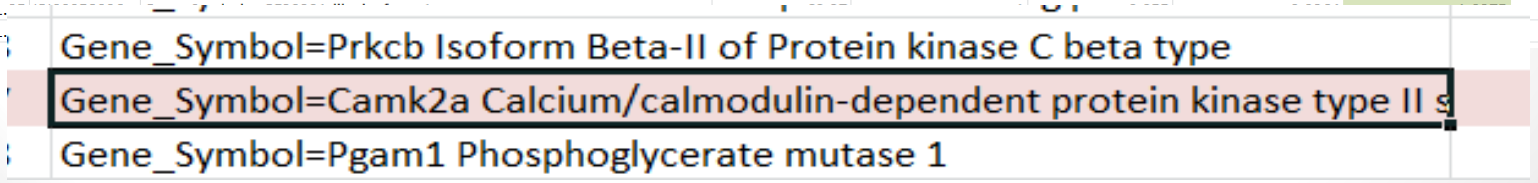
NAc Shell and Core

iTRAQ Unbiased Analysis of Proteome

Utilizes a multiplexed isobaric chemical tagging reagent which allows multiplexing of two to eight protein samples and produces identical MS/MS sequencing ions for all eight versions of the same derivatized tryptic peptide.

-Quantified 1735 proteins
-Compared chronic cocaine to saline treatment in both NAc shell and core

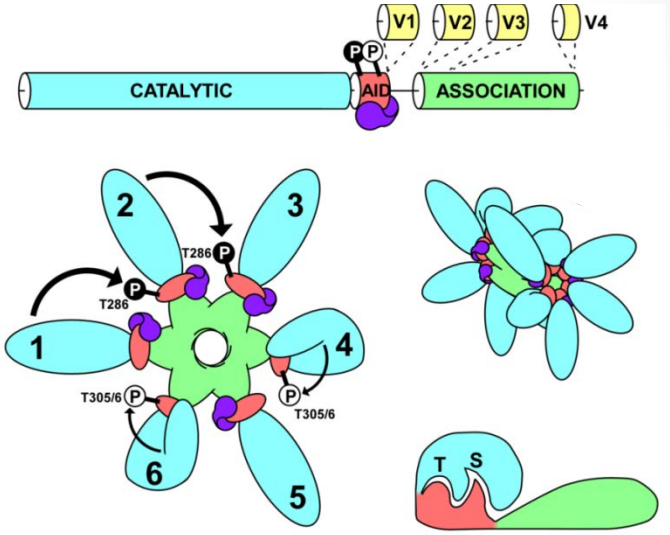
22.13	IPI00189278	Gene_Symbol=Prkcb Isoform Beta-II of Protein kinase C beta type	39.08	7	1.7378	0.1701	1.8197
22.1	IPI00192337	Gene_Symbol=Camk2a Calcium/calmodulin-dependent protein kinase type II s	64.44	9	3.9446	0.9133	4.4463
21.69	IPI00421428	Gene_Symbol=Pgam1 Phosphoglycerate mutase 1	61.02	10	1.7539	0.1657	1.6904
21.6	IPI00763802	Gene_Symbol=Cyfip2 Putative uncharacterized protein Cyfip2	24.94	14	1.5417	0.0785	1.3932
21.46	IPI00324741	Gene_Symbol=Pdia3 Protein disulfide-isomerase A3	39.21	11	1.2589	0.492	1.2359
21.44	IPI00231302	Gene_Symbol=Nefl Neurofilament light polypeptide	45.57	13	2.0324	0.0225	0.4656

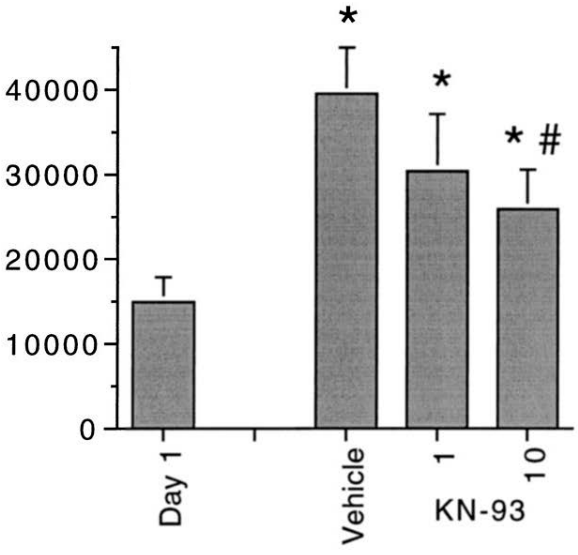


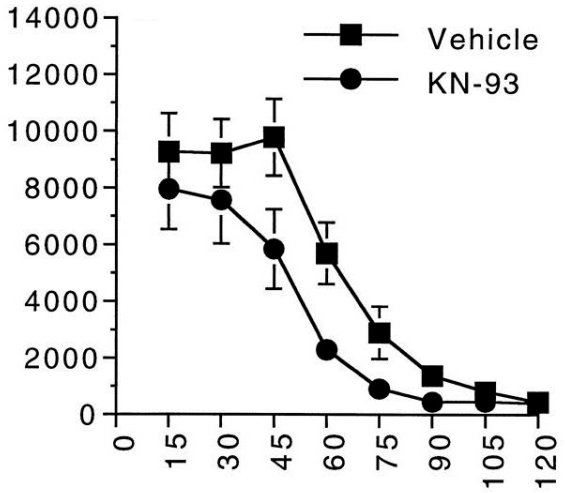
Calcium/Calmodulin-Dependent Protein Kinase II

-Encoded by 4 genes (α, β, γ, δ), multiple splice variants and modifications

-Important for AMPA receptor function, spine formation, synaptic plasticity, memory, and **DRUG RESPONSES**







Pierce et al., *JPET* 1998

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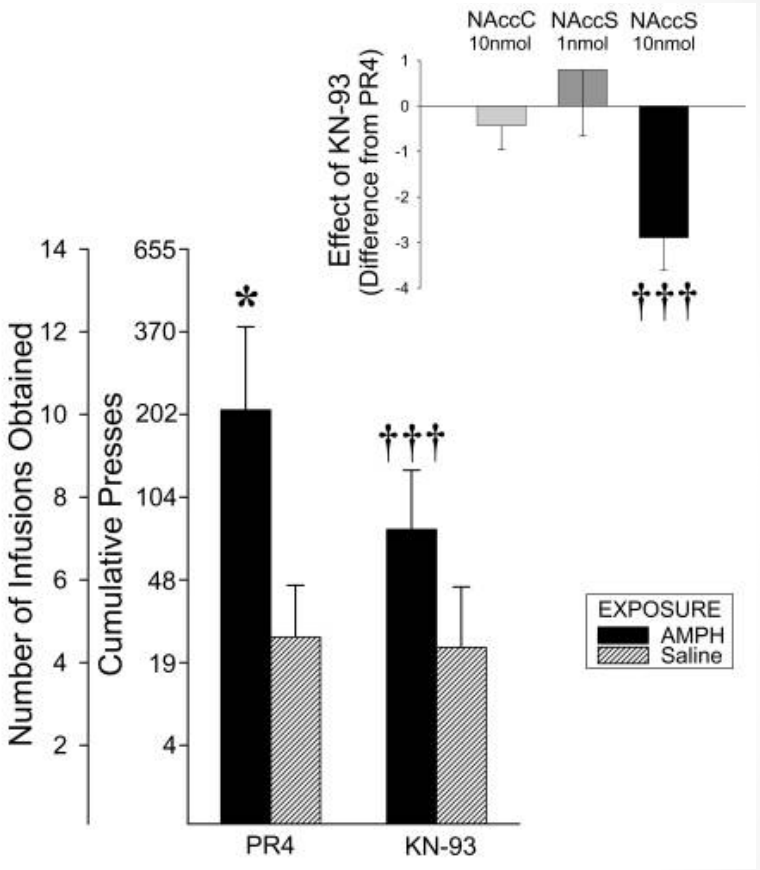
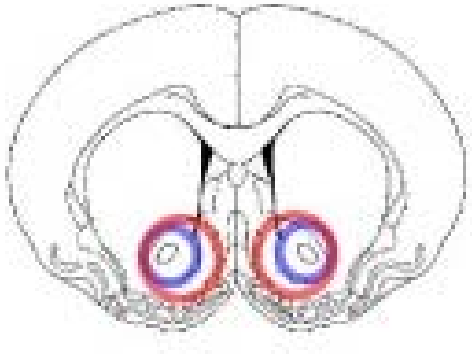
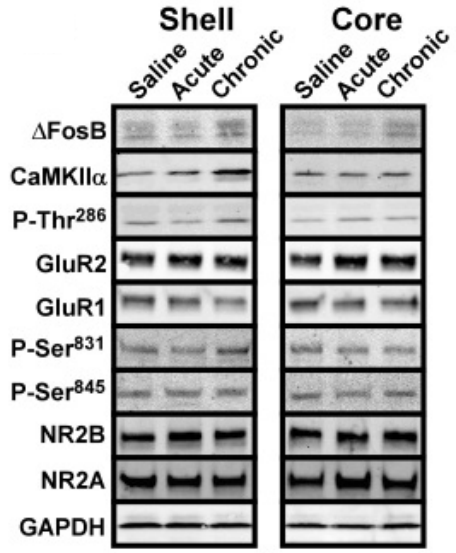
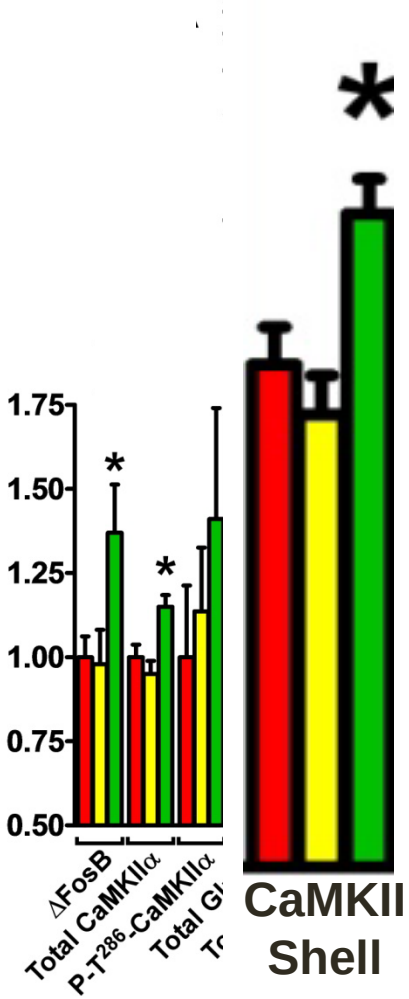


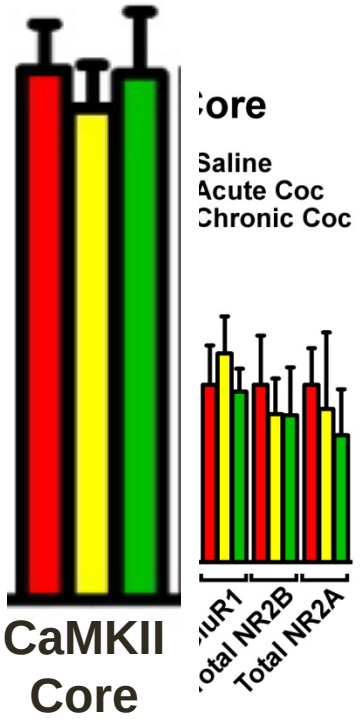
Loweth et al., *Neurosci. Let.* 2008

Does Cocaine Regulate CaMKII Espression?



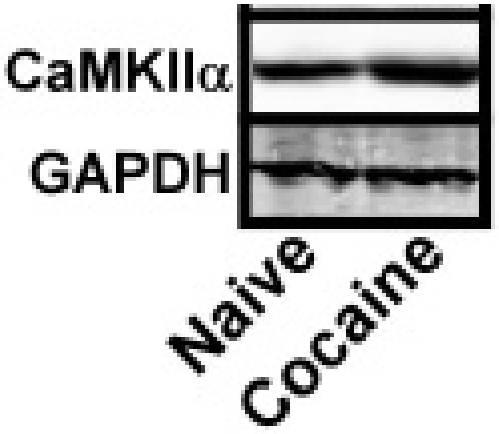


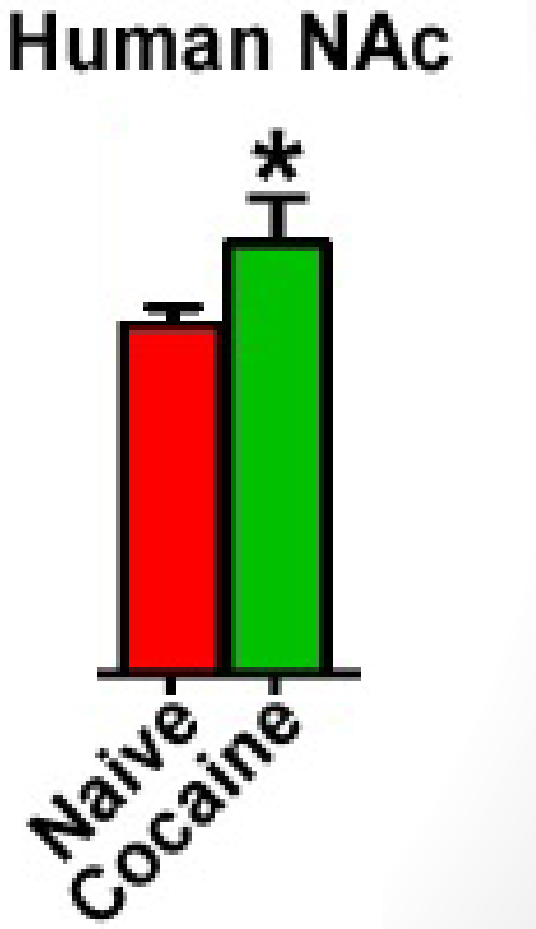




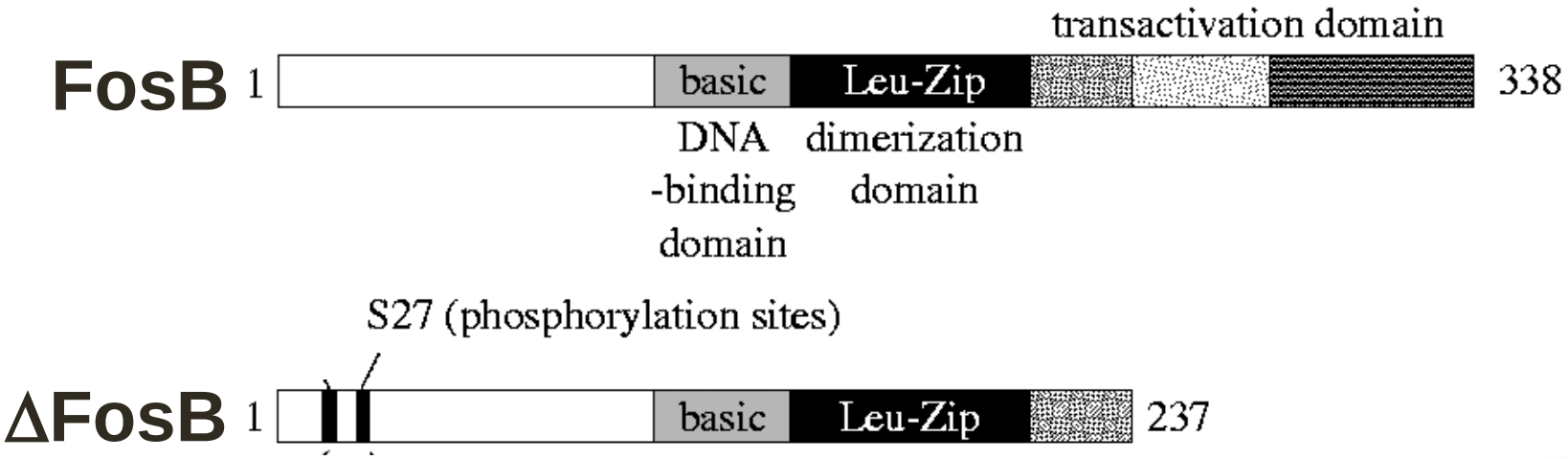
Does Cocaine Regulate CaMKII Espression?

-Accumbens from human cocaine addicts and matched controls
-Canadian population from Gustavo Turecki





ΔFosB

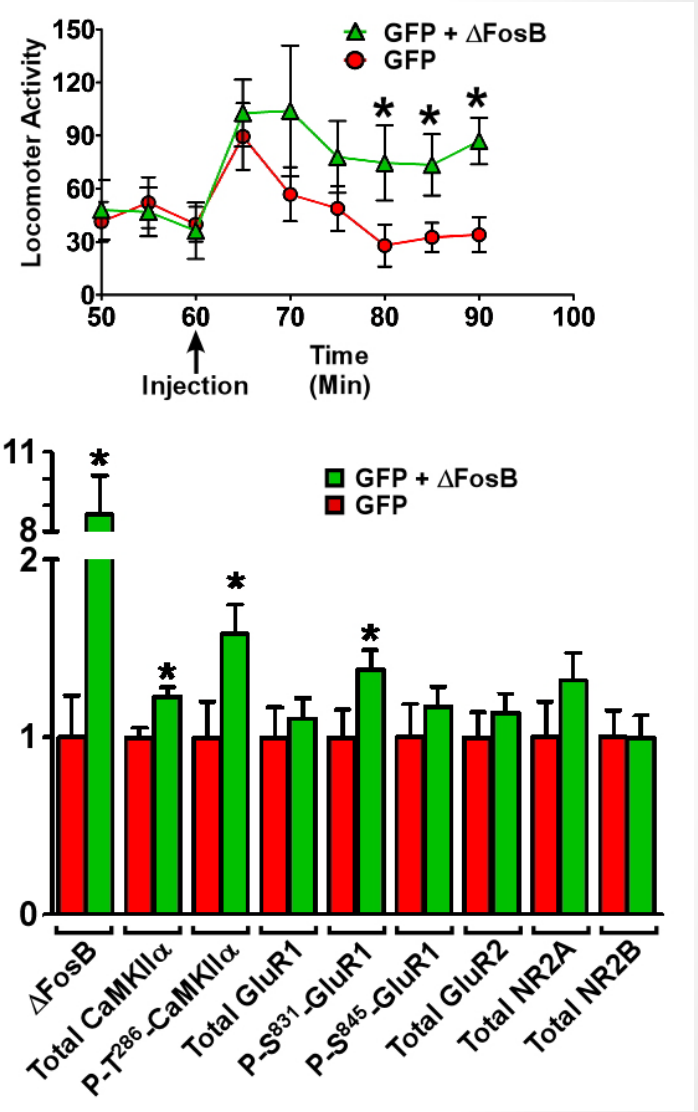


Nestler, 2008

- Encoded by FosB gene
- Fos proteins heterodimerize with Jun proteins to make AP-1 complexes
- Bind to AP-1 recognition sites in promoter regions of a variety of genes

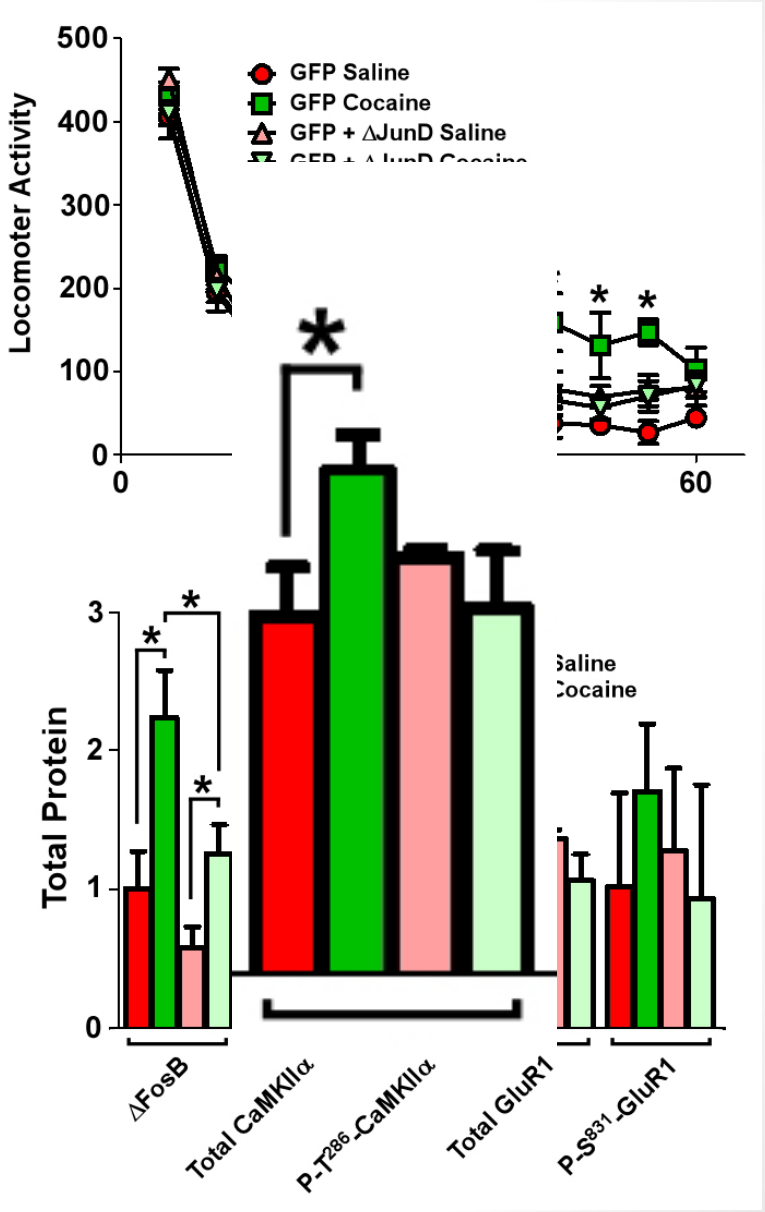
Does ΔFosB Mediate Increases in CaMKII *in vivo*?

-Use AAV to overexpress GFP and ΔFosB or GFP alone into rat NAc shell
-Observe cocaine-driven locomoter activity
-Punch green area from brain slice and Western Blot for proteins of interest



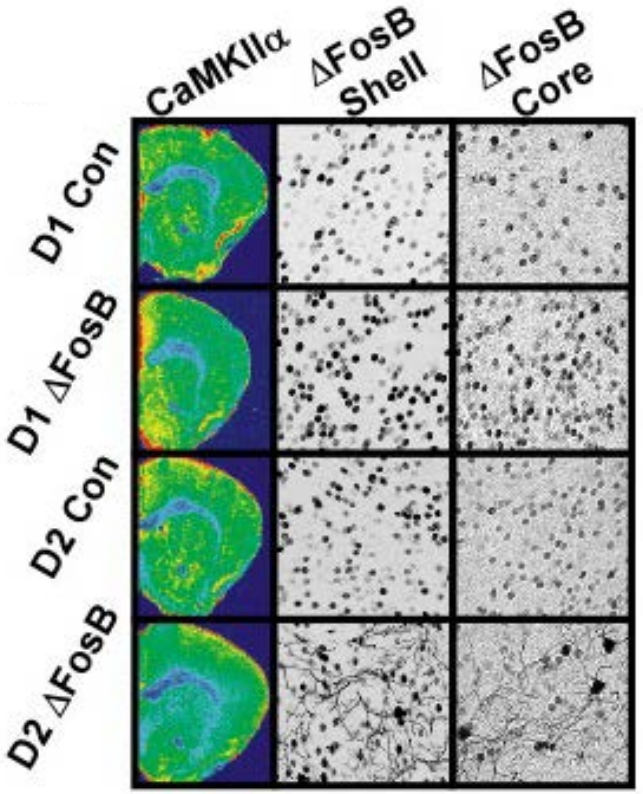
Is Cocaine Regulation of CaMKII ΔFosB-Dependent?

-Use AAV to overexpress GFP and ΔJunD or GFP alone into rat NAc shell
-Observe cocaine-driven locomoter sensitization
-Punch green area from brain slice and Western Blot for proteins of interest



Is ΔFosB Induction of CaMKII Cell-Type Specific?

-Transgenic mice overexpress ΔFosB in D1 or D2 neurons for 8 weeks
-Perfuse and count FosB positive cells
-Quantify CaMKII by Licor



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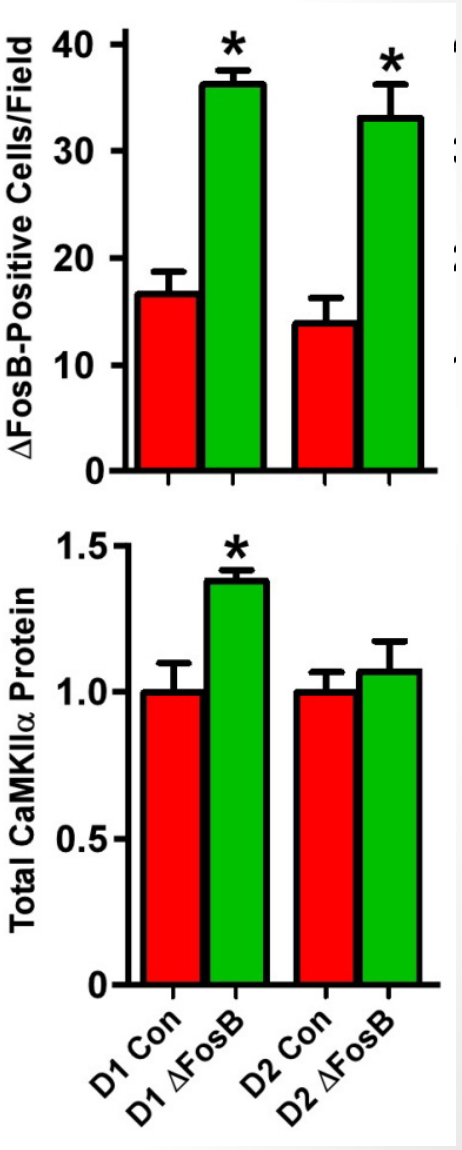
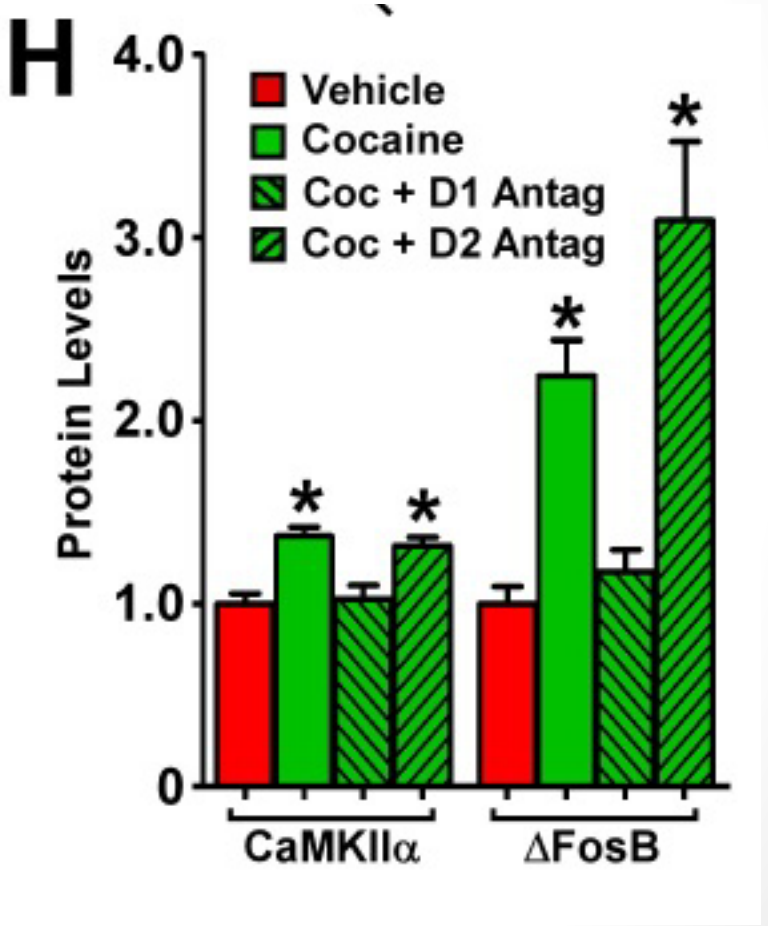


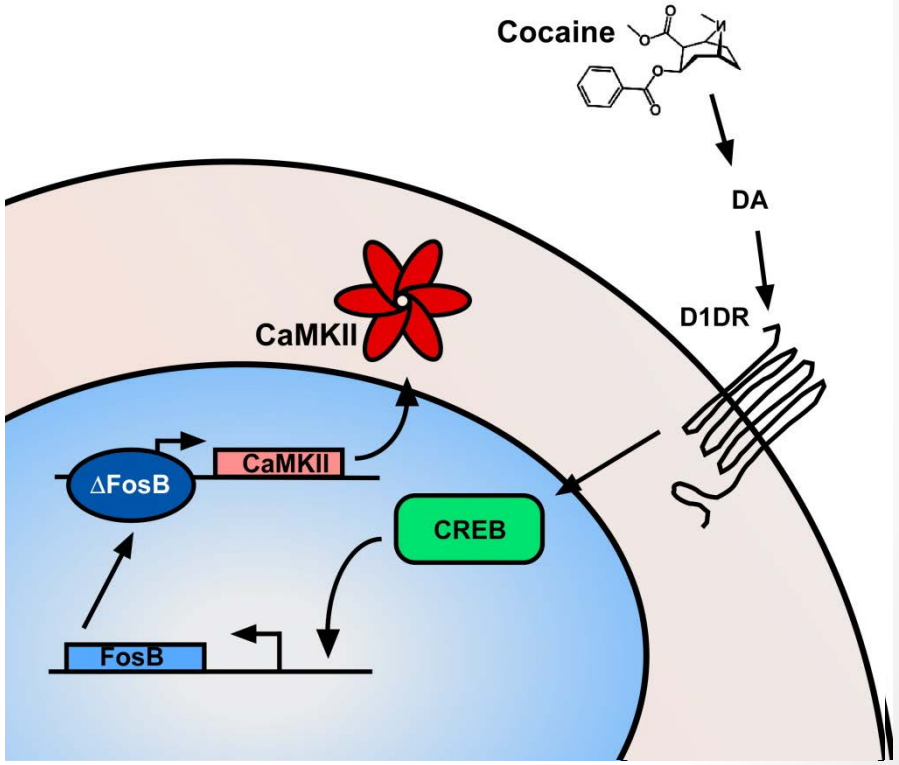
Does Cocaine Induction of CaMKII Require D1DR?

-Adult male rats injected 7 days 20 mg/kg cocaine ip
-30 min before cocaine, injected 0.5 mg/kg SCH 23390 or eticlopride

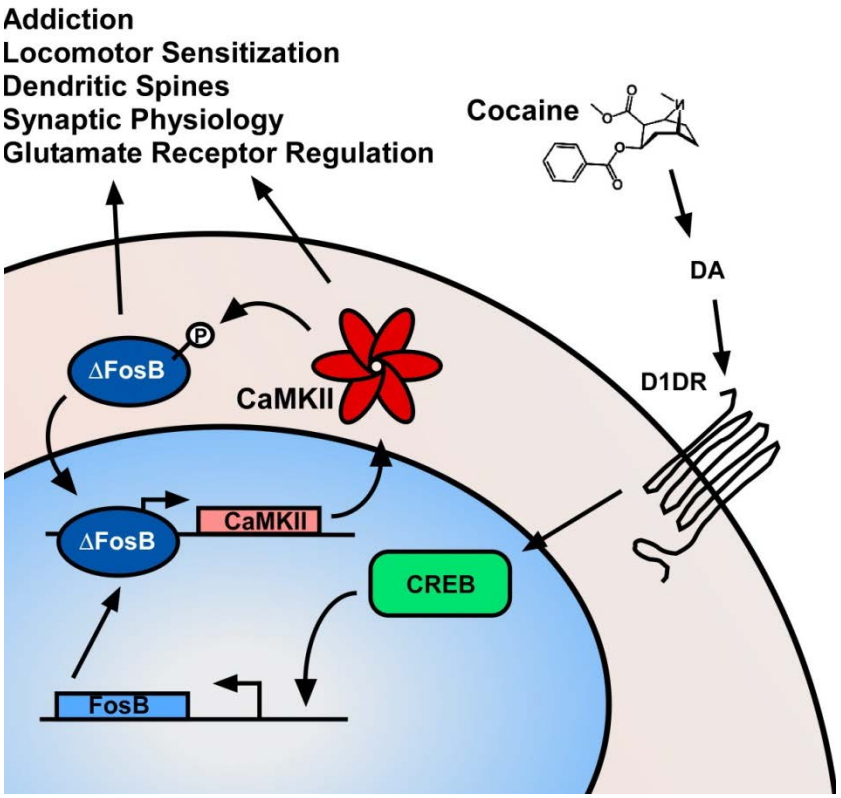


Summary I

- CaMKII is induced in the NAc shell by a variety of chronic cocaine exposure paradigms.
- ΔFosB is necessary and sufficient for this induction.
- ΔFosB binds the CaMKIIα promoter and induces CaMKII in NAc shell but not core.
- D1 vs D2 specificity.



Current Model



D1 vs D2 specificity

Proteomics of D1-specific ΔFosB Expression

iTRAQ Unbiased Analysis of Proteome

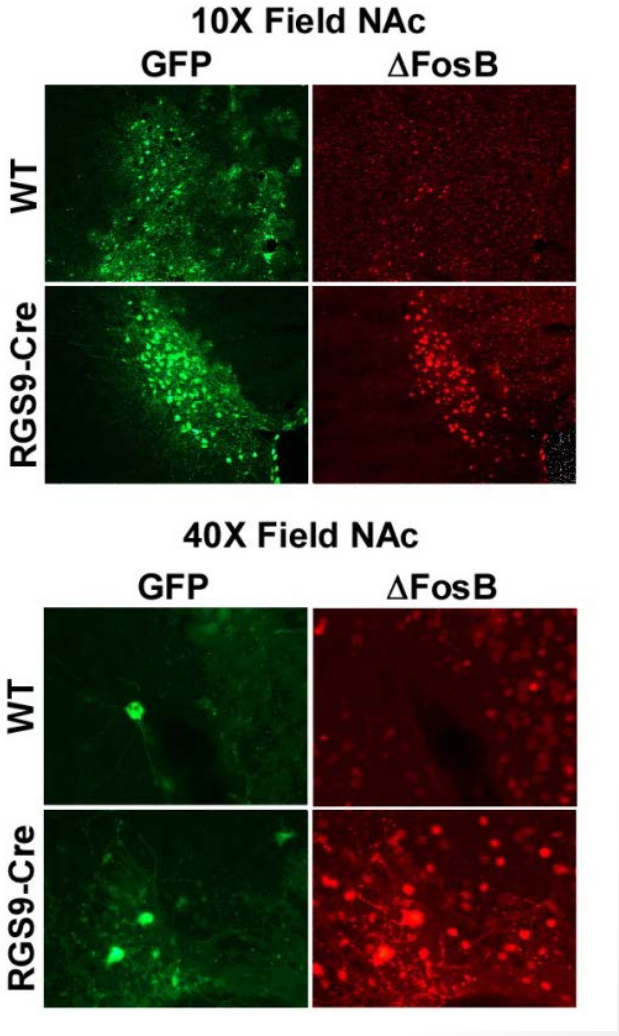
NAc isolated from mutant mice expressing ΔFosB specifically in D1 MSNs for 8 weeks.

-Used 4 biological replicates from each condition
-Quantified 1185 proteins
-Many regulated proteins were associated with the synapse

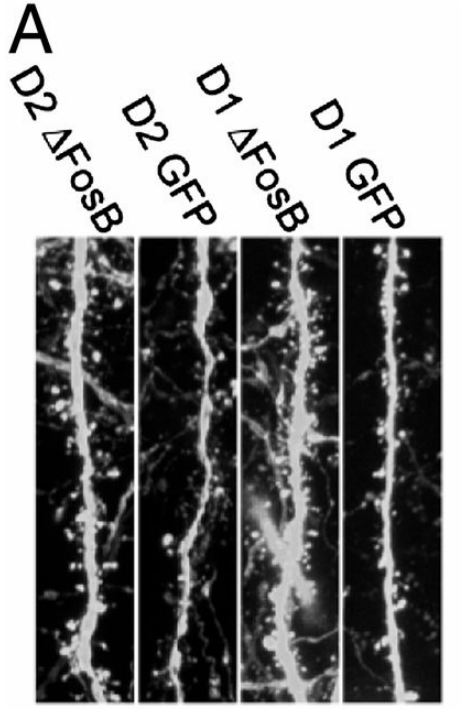
Synaptic Regulatory Proteins
Syntaxin-12
Glutamate receptor 2
CB1 cannabinoid receptor-interacting protein 1
cAMP-dependent protein kinase type I-alpha regulatory subunit
cAMP-dependent protein kinase type II-alpha regulatory subunit
Casein kinase II subunit beta
Calcium/calmodulin-dependent protein kinase type II subunit alpha
Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit gamma isoform
Calmodulin
cAMP-dependent protein kinase type II-beta regulatory subunit

What are ΔFosB's Cell-Type Specific Effects?

-HSV-GFP-Lox-Stop-ΔFosB
-Makes GFP in all infected cells
-Makes ΔFosB only in Cre-positive cells
-Use D1-Cre and D2-Cre to achieve specific expression



ΔFosB Induces Immature Dendritic Spines only in D1 Cells



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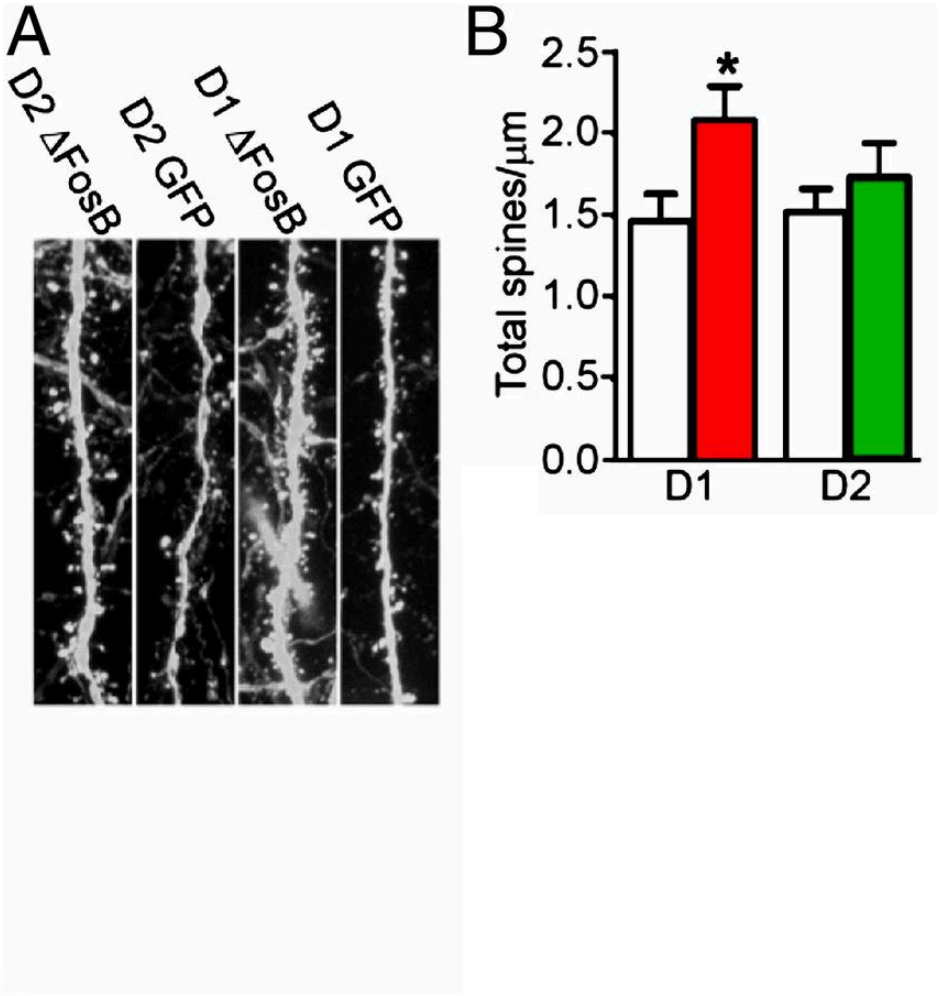

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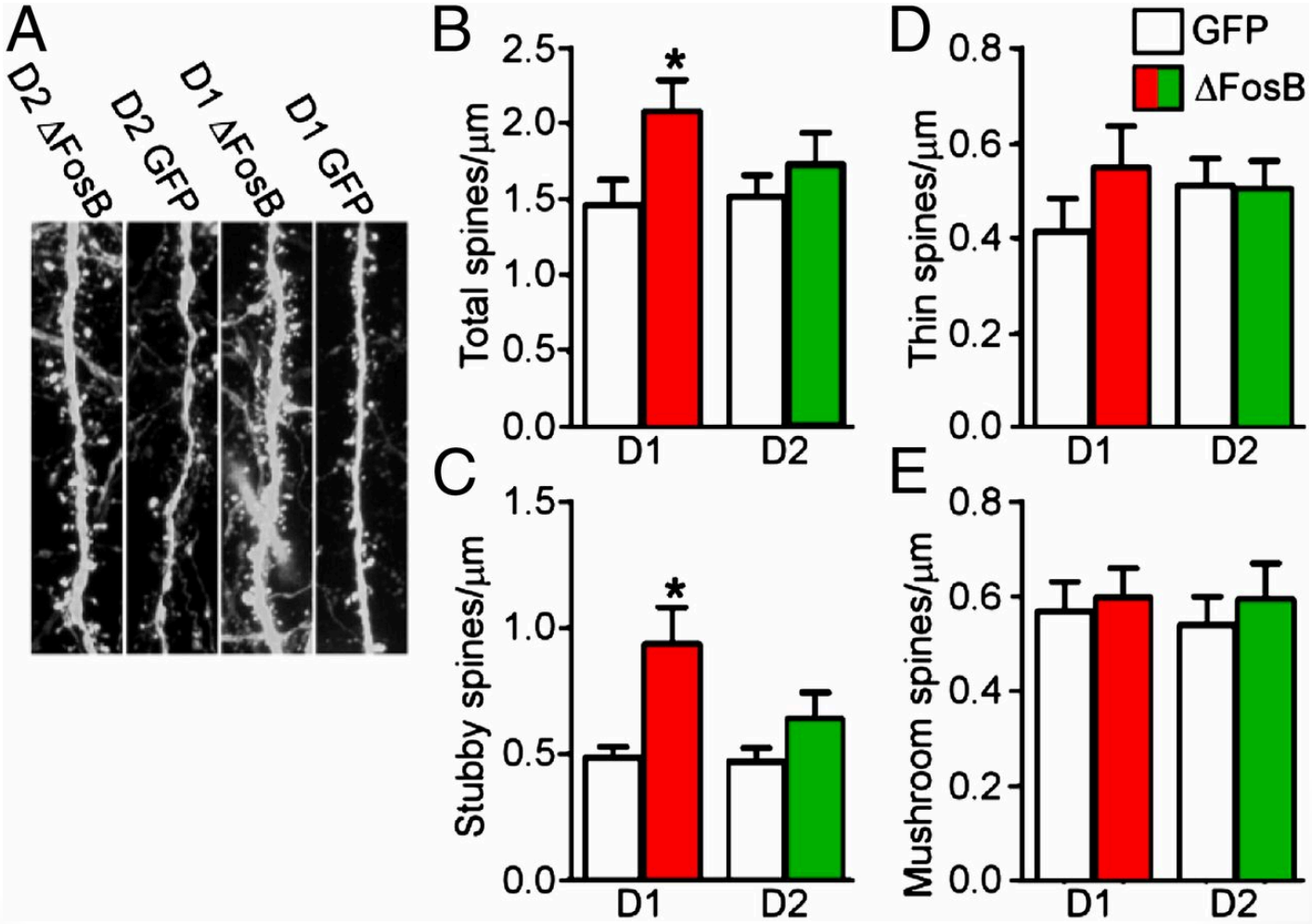
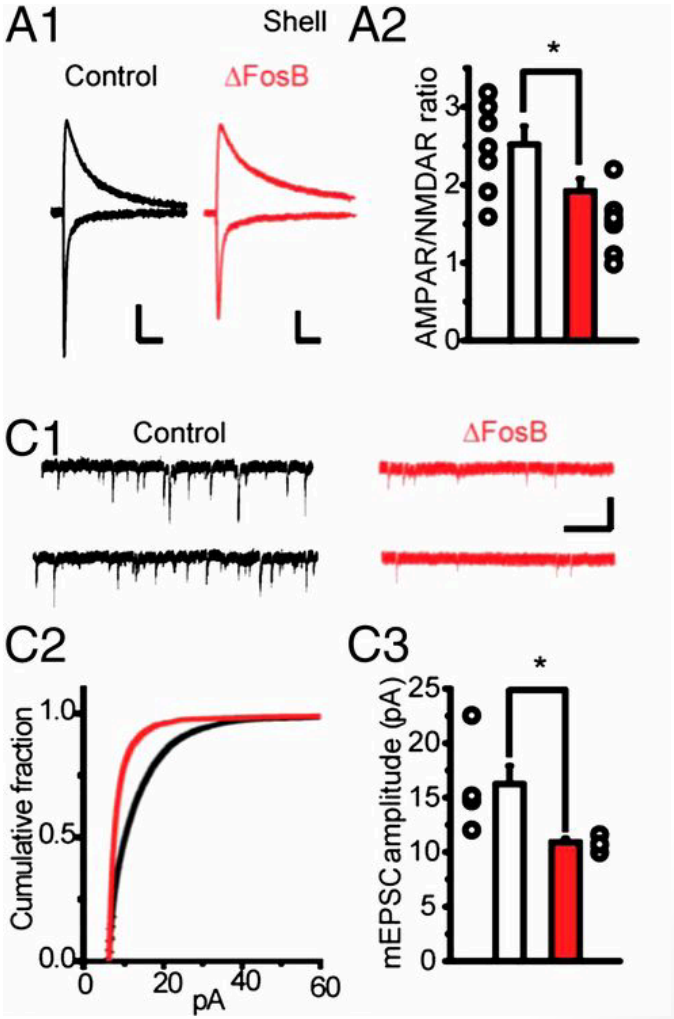


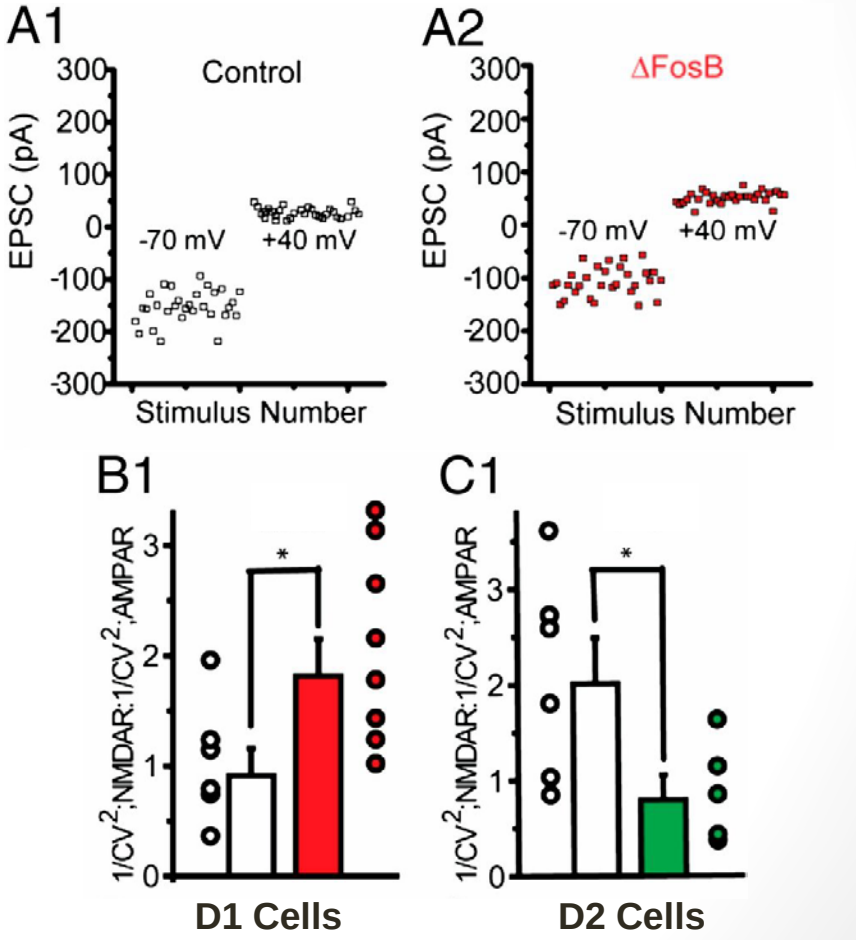
ΔFosB Decreases Average Synaptic Strength in D1 Cells

-HSV-GFP-ΔFosB in D1- or D2-Tomato mice
-Record synaptic function from ΔFosB and control cells
-AMPA/NMDA ratio indicates strength and number of functional synapses
-mEPSC amplitude indicates synaptic strength



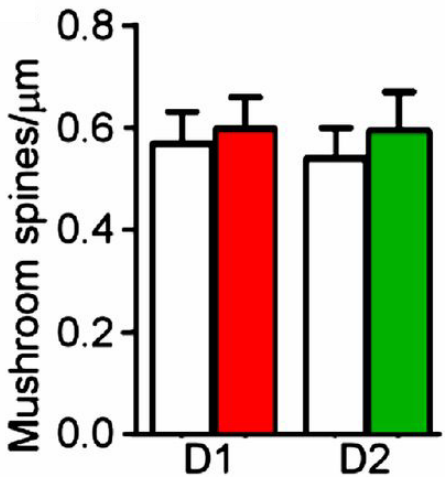
ΔFosB Increases Silent Synapses only in D1 Cells

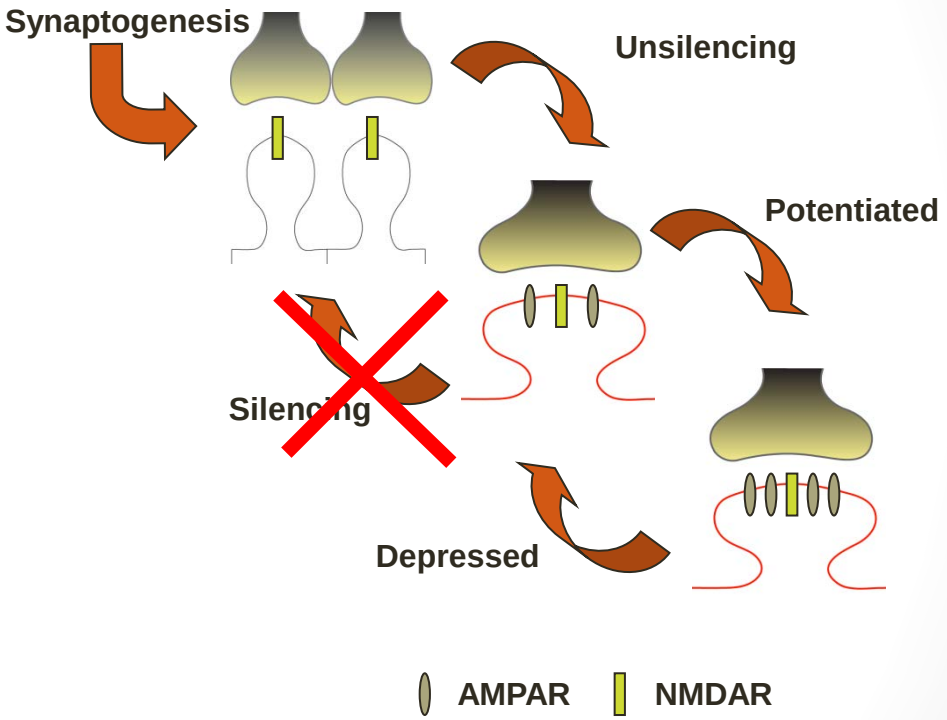
-HSV-GFP-ΔFosB in D1- or D2-Tomato mice
-Record synaptic function from ΔFosB and control cells
-Ratio of $1/CV^2$ for NMDA and AMPA receptors directly correlates to number of silent synapses



ΔFosB Increases Silent Synapses only in D1 Cells

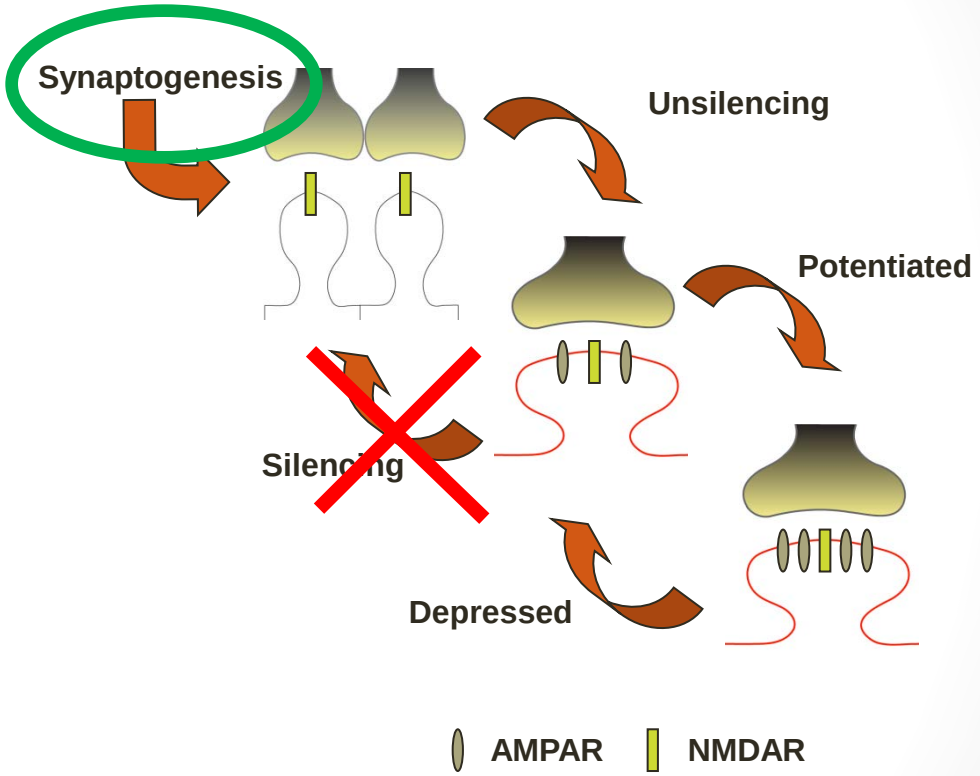
-Silencing of existing synapses?





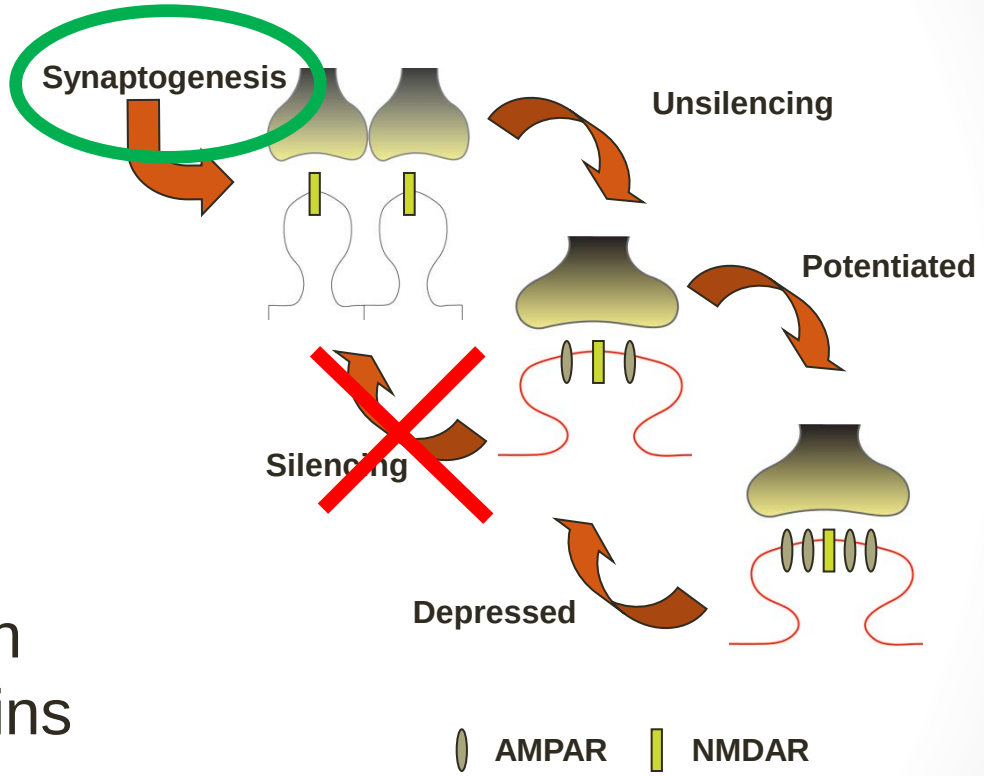
ΔFosB Increases Silent Synapses only in D1 Cells

-Silencing of existing synapses?
-Increased synaptogenesis?



ΔFosB Increases Silent Synapses only in D1 Cells

-Silencing of existing synapses?
-Increased synaptogenesis?
-iTRAQ:
- -↑ synaptojanin
- -↑ synaptopodin
- -↑ myelin proteins
- -↓ neurexin-1



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- Vincent Vialou
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NIDA
NATIONAL INSTITUTE ON DRUG ABUSE

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