IDENTIFICATION OF ALTERATIONS IN SYNAPTIC PROTEIN COMPOSITION IN CORTICO-LIMBIC-STRIATAL BRAIN REGIONS AFTER CHRONIC COCAINE EXPOSURE IN RATS

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Andrade, E.C.[a], Colangelo, C.[b], Wilczak-Havill, K.[b], Taylor, J.R.[c], Williams, K.R.[b], Nairn, A.C.[a,c]

Departments of [a] Pharmacology, [b] Molecular Biophysics and Biochemistry, [c] 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.

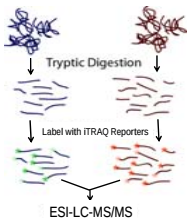
Persistent morphological alterations as well as number of dendritic spines are associated with long-lasting changes at the molecular level (Robinson, T.E. & Kolb, B. 1999). Repeated exposure to drugs of abuse also appears to alter the amount and even types of genes expressed in several brain regions 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.

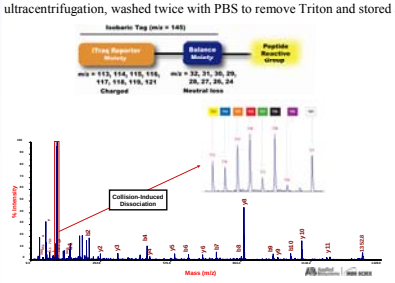
This study aims to look at protein changes associated with chronic exposure to cocaine which underlie both the behavioral and structural plasticity. We hypothesized that the postsynaptic density (PSD), which is a dynamic multi-protein complex that links neurotransmission with intracellular signaling 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 Sprague-Dawley rats (n=12, each group, 2 animals pooled per sample) were treated once daily with an intraperitoneal (i.p) injection of saline or 15mg/kg cocaine either acutely or for 14 days. Animals were sacrificed 24 hours after the final cocaine injection for the acutely and chronically treated animals. Another group was exposed for 14 days, but were sacrificed one week after the final cocaine injection. Prefrontal cortical, nucleus accumbens, hippocampal, and striatal regions were isolated using a rat brain matrix. Locomotor activity was recorded following cocaine administration for 14 days when the animals were treated chronically.

PSD preparation: Postsynaptic density (PSD) fractions from brain regions of interest were isolated as described previously with procedural modifications (Carlin *et al*. 1980). Briefly, tissue was isolated and 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 synaptoneurosomes. This was applied to a Percoll gradient (3%, 10%, 15%, 23%) and ultracentrifuged. The interface between 15% 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, 1% Triton), the PSD fraction was collected by ultracentrifugation, washed twice with PBS to remove Triton and stored at -80°C.





iTraq analysis: iTraq technology uses a chemical tagging reagent to which allows multiplexing of eight samples and produces identical MS/MS sequencing ions for all eight versions of the same derivatized tryptic peptide. Quantitation is achieved by comparison of the peak areas and resultant peak ratios for the four MS/MS reporter ions, which range from 113 to 121 Da. For our experiments, 20-50 ug of control and treated samples from cortical, accumbal, hippocampal and striatal PSDs were digested with Lys C and trypsin and then labeled. After labeling the samples were combined and separated into 20 fractions via cation-exchange chromatography. Each fraction was dried and resuspended in 10 µl 0.1% formic acid in preparation for RP-LC with the LC Packing's Ultimate workstation allowing us to pre-concentrate the 10 µl samples on a Waters 5 mm C18 Symmetry 300 trap column. The individual peptides were separated at a flow rate of 450 nl/min on an in-line 100 µm x 15 cm Waters Atlantis C18 column equilibrated with 0.5% acetic acid, 5% acetonitrile and eluted with a 60 min acetonitrile gradient. Electrospray Ionization of the eluent was followed by data-dependent acquisition on a Applied Biosystems API Q-Star XL mass spectrometer. Data analysis was performed using MASCOT database search engine to determine protein identifications and ProQuant software to determine iTRAQ ratios. Data results were analyzed with the Trans Proteomic Pipeline developed at the Seattle NHLBI Proteomics Center.

RESULTS

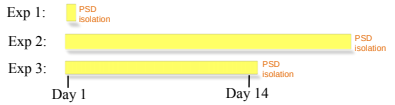


Figure 1: Rats were given either 1 day of cocaine (15 mg/kg) or saline and sacrificed 24 hours later, 14 days and sacraficed 1 week later, or 14 days and sacraficed 24 hours later. PSDs were isolated from the prefrontal cortex, nucleus accumbens, striatum, and the hippocampus.

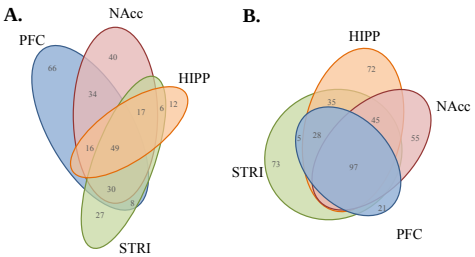


Figure 2: Number of proteins determined by iTRAQ. (a) 207 proteins, 200 proteins, 126 proteins, and 110 proteins were identified in the prefrontal cortical (PFC), accumbal (NAcc), striatal (Stri), and hippocampal (Hipp) PSDs, respectively from experiment 1. (b) 182 proteins, 256 proteins, 314 proteins, and 300 proteins were identified in the PFC, NAcc, Stri, and Hipp PSDs, respectively from experiment 2.

Protein Name	PFC	Nacc	Stri	Hippo
17 kDa protein	0.21	0.59		0.95
40S ribosomal protein S13	0.93	0.79	1.00	0.73
60S ribosomal protein L10a	0.82	0.75	1.31	0.89
Actin	0.93	0.75	1.07	0.69
Guanine nucleotide-binding protein G(o)	0.84	0.74	0.94	0.71
Brain acid soluble protein 1	0.78	0.72	1.14	0.67
Calmodulin	1.22	1.00	1.25	0.78
Cell cycle exit & neuronal differentiation protein 1	0.63	0.85	1.32	0.98
coiled-coil domain containing 109A	1.09	0.52		0.78
Contactin-1 precursor	0.88	0.78	1.07	0.73
Disks large homolog 4	0.82	0.79	1.25	0.76
Dynein light chain 1, cytoplasmic	0.78	0.80		0.79
ERC protein 2	0.83	0.76	1.46	
ganglioside-induced diff.-associated-protein 1	0.71	0.66	1.05	
Gap junction alpha-1 protein	0.94	0.76	1.34	0.92
Gap43 Neuromodulin	0.84	0.74	1.00	0.76
Guanine nucleotide-binding protein G(i)/G(S)/G(T)	0.79	0.78	1.08	
Hemoglobin subunit alpha-1/2	1.08	0.58	0.86	0.77
Hemoglobin subunit beta-2	1.04	0.64	0.68	0.67
Immt 87 kDa protein	0.70	0.76	1.13	0.88
Ras GTPase-activating protein SynGAP	0.75	0.83	1.21	0.75
Syntaxin-binding protein 1	0.71	0.72	0.96	0.90
Limbic system-associated membrane protein	0.76	0.65		
metaxin 1 isoform 1	0.54	0.57		0.94
Ndufa8 20 kDa protein		0.47	1.09	0.69
Ogdh 118 kDa protein	0.94	0.61		0.76
Plp1 Myelin proteolipid protein	0.78	0.76	1.30	0.96
Prohibitin	0.53	0.50	1.02	
Rac1 Ras-related C3 botulinum toxin substrate	0.78	0.70	0.93	
Rps5 Putative protein		0.76	1.28	
Sideroflexin-5	0.90	0.70	1.40	0.99
Sorting and assembly machinery component 50	0.72	0.75	1.21	
Synaptotagmin-1	0.77	0.70	0.93	
Syntaxin-1B	0.74	0.68	0.96	
Thy-1 membrane glycoprotein	0.93	0.80	0.96	0.61
Ugcrb Putative protein		0.46	1.05	0.76
Voltage-dependent anion-selective channel-1	0.57	0.67	1.19	0.82
Voltage-dependent anion-selective channel-2	0.57	0.64	1.11	0.83
Voltage-dependent anion-selective channel-3	0.59	0.65	1.22	0.79

Figure 3: Proteins determined by iTRAQ analysis to change in the PSD following acute cocaine exposure. PSDs from the PFC, NAcc, Stri, and Hipp (n=4, with 2 animals pooled per sample) were isolated 24 hours after acute exposure to cocaine (15 mg/kg) or saline. In total, PSDs from the PFC showed decreases in 74 proteins and increases in 5 following acute exposure; PSDs from the NAcc showed decreases in 114 proteins; PSDs from the Stri showed decreases in 8 proteins and increases in 27 proteins; PSDs from the Hipp showed decreases in 46 proteins and increases in 3 proteins. Represented are proteins that were found to significantly change in at least two brain regions. For simplicity, metabolic proteins have been omitted.

Protein Name	PFC	Nacc	Stri
15 kDa protein	0.72	1.32	0.82
40S ribosomal protein S25	0.82	1.72	0.78
60S ribosomal protein L10	0.91	1.35	0.77
Calmodulin	0.71	1.32	0.75
Cam Kinase II	0.78	1.03	0.78
Dynein light chain	0.66	0.79	0.92
Neuromodulin	0.60	0.76	1.03
Hemoglobin subunit alpha-1/2	0.74	0.65	0.93
hypothetical protein LOC681418	1.40	1.53	0.77
Alpha-internexin	0.74	0.74	0.83
Disks large-associated protein 3	0.77	1.08	0.76
Synapsin-1	0.77	0.83	1.05
Myelin proteolipid protein	1.44	1.08	1.28
Ndufa8 Aa2-258	1.35	1.46	
Ndufb9 protein		1.88	0.70
Neurofilament light polypeptide	0.72	0.68	
Prohibitin	1.65	1.70	0.73
Prohibitin-2	1.72	1.34	0.74
Putative protein Ndufc2	1.29	1.73	0.84
Rho guanine nucleotide exchange factor 2		1.34	0.77
Sideroflexin-5	1.25	1.87	0.70

Figure 3: Proteins determined by iTRAQ analysis to change in the PSD following chronic cocaine exposure. PSDs from the PFC, NAcc, Stri (n=4, with 2 animals pooled per sample) were isolated one week after chronic exposure to cocaine (15 mg/kg) or saline. In total, PSDs from the PFC showed decreases in 39 proteins and increases in 17 following chronic exposure; PSDs from the NAcc showed decreases in 12 proteins and increases in 60 proteins; PSDs from the Stri showed decreases in 50 proteins and increases in 5 proteins. Represented are proteins that were found to significantly change in at least two brain regions. For simplicity, metabolic proteins have been omitted.

CONCLUSIONS

• The PSDs obtained from the prefrontal cortex, nucleus accumbens, 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 protein synthesis, cytoskeletal and signaling proteins.

• While PSDs from the 4 different regions contain a number of distinct proteins, there is a great deal of overlap when comparing two or even three regions.

• In the nucleus accumbens, most of the significant changes found in the PSD following acute exposure to cocaine are reduction when compared to control; however, most changes in this brain region after chronic exposure to cocaine followed by 1week are increases. This difference may be do to short-term structural and functional changes following acute exposure versus the more lasting changes expected after chronic exposure.

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