Identification of alterations in synaptic protein composition following chronic cocaine exposure in mice

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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). In our study, we have begun the characterization of brain region-specific differences in the PSD proteome.

METHODS

PSD preparation: Postsynaptic density (PSD) from brain regions of interest were isolated as described previously with procedural modifications (Carlin *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 synaptoneurosomes. 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.

Multidimensional Protein Identification Technology (MudPIT): PSD composition was determined using MudPIT analysis. Briefly, 50 µg of sample was tryptically digested and loaded onto a strong cation exchange with reverse phase column. Eluted fractions using increasing pI were then subjected to LC-MS-MS for peptide identification.

Cocaine Sensitization: Male C57 mice (n=3, each group) were treated once daily with an intraperitoneal (i.p) injection of saline, 10mg/kg, 20mg/kg, or 30mg/kg cocaine for 22 days. Locomotor activity was monitored and recorded using a Digiscan Micro Analyzer (AccuScan Instruments) by quantifying photocell beam interruptions for 60 minutes following a 30 minute habituation period then injection over the first 10 days of cocaine exposure. Animals were sacrificed 24 hours after final cocaine injection and cortical, hippocampal, and striatal regions were isolated.

2-Dimensional Differential Fluorescent Gel Electrophoresis (DIGE): DIGE was performed using Ettan DIGE (Amersham Biosciences). 50 µg of exposed PSD sample from different brain regions was labeled with Cy5. Control PSD was isolated from age-matched, naïve animals and 50 µg was labeled with Cy3. 25 µg of each sample was pooled and labeled with Cy2 as an internal control. Labeled samples were pooled and isoelectric focusing was performed using a pI range of 3-10. SDS-PAGE on a 12% gel was performed for the second dimension. Dye ratios were determined using DeCyder (Amersham Biosciences). Spots corresponding to ≥1.5 fold changes were excised and subjected to gel tryptic digestion. High abundance proteins were determined using Micromass Tof-Spec SE, whereas low abundance proteins were determined using Applied Biosystems 4700 Proteomics Analyzer.

RESULTS

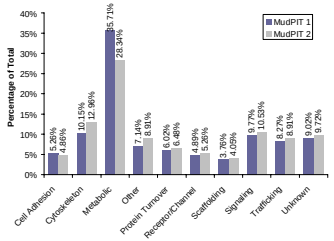

Figure 1: Classification of PSD proteome. Using both Panther Classification System (Applied Biosystems) and literature searches, proteins identified from cortical PSD samples of drug-naïve C57 males using MudPIT are summarized by functional class [MudPIT 1, (266 proteins identified)]. Replication of the first experiment shows similar functional composition of the PSD [MudPIT 2, (247 proteins identified)]. Of the proteins identified in the two experiments, 126 proteins were common in both.

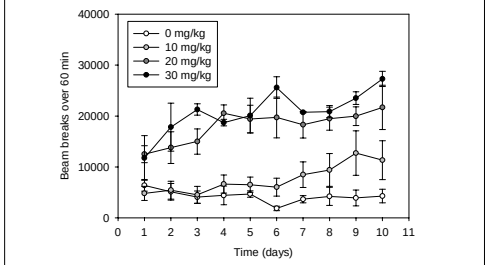

Figure 2: Cocaine sensitization. C57 males exposed to saline, 10 mg/kg, 20 mg/kg, or 30 mg/kg (n=3, each group) show dose-dependent increases in locomotor activity following cocaine injection (i.p.) over the first 10 days of cocaine exposure.

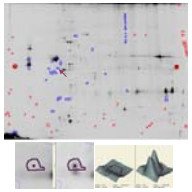

Figure 3: Region-specific differences after chronic cocaine exposure. DIGE was performed to look at brain region-specific changes following cocaine-induced behavioral sensitization. PSD samples from cortical, hippocampal, or striatal regions after chronic exposure to 30 mg/kg cocaine were run on DIGE. A representative gel (above) shows ≥ 3.0 fold changes (increases from control in blue, decreases from control in red) in cortical PSD from 30 mg/kg exposed animals. Proteins identified as changed from control in cortical, hippocampal, or striatal PSD samples are represented in the table to the right.

Cortical PSD

Description	Exposed/unexposed ratio
actin	1.16
ATP synthase	2.03
ATP synthase, H+ transporting mitochondrial F0 complex	2.08
ATP 5b	2.27-2.35
NADH-ubiquinone oxidoreductase 25kDa subunit	2.7
brain abundant signal protein 1 (BASP1)	2.63-2.89
voltage-dependent anion channel protein 1 (VDAC-1)	2.13-2.25

Hippocampal PSD

Description	Exposed/unexposed ratio
actin	-1.63 - -1.53
beta tubulin	1.63
contactin 1	1.82
spectrin alpha 2	2.06-3.08
pyruvate dehydrogenase (lipoamide) beta	-2.13- -1.8
sirtuin 2 (SIR2-like protein 2)	-1.92
succinate dehydrogenase flavoprotein subunit	-1.69
aconitase 2	-1.57
ubiquinol-cytochrome c reductase complex core protein 1	1.52
NADH-dehydrogenase (ubiquinone) Fe-S protein 1	1.62-1.79
glycerol-3-phosphate dehydrogenase	1.66
ATPase, H+ transporting, V1 subunit A, isoform 1	1.7-1.86
NADH-dehydrogenase (ubiquinone) flavoprotein 1	1.7
NADH-dehydrogenase (ubiquinone) 1 alpha complex 10	1.72
NADH-ubiquinone oxidoreductase 30 kDa subunit	1.82
mitofilin	2.05
ATP synthase, H+ transporting, mitoch. F1 complex	3.44
ATP synthase beta subunit	2.88-3.08
heat shock protein 8	1.54
40S ribosomal protein SA	1.56
heat shock protein A	1.7

Hippocampal PSD (continued)

Description	Exposed/unexposed ratio
voltage-dependent anion channel 1 (VDAC-1)	1.54
guanine nucleotide-binding protein, beta-2 subunit	-2.07- -1.62
guanine nucleotide-binding protein G(o), alpha subunit 2	-1.94
Septin 5	1.72-2.8
synapsin II	2.42

Striatal PSD

Description	Exposed/unexposed ratio
spectrin alpha 2	1.52
beta actin	1.61-2.36
capping protein alpha 1 subunit	1.79
Ina protein	1.98-2.72
neurofilament, light polypeptide	2.22-2.6
neurofilament 3, medium	2.6-3.32
succinate dehydrogenase Fp subunit	-1.87
ATPase, H+ transporting, V1 subunit B, isoform 2	1.53
ATPase, H+ transporting, V1 subunit A, isoform 1	1.63
ubiquinol-cytochrome-c reductase complex core protein 2	1.67
ubiquinol-cytochrome-c reductase complex core protein 3	1.73
NADH dehydrogenase (ubiquinone) Fe-S protein 1	1.75-1.93
ubiquinol-cytochrome-c reductase complex core protein 1	1.75-2.81
NADH dehydrogenase (ubiquinone) Fe-S protein 8	2.05
mitofilin	1.91-1.96
cell division cycle 10 (cdc10) homolog	2.54
heat shock protein 8	2.02-2.12
heat shock 70kD protein 5	2.1
VDAC-1	1.67-1.89
postsynaptic density protein 95 (PSD95)	1.98
septin 11	2.52
CDCrel-1 homolog mouse	2.57

CONCLUSIONS

- The PSDs obtained from neurons of the cerebral cortex is composed of a wide variety of molecular species. Functionally, the most represented class of proteins are metabolic, followed by cytoskeletal proteins, and proteins involved in intracellular signaling.

- 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 hippocampal and striatal neurons are consistent with structural alterations seen after chronic exposure to cocaine and may be associated with cocaine-induced neuroplasticity.

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