Molecular Correlates of Cognitive Dysfunction in Animal Models of Addiction – Finding the Targets

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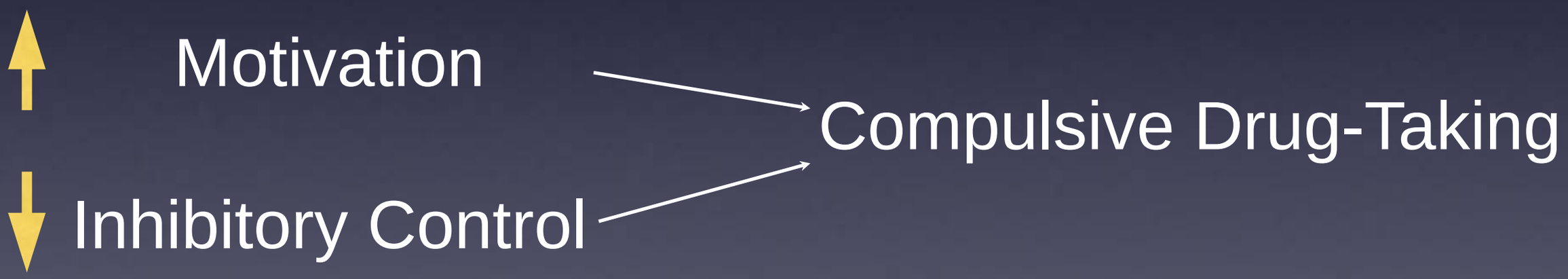
Impaired Cognitive-Motivational Processes in Addiction

Addicts display behavior characterized by:

- Cognitive/decision-making deficits
- Augmented reward-associated motivation



Jentsch & Taylor 1999; Olausson et al. 2007

Chronic Cocaine Exposure in Primates

Young adult Vervet monkeys

Treatment: Cocaine 2 mg/kg, 14 days

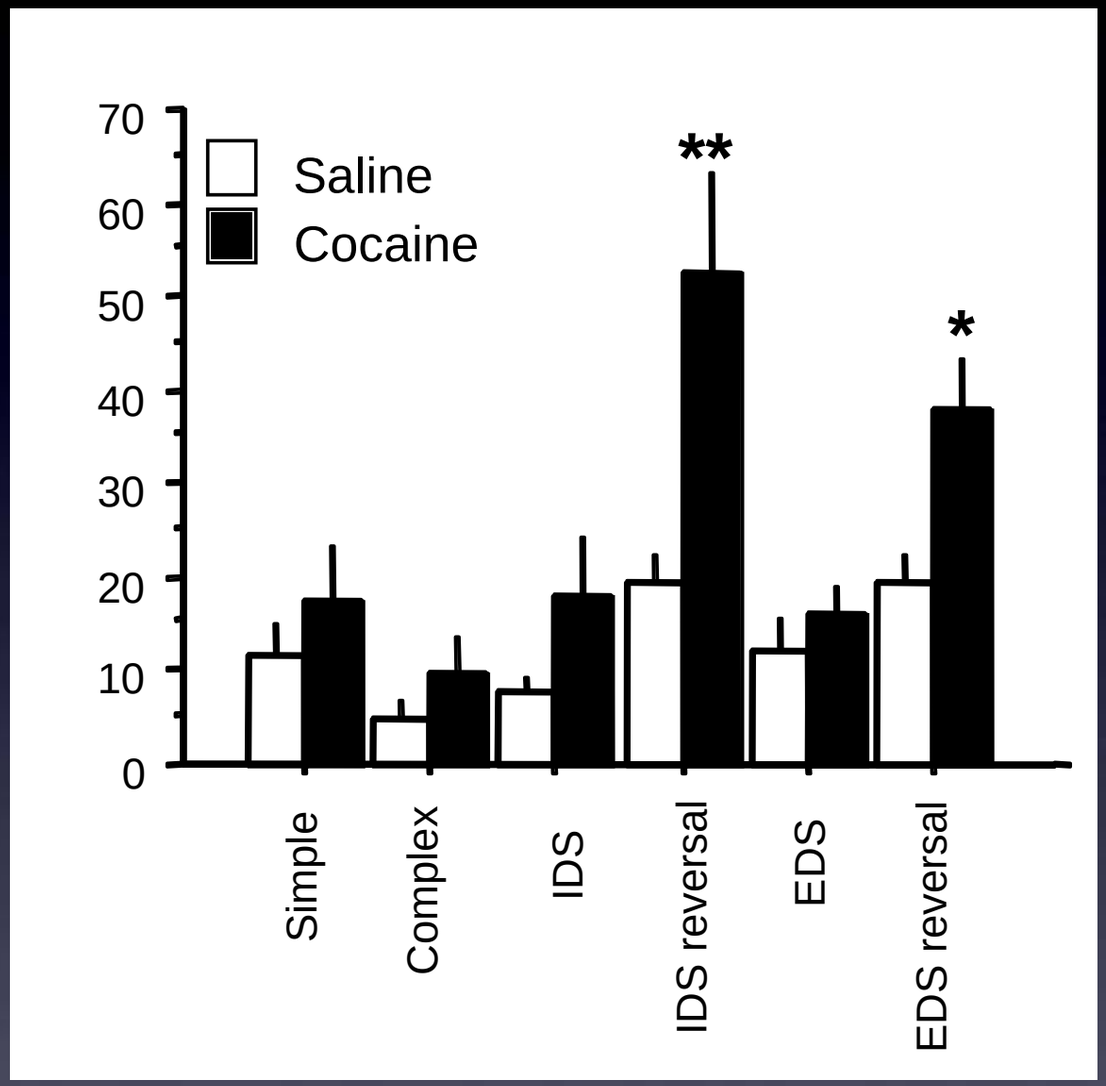
Behavioral testing (14-20 days after the last treatment):

Biochemistry:

- Animals sacrificed 21 days after final injection.
- Synaptosomes prepared from cortical and striatal regions.
- DIGE & iTRAQ analyses in biological triplicates

Cocaine-Induced Cognitive Deficits

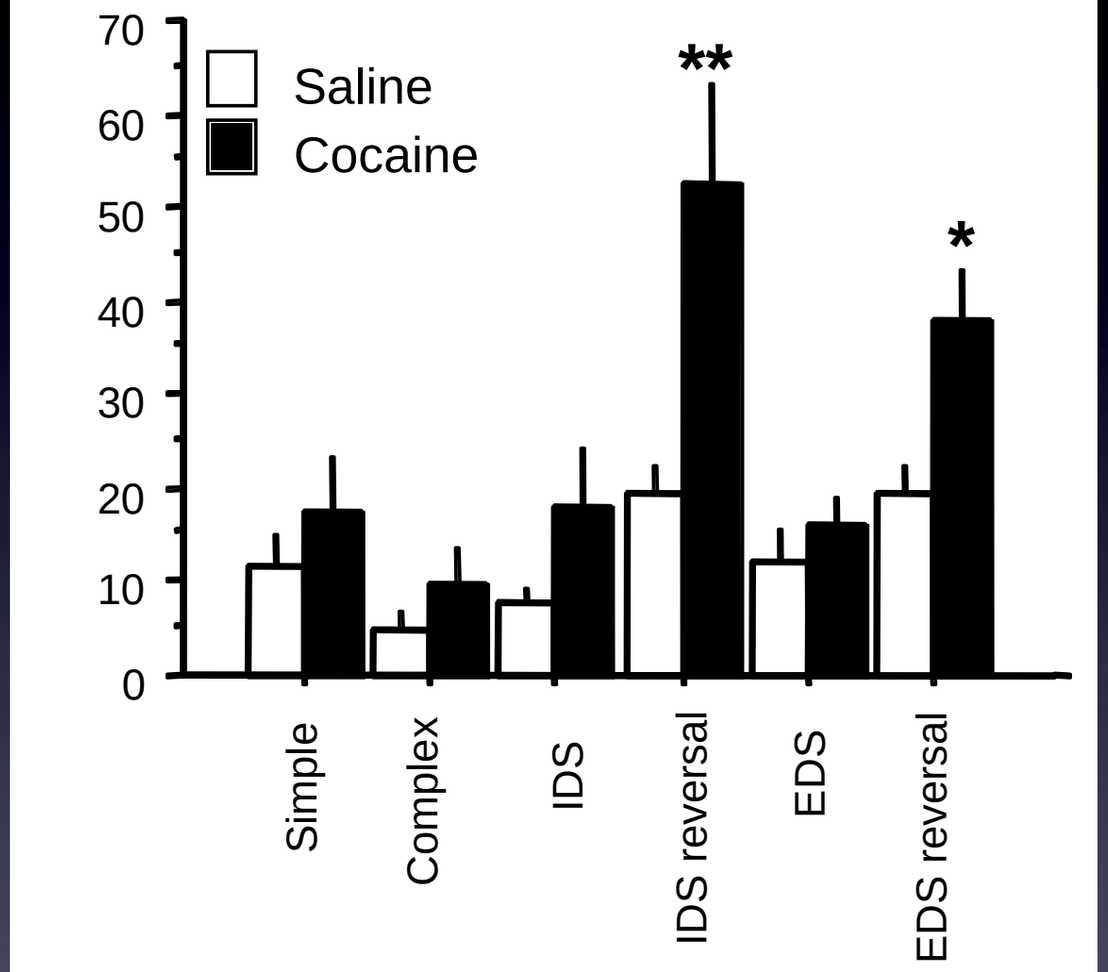
Attentional Set-Shifting (Wisconsin card sorting)

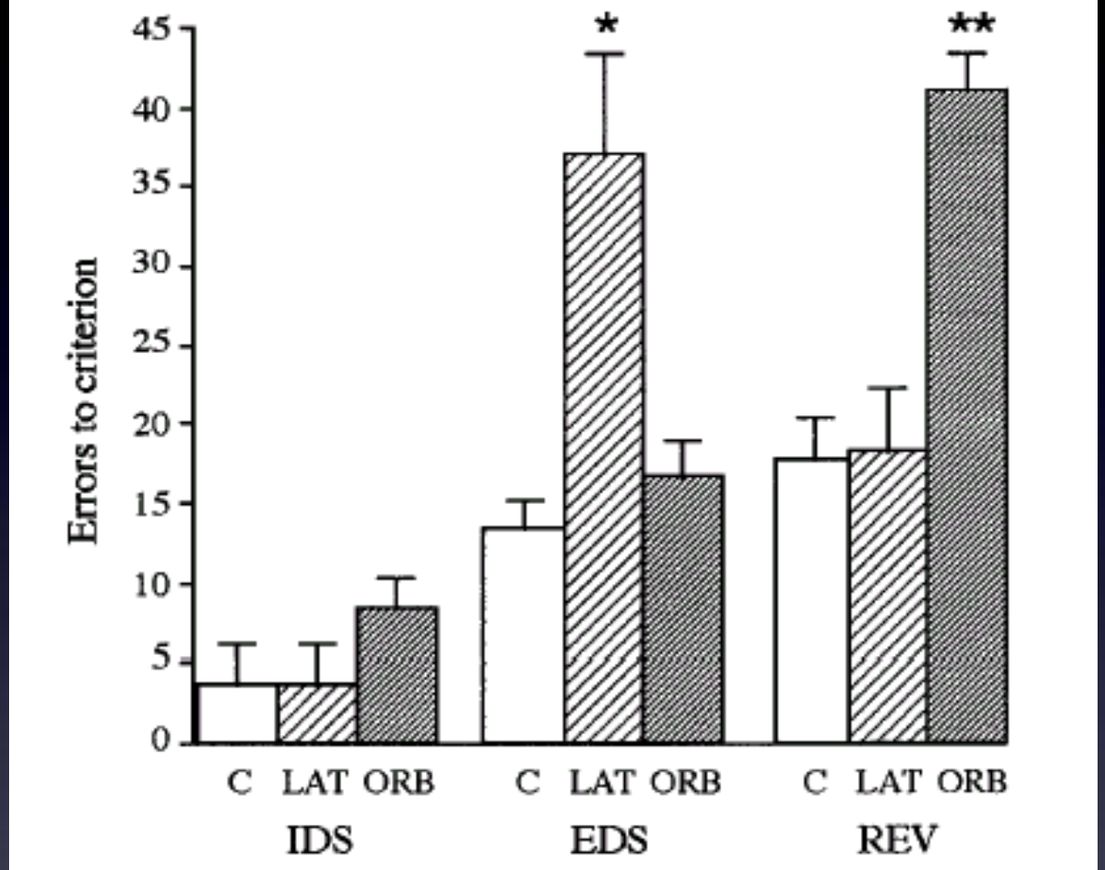


Prior chronic cocaine exposure impairs reversal learning

Cocaine-Induced Cognitive Deficits

Attentional Set-Shifting (Wisconsin card sorting)





Dias et al. 1996

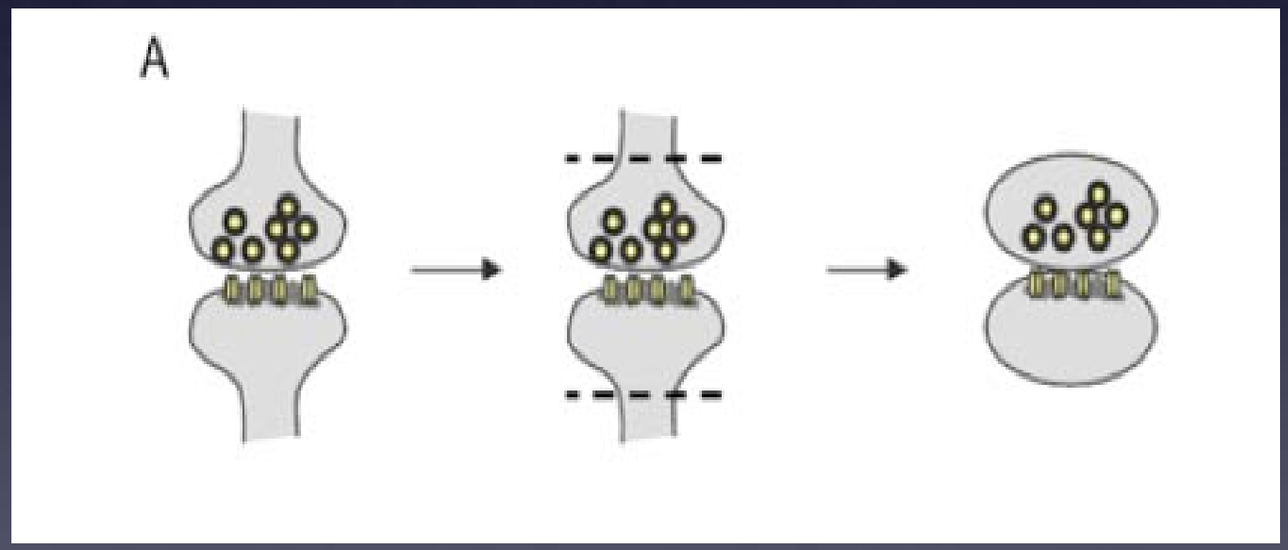
Prior chronic cocaine exposure impairs reversal learning

Cocaine-induced deficits are identical to those of OFC lesions

Effect of Cocaine on the Synaptic Proteome

Since the synapse is a critical site of plasticity and adaptation, we have analyzed synaptic protein expression using proteomic techniques

Synaptoneurosomes



Proteomic Analysis

DIGE and iTRAQ of Synaptoneurosomes

Cortical regions:

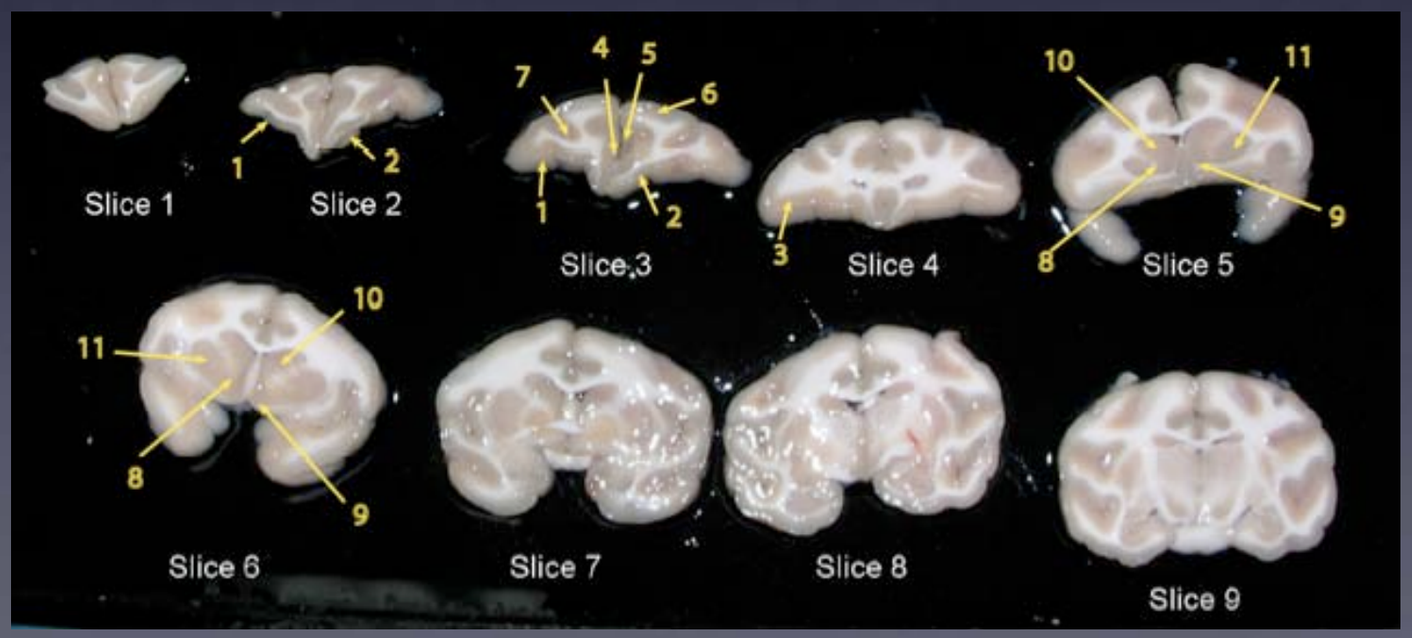
Area 11/12 (lateral OFC)
Area 13/14 (medial OFC)
PrCO/OFO (posterior OFC)
Area 24 (Anterior Cingulate)
Area 9/46 (Dorsolateral PFC)
Prelimbic/Infralimbic Cortex (medial PFC)

Striatal regions:

Caudate
Putamen
Nucleus Accumbens

Samples:

Pooled 2 monkeys/region
Biological triplicates



Proteomic Analysis

Pathway Analyses of the OFC

- Genego MetaCore and Ingenuity IPA

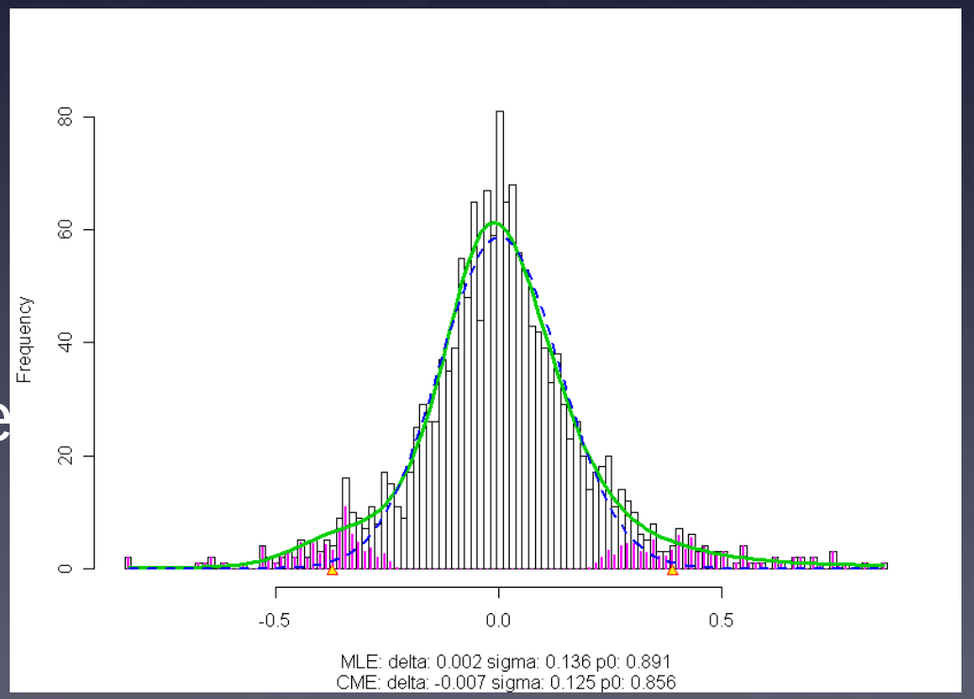
- 1.3x starting cut-off (98 altered proteins), p≤0.05, False Discovery Rate=0.2

- 49 remaining proteins included in the analysis

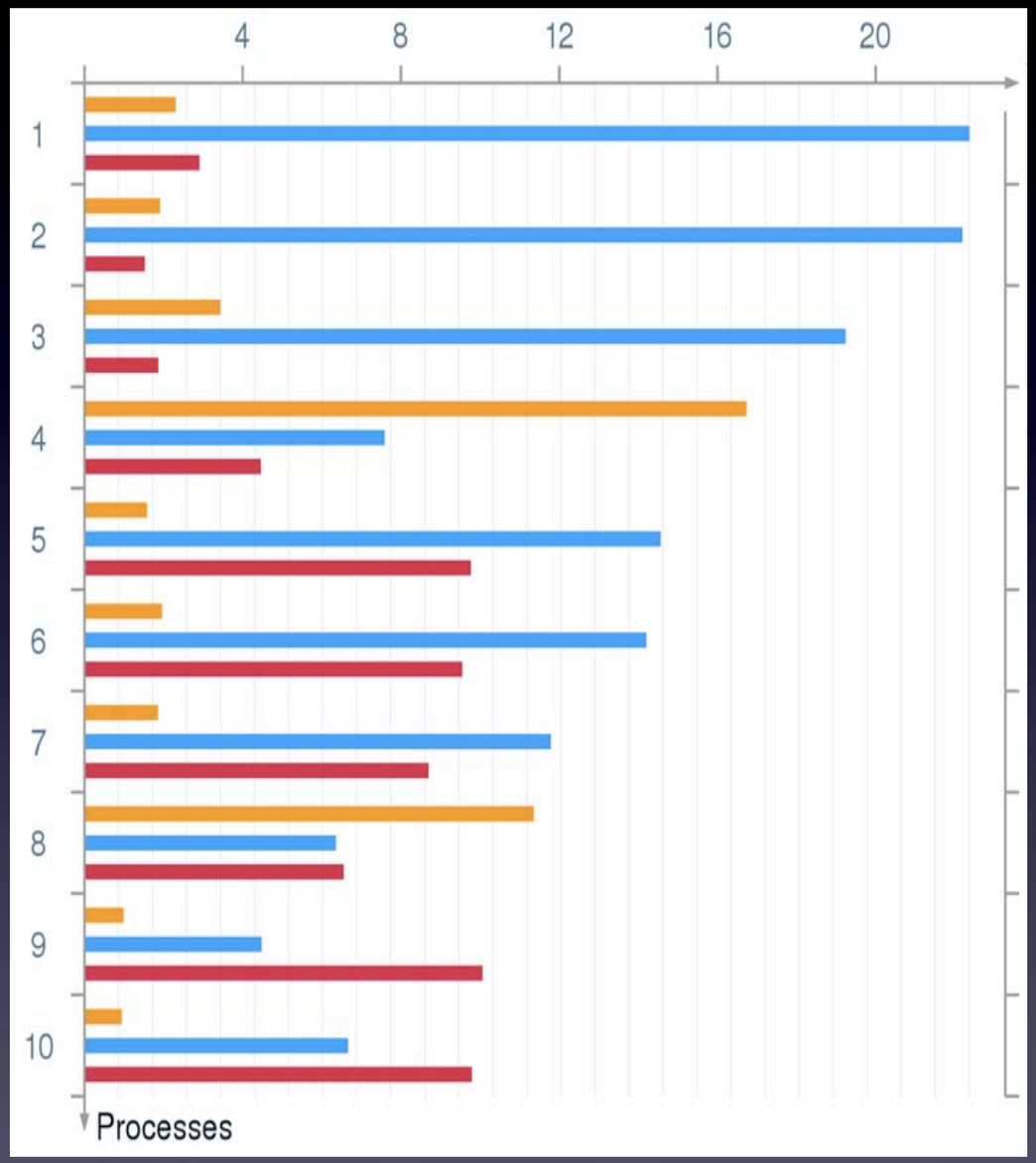
- >1.45x regulation (1.45-3.83x)

- 25 downregulated and 24 upregulated

- Focus on transcriptional regulation for identification of common cocaine-regulate processes



OFC Compared to mPFC and dlPFC



1. Energy derivation by oxidation of organic compounds

2. Generation of precursor metabolites and energy

3. Main pathways of carbohydrate/glucose metabolism

4. Organelle organization and biogenesis

5. Establishment of localization

6. Localization

7. Transport

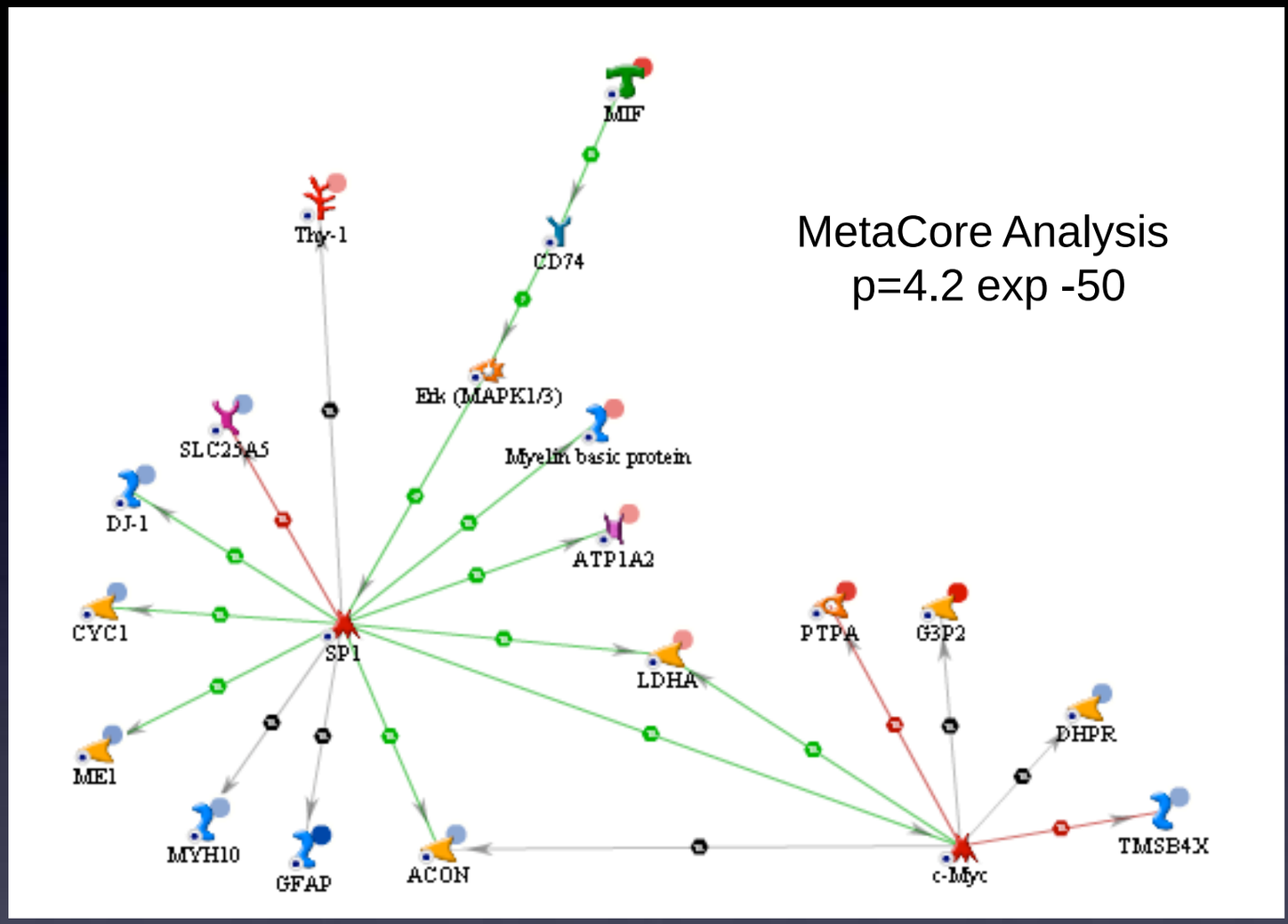
8. Cytoskeletal organization

9. Intracellular transport

10. Establishment of cellular localization

Enrichment analysis: Prior cocaine strongly regulates proteins involved in glucose and energy metabolism in the OFC compared to mPFC or dlPFC

Altered Sp1 Transcription in the OFC



MetaCore Analysis
p=4.2 exp -50

85% of Sp1-regulated proteins are downregulated in the OFC

Sp1 and Dopamine Neurotransmission

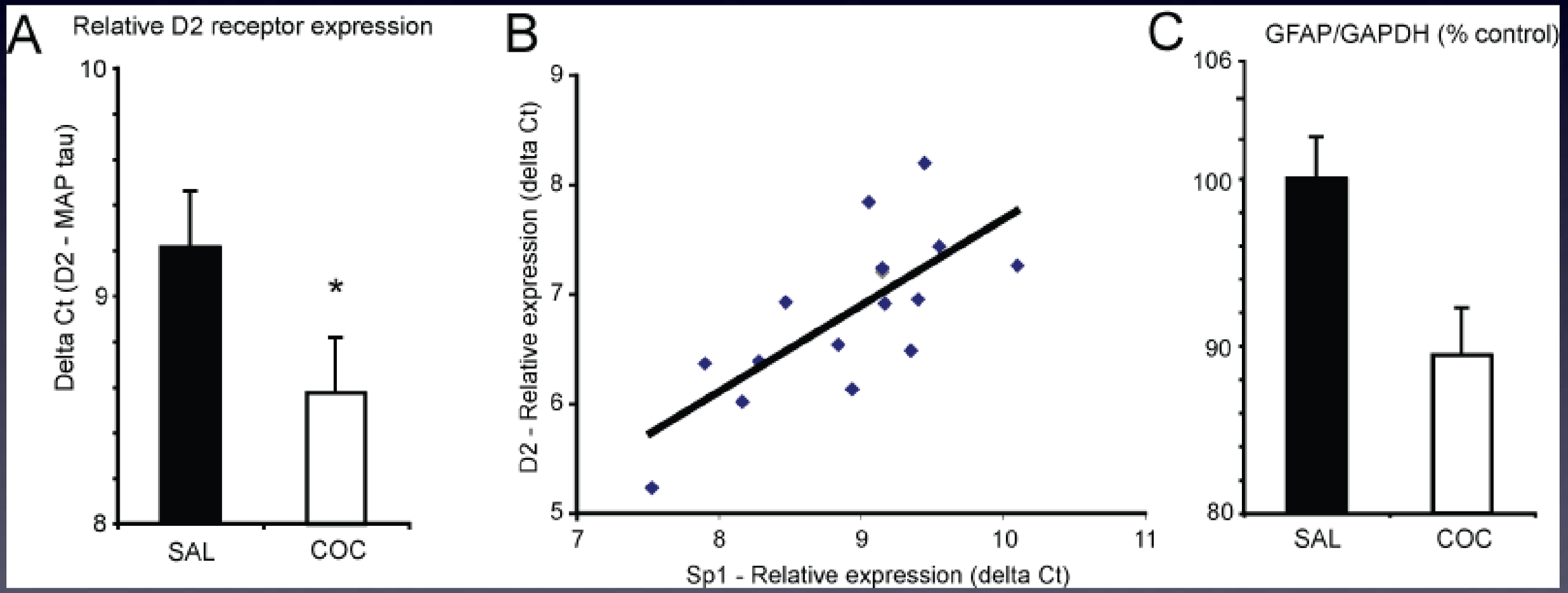
SpI is expressed in both striatum and cortex

Sp1 is a DNA-binding protein which interacts with a large number of gene promoters containing GC-box elements

Sp1 regulates proteins involved in dopamine neurotransmission including dopamine transporters, MAO, D1 and D2 receptors

Decrease in Sp1 Activity?

Reduced levels of the Sp1 targets D2 receptors and GFAP in the OFC

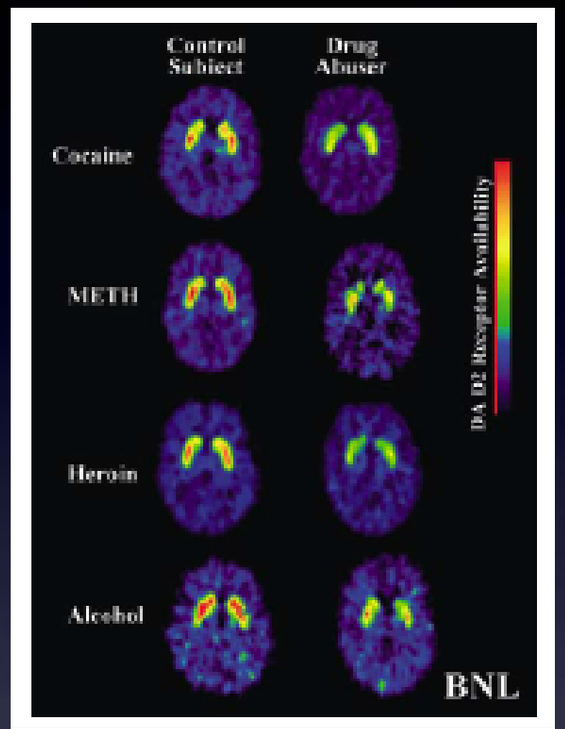


Secondary Confirmations - D2 Receptors

Target	mRNA	COC IO FC	COC mOFC	COC ACg	COC Cau
D1		=	=	Down	=
D2	**Down**	**Down?**	**Down?**	**Down**	**Down**
Sp1	=	=	=	=	=
GFAP		Down	=	=	Up
TrkB		=	=		Up
ERK		=	=	=	=
pERK/ERK		=	Down	=	=
DARPP-32					Up
pAKT/AKT		Up?	AKT down		Up?
CaM		=	Down	=	Up?
Neurogranin		=	Down		=
Cytochr ome C			Down	Down?	Down
a-synuclein					=
PSD-95				Up	Up

Consistent decrease in D2 receptor levels after cocaine exposure

Decreased Dopamine D2 Receptor Levels in Addicts; Relevance to Reversal Learning



Dopamine in drug abuse and addiction: results from imaging studies and treatment implications

ND Volkow[1,2,3,5], JS Fowler[4], G-J Wang[3] and JM Swanson[6]

[1]National Institute on Drug Abuse, Bethesda, MD, USA; [2]National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA; [3]Medical Department, Brookhaven National Laboratory, Upton, NY, USA; [4]Chemistry Department, Brookhaven National Laboratory, Upton, NY, USA; [5]Department of Psychiatry, State University of New York at Stony Brook, Stony Brook, NY, USA; [6]Department of Pediatrics, University of California, Irvine, CA, USA

Dopamine D_2/D_3 Receptors Play a Specific Role in the Reversal of a Learned Visual Discrimination in Monkeys

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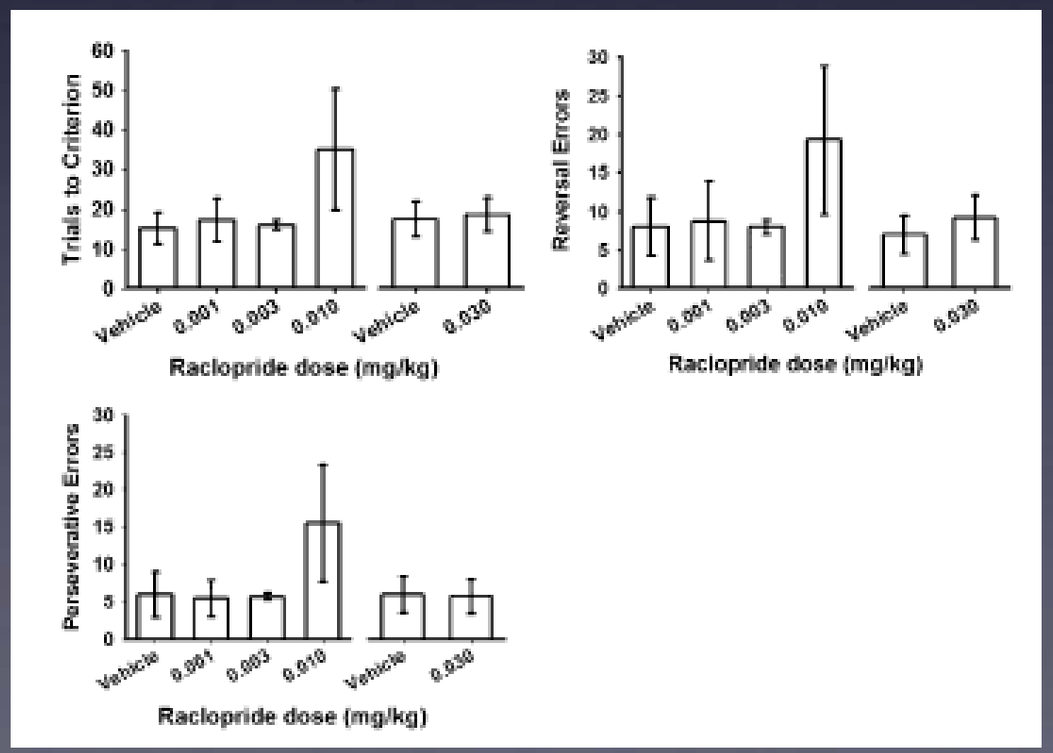
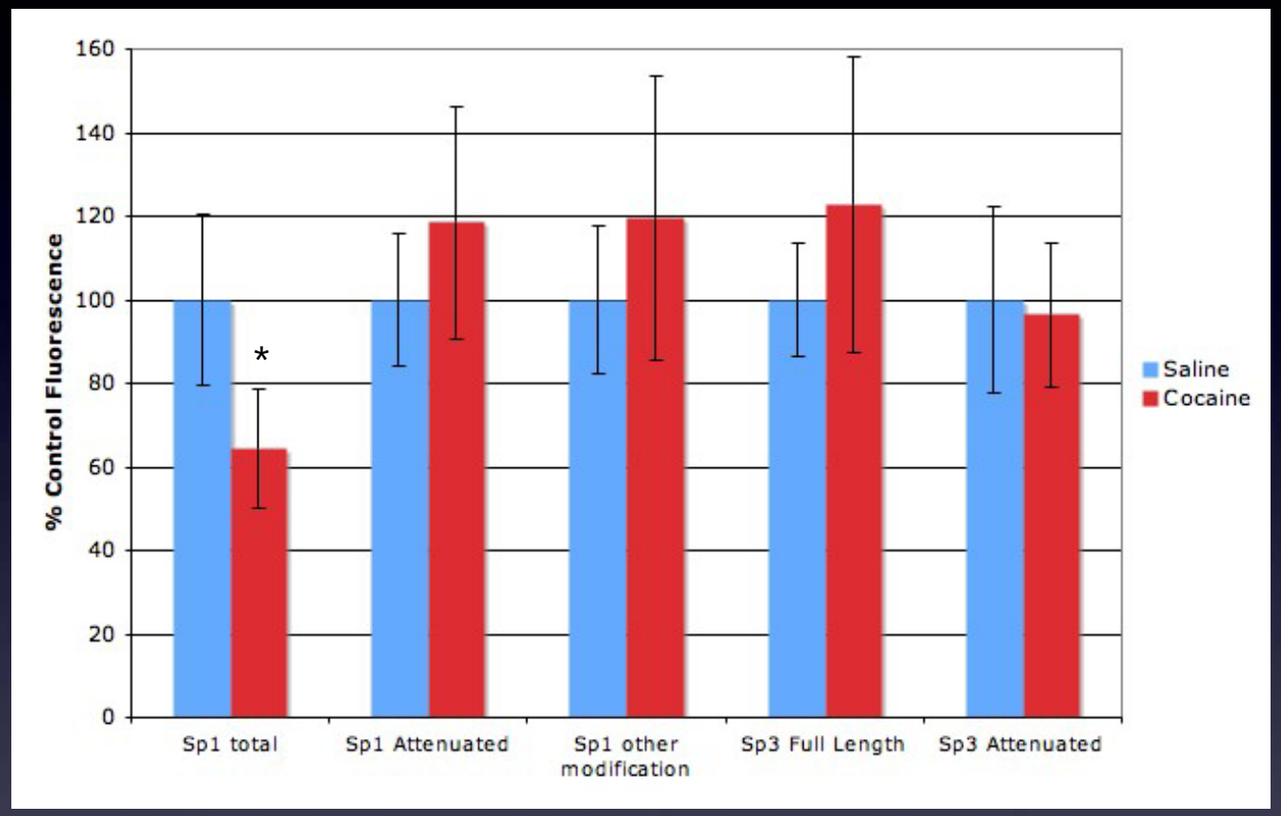
[1]Department of Psychiatry and Biobehavioral Sciences, University of California at Los Angeles, Los Angeles, CA, USA; [2]Department of Molecular and Medical Pharmacology, University of California at Los Angeles, Los Angeles, CA, USA; [3]The Brain Research Institute, University of California at Los Angeles, Los Angeles, CA, USA; [4]Department of Psychology, University of California at Los Angeles, Los Angeles, CA, USA

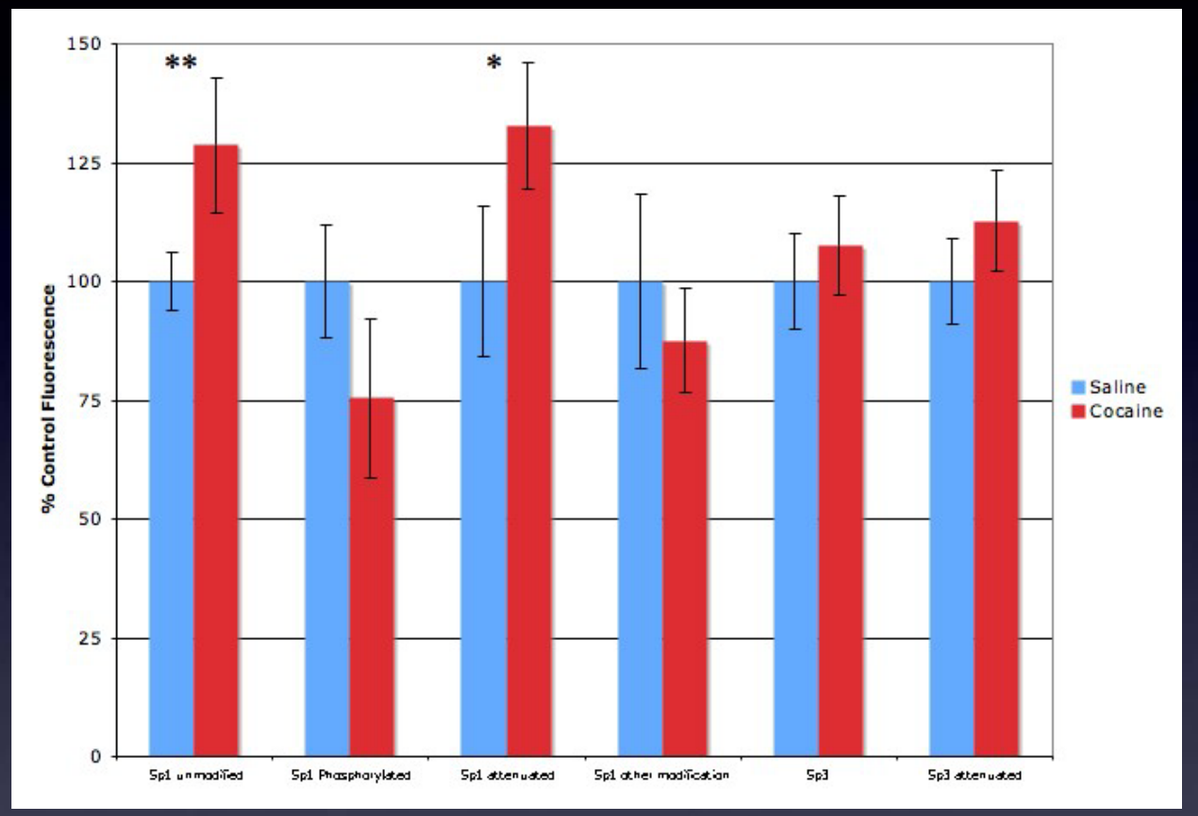


Alterations in Sp1 but not Sp3 in COC-Exposed Rats

Nuclear Fraction



Cytosolic Fraction

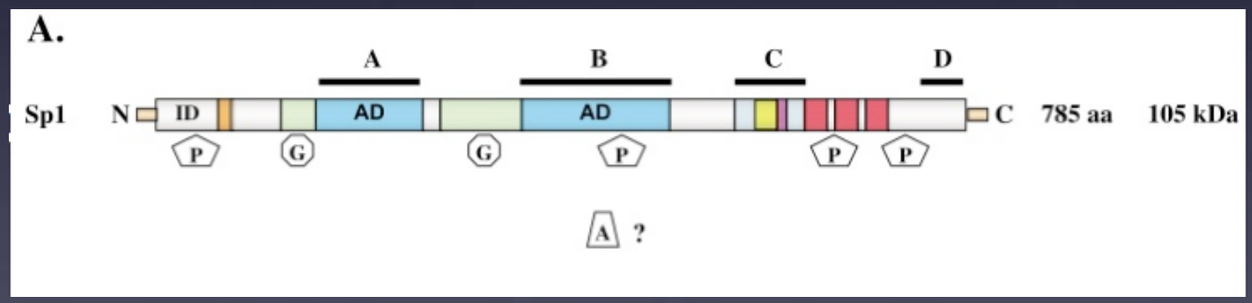


- Decreased Sp1 levels in the nucleus
- Increased Sp1 levels in the cytosol
- Does chronic cocaine lead to redistribution of Sp1?

Possible Mechanism of Sp1 Redistribution

O-linked glycosylation of Sp1 leads to its transport to the nucleus (Majumdar et al. 2003; Dauphinee et al. 2005)

Requires PP2A-mediated dephosphorylation of Ser59

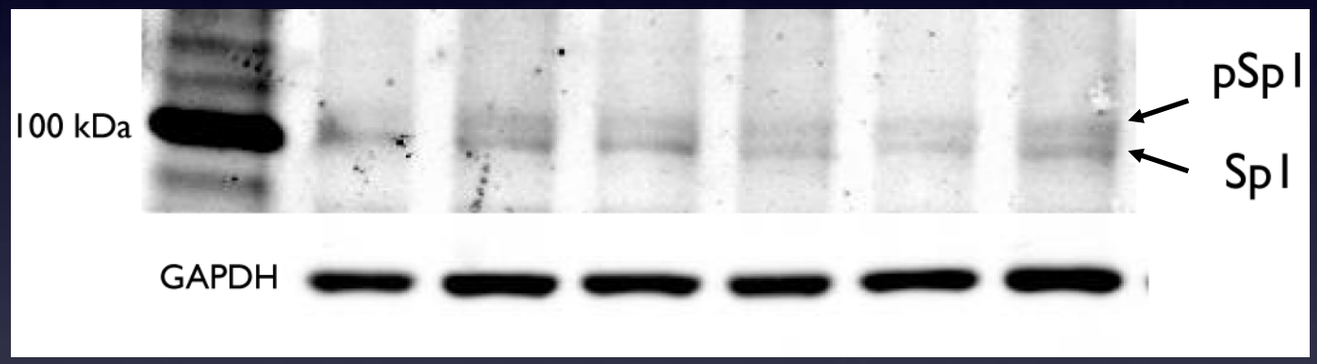


Reduced Sp1 glycosylation after Cocaine?

Post-Translational Modifications?

Analysis of Sp1 phosphorylation and glycosylaton in OFC of cocaine-exposed monkeys underway

Immunoprecipitation of Sp1



Immunoprecipitation of Sp1 and blotting using kinase subtrate-specific antibodies in dlPFC of COC-exposed monkeys

Ongoing Work

1) Overexpression of Sp1 in the OFC in cocaine-exposed animals to reverse deficits (collaboration with Ralph DiLeone)

2) Post-translational modifications of Sp1 in cocaine-treated monkeys?
(Orbitrap MS of immunoprecipitated Sp1 - Erol Gulcicek & Kathy Stone)

3) Complete the data for the dlPFC and ACg (iTRAQ - Chris Colangelo)

4) Integrated data analysis (Biostatistics and Bioinformatics Cores)

5) Preparing manuscripts:

A. PCP exposure to primates (behavior and proteomic analyses)
B. Cocaine exposure to primates (behavior and proteomic analyses)

Thank You!

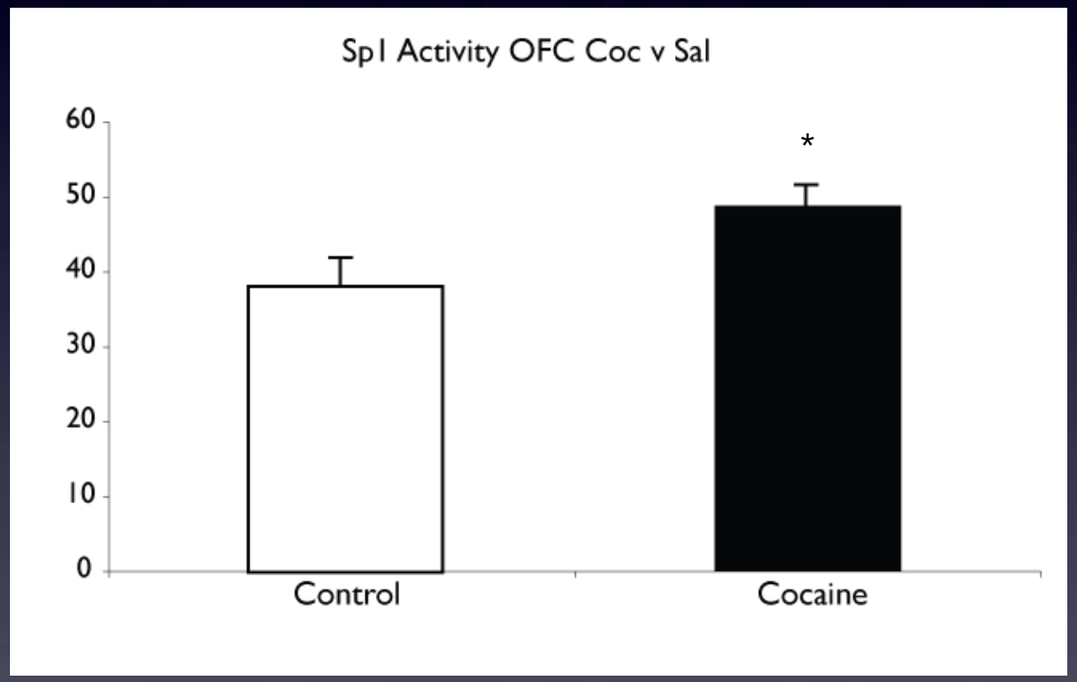
Post-Translational Modifications?

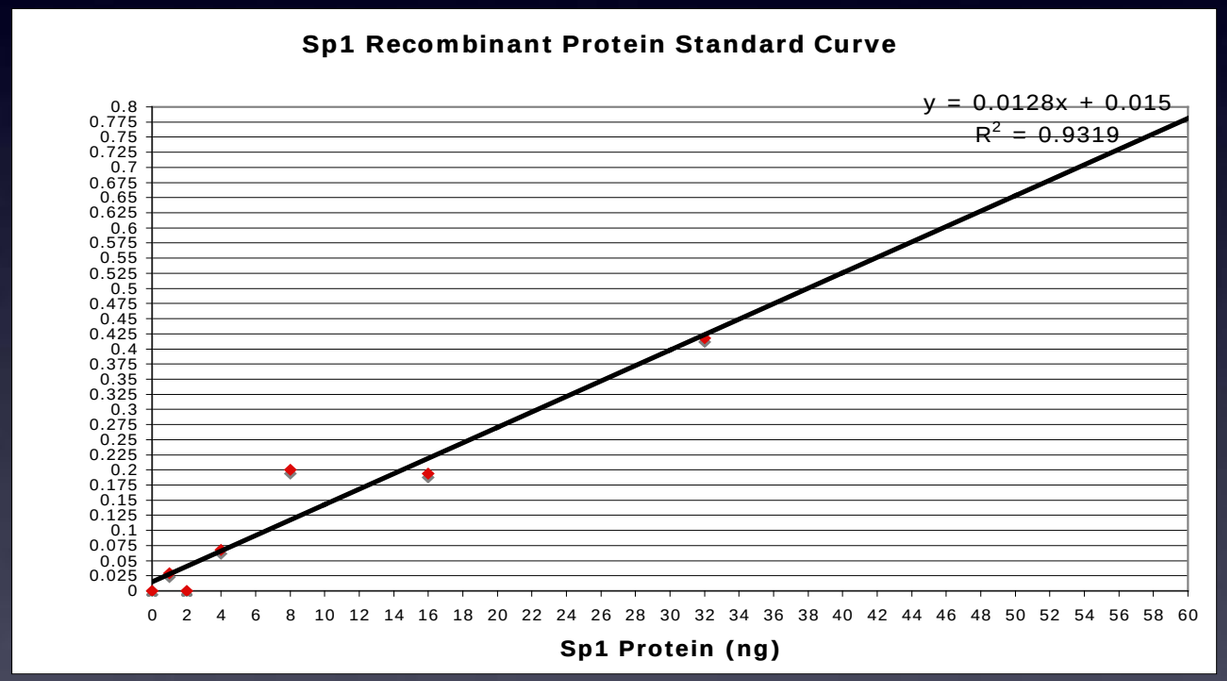
Known Modification Sites on Sp1

Position	Modification	Enzyme Activity	Consequence	Reference
Serine 59	Dephosphorylation	PP2A	increased association with chromatin; mediates glycosylation	Vicart et. al. 2006
Serine 103	Phosphorylation	ataxia telangiectaia-mutated kinase	unknown	Olofsson et. al. 2007
Threonine 366	Phosphorylation	PKA	enhancement of transcription through Sp1 DNA binding	Rohiff et. al. 1997
Threonine 453	Phosphorylation	MAPK (ERK 2)	increased Sp1-DNA binding; enhancement of basal transcripion	Milanini-Mongiat et. al. 2002
Serine 484	Glycosylation	O-linked N-acetylglucosamine transferase	Prevents Sp1-TAF110 (cofactor) association	Roos et. al. 1997
Threonine 579	Phosphorylation	casein kinase II	decreased Sp1-DNA binding	Armstrong et. al. 1997
Aspartic Acid 590	Proteolytic cleavage	caspase 3	Sp1 binds DNA; no TF activity	Rickers et. al. 1999
Threonine 668	Phosphorylation	PKC zeta	increased Sp1-DNA binding when Thr668, Thr670 and Thr 681 all phosphorylated	Tan et. al. 2008
Serine 670	Phosphorylation	PKC zeta	increased Sp1-DNA binding when Thr668, Thr670 and Thr 681 all phosphorylated	Tan et. al. 2008
Threonine 681	Phosphorylation	PKC zeta	decreased Sp1-DNA binding alone; increased Sp1-DNA binding when Thr668, Thr670 and Thr 681 all phosphorylated	Tan et. al. 2008
Threonine 681	Dephosphorylation	PP2A	increased Sp1-DNA binding	Vicart et. al. 2006
Threonine 739	Phosphorylation	MAPK (ERK1/2)	increased DNA binding; enhancement of basal transcripion	Milanini-Mongiat et. al. 2002

Prior Repeated Cocaine Exposure Increases Sp1 DNA Binding Activity in OFC

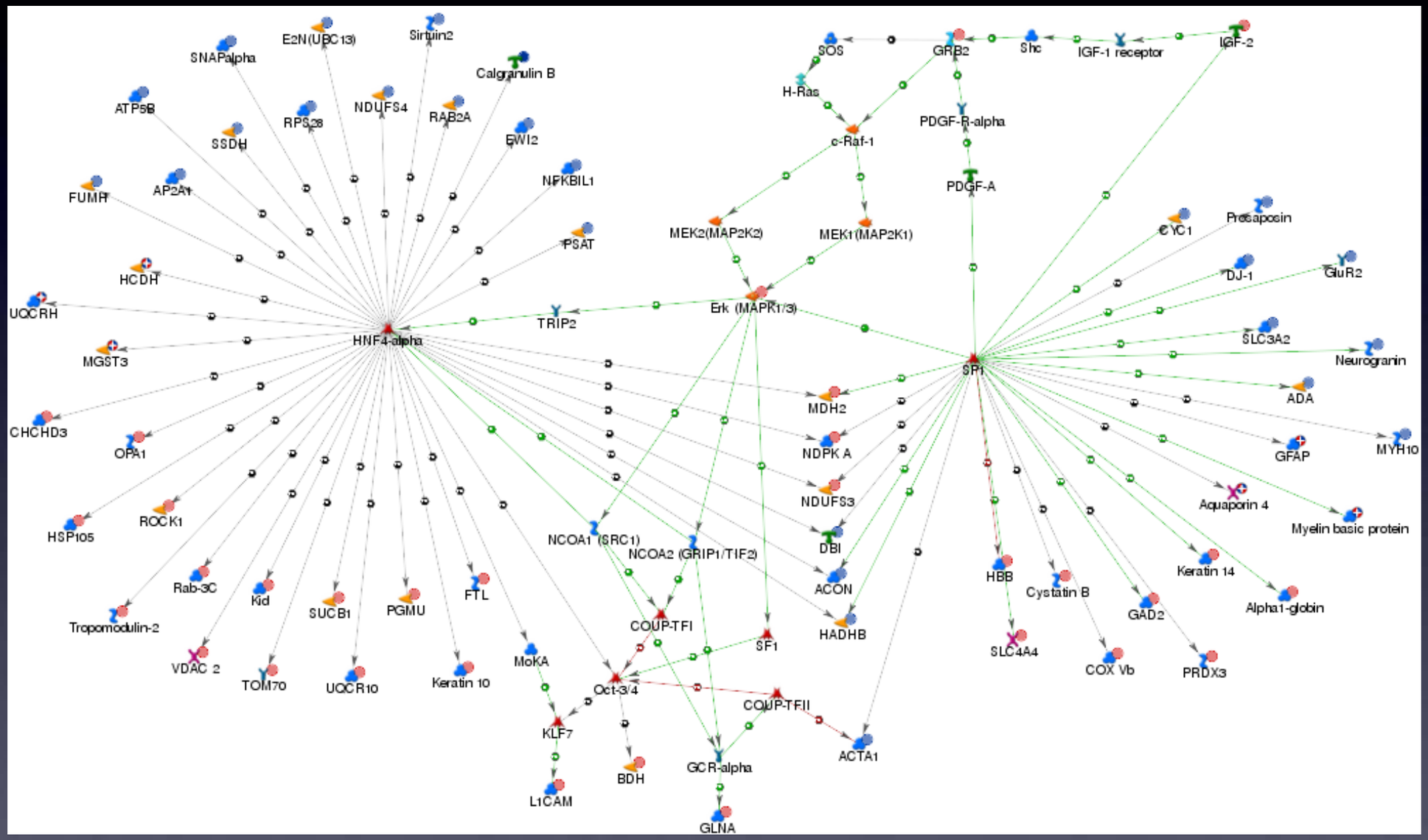
Transcription factor ELISA (ActiveMotif)





Suppression of activity by post-translational modifications?

Transcriptional Network Analysis of Altered Synaptic Proteins



HNF4alpha and Stimulatory Protein I (Sp1) appears highly integrated in the proteomic map