



# Identification of alterations in synaptic protein composition in cortico-limbic-striatal brain regions after chronic cocaine exposure in mice

## cocaine exposure in mice

394.11

Andrade, E.C.<sup>a</sup>, Colangelo, C.<sup>b</sup>, Berberich, M.<sup>b</sup>, Taylor, J.R.<sup>c</sup>, Williams, K.R.<sup>b</sup>, Nairn, A.C.<sup>a,c</sup>

Departments of <sup>a</sup> Pharmacology, <sup>b</sup> Molecular Biophysics and Biochemistry, <sup>c</sup> Psychiatry, Yale University School of Medicine, New Haven, CT 06508, USA

## INTRODUCTION

Long-lasting neuroadaptations in intracellular signaling pathways and synaptic morphology are thought to underlie drug-induced plasticity in addiction. Such changes resemble those implicated in learning and memory. Consequently, it has thus been suggested that drugs of abuse may usurp the molecular machinery required for learning in brain reward centers, resulting in an aberrant form of plasticity. In animal models, chronic exposure to a variety of drugs of abuse can produce locomotor activity hyperactivity (for review, Robinson, T.E. & Berridge, K.C. 2000). The induction of behavioral sensitization is associated with cocaine-induced neuroplasticity in brain regions known to be involved in addiction (Li *et al.* 2004). This behavioral plasticity may contribute to the increased drive and motivation for drug, a core symptom of addiction.

Persistent morphological alterations as well as number of dendritic spines are associated with long-lasting changes at the molecular level (Robinson, T.E. & Kolb, B. 1999). Repeated exposure to drugs of abuse also appears to alter the amount and even types of genes expressed in several brain regions known to be involved in drug addiction (for review, Nestler, E.J. 2004). However, large-scale studies of drug-induced molecular alterations at the protein level are lacking.

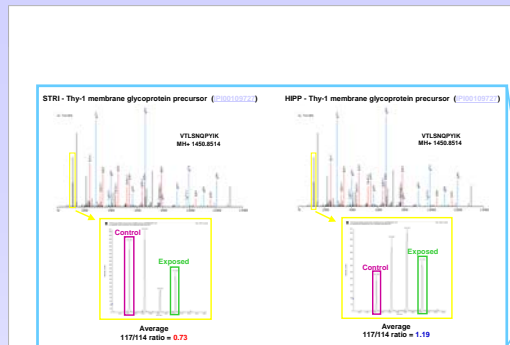
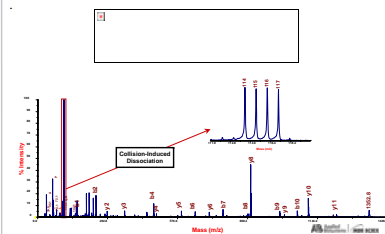
This study aims to look at protein changes associated with chronic exposure to cocaine which underlie both the behavioral and structural plasticity. We hypothesized that the postsynaptic density (PSD), which is a dynamic multi-protein complex that links neurotransmission with intracellular signaling molecules, is critical for these persistent cocaine-induced synaptic alterations. Previous characterizations of the PSD proteome from whole brain reveals a complex organelle which consists of between 250 and 500 proteins (Li *et al.* 2004, Jordan *et al.* 2004, Yoshimura *et al.* 2002, Collins *et al.* 2005). In our study, we have begun the characterization of brain region-specific differences in the PSD proteome following cocaine exposure.

## METHODS

**Chronic Cocaine Exposure:** Male C57 mice (n=6, each group) were treated once daily with an intraperitoneal (i.p) injection of saline or 30mg/kg cocaine for 22 days. Animals were sacrificed 24 hours after the final cocaine injection and cortical, hippocampal, and striatal regions were isolated. Locomotor activity was recorded following cocaine administration for the first ten days when sensitization was observed at this dose (data not shown).

**PSD preparation:** Postsynaptic density (PSD) from brain regions of interest were isolated as described previously with procedural modifications (Carlini *et al.* 1980). Briefly, tissue was homogenized using a Dounce tissue grinder in 0.32M sucrose, 20mM HEPES, pH 7.4 with protease and phosphatase inhibitors. Nuclear and unhomogenized cell contaminants were removed by low-speed centrifugation, followed by a high-speed centrifugation to obtain pellet containing synaptosomes. This was applied to a Percoll gradient (3%, 10%, 23% and ultracentrifuged. The interface between 10% and 23% was collected and subjected to hypotonic lysis (20mM HEPES, pH 7.4, 1.0mM DTT). Subsequently, the synaptic plasma membrane fraction was collected by ultracentrifugation. Following a detergent treatment (0.32M sucrose, 20mM HEPES, pH 7.4, 0.5% Triton), the PSD fraction was collected by ultracentrifugation and stored at -80°C.

**Western blotting analysis:** PSD and unfractionated samples from animals treated with either 30 mg/kg cocaine (n=6) and saline (n=6) were isolated from the cortex, striatum, and hippocampus. Samples were run on 8-16% gels and transferred onto nitrocellulose membrane. Antibodies against Na<sup>+</sup>/K<sup>+</sup> - ATPase alpha 3 subunit and Prohibitin were used and blots analyzed and quantified using Odyssey Infrared Imaging System (LiCor, NE, USA). Samples were normalized using bands from post-transfer gels stained with SimpleBlue Safe Stain (Invitrogen, CA, USA).



### Secondary analysis of iTRAQ results with Western blotting

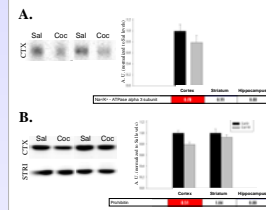


Figure 2: Proteins identified by iTRAQ analysis to change in the PSD following chronic cocaine exposure were confirmed by immunoblotting. (a) Na<sup>+</sup>/K<sup>+</sup> -ATPase alpha 3 showed a 0.80 decrease in cocaine-exposed cortical PSDs (n=6) when compared to saline-treated cortical PSDs (n=6). (b) Prohibitin showed a 0.80 decrease in cocaine-exposed striatal PSDs (n=6) when compared to saline-treated striatal PSDs (n=6). There was no difference in prohibitin between cocaine-exposed and saline-treated striatal PSDs.

### Summary of iTRAQ analysis of cortical, striatal, and hippocampal PSDs from chronic cocaine exposed mice

Protein Name	Ctx Exp/Cnt	STR1 Exp/Cnt	Hipp Exp/Cnt	Category
Splice isoform 1 of Tensin-8 precursor	1.56	0.99	1.00	Cell Adhesion
Connexin 1 precursor	1.25	1.17	0.98	
Tubulin alpha-4 chain	1.25	0.97	0.97	Cytoskeletal
Tubulin beta-4 chain	0.86	0.76	1.00	
Tubulin polymerization-promoting protein	0.96	0.78	1.01	
Cytoskeleton-associated protein 4	0.85	1.06	1.04	
Neurofilament light L protein	0.92	0.94	1.12	
Quaraine nucleotide-binding protein (QSOX1/ST) beta subunit 2	1.31	1.24	1.04	GPCR Signaling
Quaraine nucleotide-binding protein (QSOX1/ST) alpha subunit	1.31	1.09	1.12	
Ras-related protein Rap A	0.97	0.77	1.07	GTPase Regulation
RA-related G12 binding substrate 1	1.15	1.16	0.92	
SMBP antigen	0.45	1.03	1.01	Immune
P22-RACK	0.99	0.72	0.97	
Thy-1 membrane glycoprotein precursor	1.13	0.73	1.20	Lipid Raft
Brain acid soluble protein 1	1.00	0.91	1.22	
Prohibitin	0.57	1.04	0.88	
Mitochondrial glutamate carrier 1	0.63	1.05	0.84	Metabolic
ATP synthase a chain, mitochondrial	1.30	0.92	0.90	
Cytochrome c oxidase polypeptide VIa	1.02	1.03	1.44	
Dihydropyridyl dehydrogenase	0.91	1.23	0.99	
ATP synthase b subunit	0.95	0.90	0.96	
ATPase, H <sup>+</sup> transporting, V1 subunit B	1.10	1.10	0.96	
Cytochrome c oxidase, subunit VIa	1.06	0.98	1.43	
MADH-ubiquinone oxidoreductase 13 kDa subunit	1.12	0.63	1.20	
MADH-ubiquinone oxidoreductase PS2W subunit	1.04	0.60	1.19	
Voltage-dependent anion-selective channel protein 2	1.15	0.80	0.93	
Voltage-dependent anion channel 3	0.42	1.27	0.93	
Phosphate carrier protein	0.52	1.18	0.76	
ATP synthase coupling factor 6	0.97	0.85	1.00	
MADH-ubiquinone oxidoreductase MLR0 subunit	1.02	1.18	1.00	
Splice isoform 1 of Sideroflexin-3	0.75	0.82	0.86	
ADP/ATP translocase 2	1.00	1.28	0.82	
MADH-ubiquinone oxidoreductase 49 kDa subunit	0.89	0.65	0.99	
Ubiquitin-cytochrome c, mitochondrial complex 11 kDa	0.46	0.47	1.01	
ATP synthase alpha chain, mitochondrial precursor	0.62	1.05	0.87	
MADH-ubiquinone oxidoreductase subunit B14.5a	1.19	0.95	1.23	
Cytochrome c oxidase subunit 2	0.90	0.96	1.00	
Cytochrome c oxidase polypeptide VIc	1.02	1.02	1.20	
Pyruvate dehydrogenase E1 component beta subunit	0.89	0.99	0.99	
Ubiquitin-cytochrome c, mitochondrial binding protein	1.22	0.73	0.99	
Aryl carrier protein, mitochondrial precursor	1.26	0.71	1.00	
MADH-ubiquinone oxidoreductase B2 subunit	1.20	1.15	1.00	
Acetyl-CoA acetyltransferase	1.43	0.83	1.05	
Acetyl-CoA oxidase (beta-oxaloacetyl) A	1.04	1.02	0.90	
MADH-ubiquinone oxidoreductase 23 kDa subunit	1.26	0.96	0.75	
Ubiquitin-cytochrome c, redoxin complex, ubiquitin-binding protein-GPC	1.11	0.71	0.93	
Cytochrome c oxidase subunit VIb isoform 1	0.70	0.96	1.24	
MADH-ubiquinone oxidoreductase 19 kDa subunit	0.84	0.74	1.28	
MADH-ubiquinone oxidoreductase B16.6 subunit	1.02	1.05	1.20	
Dolichyl-diphosphooligomannosyl-protein glycosyltransferase E7 kDa subunit precursor	0.70	0.90	0.90	
Cytochrome b5 outer mitochondrial membrane	1.00	0.93	0.70	
MADH-ubiquinone oxidoreductase 13 kDa subunit	0.72	1.02	1.16	
Mitochondrial import receptor subunit TOM22	0.84	0.73	0.86	
ATP synthase b chain	0.95	0.91	1.00	
ATPase, H <sup>+</sup> transporting, V1 subunit A, isoform 1	1.00	1.16	1.04	
ATP synthase beta chain	1.01	0.98	0.92	
ATP synthase beta chain	0.50	0.89	0.83	
Splice isoform HK1.5A of Hexameric type 1	0.97	1.00	0.91	
Quaraine synthase	0.89	0.70	1.07	
actin carrier family 25	0.82	1.00	0.82	
Digondendrocyte myelin glycoprotein precursor	0.94	0.93	1.27	Myelin/Glia
peroxiredoxin 6	1.02	0.79	0.95	Neuroprotection
MS (Revised) protein L28	1.12	0.42	1.16	Protein Synthesis
Na <sup>+</sup> /K <sup>+</sup> -ATPase alpha 3 subunit	0.70	0.99	0.88	Receptor/Channel
Sodium/potassium-transporting ATPase alpha-1	0.90	1.02	0.96	
Shank1	0.92	0.74	0.97	Scaffolding
Vacuolar ATP synthase subunit E	1.10	1.10	0.95	Transporting
isoform DNAF-25b of Synaptosomal-associated protein 25	0.87	0.76	1.01	
Splice isoform A1-8 of Vacuolar protein translocating ATPase 116 kDa subunit a isoform 1	1.20	1.14	1.00	
Vacuolar ATP synthase subunit H	0.42	0.70	0.99	
Seguin 5	1.23	1.14	1.16	
Splice isoform 2 of Septin-11	1.30	1.06	1.11	
aphin E	1.20	1.13	1.19	
Vacuolar ATP synthase subunit d	1.14	0.92	1.00	
Bcl-2 inhibitor of transcription	0.49	1.14	1.01	Transcriptional Reg.
hypothetical protein	0.91	0.92	0.85	Unknown
Protein C10orf70 homolog	0.91	0.74	0.89	
K2 kDa protein	0.40	0.93	0.86	

Figure 1: Cortical, striatal, and hippocampal PSDs were isolated from animals either exposed to cocaine (30 mg/kg) (n=8, pooled) or saline (n=8, pooled) for 22 days. Using MALDI-LC-MS/MS, 271 proteins were confidently identified in cortical PSDs, while 482 and 471 were identified in the hippocampal and striatal PSDs, respectively. Summarized are proteins found in all three regions with decreases of 0.8 fold or less following cocaine exposure versus saline in red and increases of 1.2 fold or more in blue. Protein identification, cortical PSD, striatal PSD, and hippocampal PSD results as well as categorization of proteins are shown.

## CONCLUSIONS

• The PSDs obtained from the cortex, striatum, and hippocampus are composed of a wide variety of molecular species. Functionally, the most represented class of proteins are metabolic, followed by proteins involved in trafficking, as well as cytoskeletal proteins.

• There is an overrepresentation of metabolic proteins that change with chronic exposure to 30 mg/kg cocaine in cortical, hippocampal, and striatal PSDs.

• Increases in cytoskeletal proteins in the PSDs from cortical, hippocampal, and striatal neurons are consistent with structural alterations seen after chronic exposure to cocaine and may be associated with cocaine-induced neuroplasticity.

## REFERENCES

Carlini, R.K., Grab, D.J., Cohen, R.S., Siekevitz, P. Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. *J Cell Biol.* 1980 Sep;86(3):831-45.

Collins, M.O., Husi, H., Yu, L., Brandon, J.M., Anderson, C.N., Blackstock, W.P., Choudhary, J.S., Grant, S.G. Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *J Neurochem.* 2006 Apr;97 Suppl 1:16-23.

Jordan, B.A., Fernald, B.D., Bouscarr, M., Xu, C., Grigorean, G., Ziff, E.B., Neubert, T.A. Identification and verification of novel rodent postsynaptic density proteins. *Mol Cell Proteomics.* 2004 Sep;3(9):877-71.

Li, K.W., Hornshaw, M.P., Van Der Schors, R.C., Watson, R., Tate, S., Casetta, B., Jimenez, C.R., Gouwenberg, Y., Gundelfinger, E.D., Smalla, K.H., Smit, A.B. Proteomics a analysis of rat brain postsynaptic density. Implications of the diverse protein functional groups for the integration of synaptic physiology. *J Biol Chem.* 2004 Jan 9;279(2):987-1002.

Nestler, E.J. Molecular mechanisms of drug addiction. *Neuropharmacology.* 2004;47 Suppl 1:24-32.

Robinson, T.E., Berridge, K.C. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction.* 2000 Aug;95:S91-117.

Robinson, T.E., Kolb, B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci.* 1999 May;11(5):1598-604.

Yoshimura, Y., Yamauchi, Y., Shinkawa, T., Taoka, M., Donai, H., Takahashi, N., Isobe, T., Yamauchi, T. Molecular constituents of the postsynaptic density fraction revealed by proteomic analysis using multidimensional liquid chromatography-tandem mass spectrometry. *J Neurochem.* 2004 Feb;88(3):759-68.

## SUPPORT

Yale/NIDA Neuroproteomics Research Center (1 P30 DA018343-0), NIDA grant DA10044 (ACN), DA15222 (JRT)