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Genes and Pathways Co-associated with the Exposure to Multiple Drugs of Abuse, Including Alcohol, Amphetamine/Methamphetamine, Cocaine, Marijuana, Morphine, and/or Nicotine: a Review of Proteomics Analyses

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Abstract Drug addiction is a chronic neuronal disease. In recent years, proteomics technology has been widely used to assess the protein expression in the brain tissues of both animals and humans exposed to addictive drugs. Through this approach, a large number of proteins potentially involved in the etiology of drug addictions have been identified, which provide a valuable resource to study protein function, biochemical pathways, and networks related to the molecular mechanisms underlying drug dependence. In this article, we summarize the recent application of proteomics to profiling protein expression patterns in animal or human brain tissues after the administration of alcohol, amphetamine/methamphetamine, cocaine, marijuana, morphine/heroin/butorphanol, or nicotine. From available reports, we compiled a list of 497 proteins associated with exposure to one or more addictive drugs, with 160 being related to exposure to at least two abused drugs. A number of biochemical pathways and biological processes appear to be enriched among these proteins, including synaptic transmission and signaling pathways related to neuronal functions. The data included in this work provide a summary and extension of the proteomics studies on drug addiction. Furthermore, the proteins and biological processes highlighted here may provide valuable insight into the cellular activities and biological processes in neurons in the development of drug addiction.

Keywords Genes · Pathways · Substances · Proteomics · Review

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Introduction

Drug addiction is an abnormal behavior characterized by compulsive, out-of-control drug use despite serious negative consequences [1]. These behavioral abnormalities develop gradually and progressively during repeated exposure to a drug of abuse and can persist for a long period after discontinuation of use [2]. For this reason, drug addiction is also considered a form of drug-induced neural plasticity [3–5]. Studies have shown that repeated exposure to an addictive drug can alter the level or types of genes/proteins expressed in specific brain regions [6, 7]. Such alteration in expression mediates the functions of individual neurons and the related neural circuits and may be responsible for the consequent behavioral abnormalities related to addiction [8]. Thus, identification of the genes/proteins associated with the development and maintenance of drug dependence is not only critical to an understanding of the molecular mechanisms underlying addiction but also imperative for the development of new pharmacological means to reverse the addictive state, prevent relapse, or reduce the use of these drugs [9].

Various molecular techniques have been utilized to investigate the gene/protein expression changes associated

with drug addiction. Proteomics, characterized by the ability to measure dynamic changes in proteomes on a global scale, has become a powerful tool in drug addiction research and offers great promise for increasing understanding of the biochemical basis of addiction [10]. Similar to other high-throughput approaches, proteomics use in drug addiction allows investigators to study the perturbed states invoked by the abused substances without the need for a prior hypothesis. As a neuropsychiatric disorder of the central nervous system, drug dependence involves a large array of interacting proteins and is thus well suited for proteomics analysis. Although they are still evolving technologies, proteomics approaches have become useful tools for elucidating the molecular effects of addictive substances. In past years, proteomics has been used to profile the protein expression pattern in cultured neurons or different brain regions of animals or humans in response to a number of substances of abuse such as nicotine, amphetamine, alcohol, cocaine, and morphine [11–15].

Proteomics technologies and their application in drug addiction research have been reviewed in detail earlier [16, 17]. Lull et al. [16] presented a comprehensive review of the strengths and technical limitations of proteomics approaches and their application in drug addiction studies, highlighting the challenges that need to be addressed, such as animal model selection, sample quality control, neuroanatomic sample preparation, and result confirmation, as well as data analysis and interpretation. In one of our earlier reviews, we summarized the application of proteomics to drug addiction study and compared the proteins associated with exposure to nicotine, alcohol, morphine, and other addictive drugs [17]. In view of the proteins whose expression was modulated in response to the exposure to nicotine and other drugs, we identified a few biological processes that may be involved in the development of drug dependence, among which are energy metabolism, oxidative stress response, and protein modification and degradation. With the recent advancement in techniques and the improvement in its power, proteomics has increasingly been utilized in drug addiction study. More and more proteins potentially related to drug dependence have been identified, which provide us with rich information on the biological mechanisms underlying the development of this state. Thus, the primary objective of this report is to provide an updated summary of the drug-addiction-related proteins identified from proteomics studies and highlight the potential biological themes discovered.

Application of Proteomics in Drug Abuse Study

As evidenced in the literature, a comprehensive genome-wide proteome analysis in drug addiction remains beyond our capability because of its extreme complexity and the limits of the current technology. For the time being, a more feasible approach is to focus on substructures, such as the brain and its components. During recent years, proteomics has been used extensively to profile the protein expression pattern in cultures of neurons or in different brain regions of animals or humans in response to a number of substances of abuse such as alcohol, amphetamine, cocaine, morphine, and nicotine. On the other hand, the application of proteomics technology to the study of a specific addictive drug is still rather limited, which makes a thorough and detailed summary for a single substance difficult and unlikely. Thus, we attempt a collective summary of the application of proteomics techniques to studies for different abused substances such that we can have a better understanding of proteins and pathways commonly modified by multiple abused substances. In the following sections, we first discuss the current reports on the application of proteomics in studies of several addictive substances and then focus on the key proteins identified in these studies and discuss their relevance to drug addiction.

The published proteomics analyses were selected by the following steps: first, items combined by "proteomics" and a certain drug or relevant terms (e.g., nicotine/smoking/tobacco, alcohol/ethanol, amphetamine/methamphetamine, etc.) were researched in PUBMED (http://www.ncbi.nlm.nih.gov/pubmed/); second, after reviewing the abstracts of retrieved publications, we kept only the original reports on the proteomics analysis of the drug effects on the brain tissues or neuronal cells from animal models or human subjects; third, the full reports of the selected publications were reviewed to ensure that proteomics analysis was the major method adopted in the study and valid protein expression results were obtained; and fourth, from these studies, proteins reported to be significantly associated with the drug of interest were retrieved.

Alcohol

Proteomics approaches have been extensively used in alcohol addiction study with biological samples from both animal models and humans. After comparing the protein expression differences in two regions (amygdala and nucleus accumbens [NAc]) of alcohol-naïve inbred alcohol-preferring and -non-preferring rats [15, 18, 19], the investigators found that selective breeding for disparate alcohol drinking behaviors produced innate alterations in the expression of several proteins that could influence neuronal function within the NAc and amygdala. Especially, the data suggested that there may be some basic differences in the mechanisms underlying synaptic transmission in the NAc in inbred alcohol-preferring and -non-preferring rats. Bell et al. [19] also examined the protein expression changes in the

amygdala and NAc of inbred alcohol-preferring rats given either continuous or scheduled access to ethanol over 6 weeks. Proteins related to intracellular signal transduction, cytoskeletal structure, metabolism, response to stress, and synaptic transmission were potentially associated with ethanol self-administration in these two brain structures. Further, the effects of ethanol injections on protein expression in the NAc shell of alcohol-preferring, alcohol-non-preferring, and Wistar rats were analyzed [20]. The results indicated a unique response to ethanol in the NAc of alcohol-non-preferring rats. Damodaran et al. [21] found that chronic alcohol treatment for 4 weeks alters protein expression in the brains of zebrafish. They identified eight proteins that are differentially regulated in response to alcohol, e.g., voltage-dependent anion channel proteins (VDAC1 and VDAC2), heat shock protein 70 (HSP70), alpha subunit of G_o (GNAO1), and subunit A of the catalytic domain of H^+ transporting ATPase (ATP6V1A1). Park et al. [22] suggested that the different responses to alcohol in two inbred mouse strains, C57BL/6J and DBA/2J, may come from differences in the response rates and interactions of different variants of the alcohol-responsive protein family. To study the effect of ethanol on central nervous system development, Sari et al. [23] analyzed the protein expression pattern in fetal brains of C57BL/6J mice subjected to ethanol exposure. Significant downregulation of several proteins important for energy generation, apoptosis, cellular signaling, and neuronal migration was observed.

A few proteomics studies on alcoholic human brain tissues have also been reported. In one of the first such studies, Lewhol et al. [24] applied the proteomics approach to tissue extracts of human brain obtained at autopsy. They detected 182 significant changes in the superior frontal cortex, with the majority showing a lower expression in alcoholic than control brains. Further, more than 60 proteins were identified, indicating that it is possible to use autopsy human brain for proteomics analysis.

Several other regions important for neurocognitive function have also been analyzed by proteomics approaches. In the dorsolateral prefrontal cortex (PFC), two thiamine-dependent proteins, transketolase and pyruvate dehydrogenase E1 beta-subunit, were found to be differentially expressed in alcoholics [25, 26]. In addition, a large number of metabolism-related enzymes, in particular ones related to energy transduction, were found to be differentially expressed in this region. Protein expression was examined in the cerebellar vermis of uncomplicated and cirrhotic alcoholics [27]. Many differentially expressed proteins were found to be unique to the cirrhotic group, indicating that liver dysfunction might be producing significant differences in protein expression [28]. The splenium, genu, and body of the corpus callosum are all affected in alcoholic brains. Proteomics analysis identified the proteins with differential expression in each of these subregions, among them were glutamate carboxypeptidase (GCP1) and phospholipase D (PLD) [29–31]. Expression profiles also identified the region-specific proteins in these subregions. Proteomics analysis of the hippocampus identified about 20 differentially expressed proteins in the alcoholic group [32], among which was glutamine synthetase, an enzyme located predominantly in astrocytes.

From 17 proteomics studies, we collected 237 proteins reported to be associated with alcohol exposure (listed in Supplemental Table 1). Some of the representative biochemical pathways enriched among these proteins are glycolysis/gluconeogenesis, oxidative phosphorylation, 14-3-3-mediated signaling, Huntington's disease signaling, p70S6K signaling, and cAMP response element-binding (CREB) signaling in neurons.

Amphetamine/Methamphetamine

All available proteomics analyses of the effects of amphetamine/methamphetamine on the central nervous system (CNS) are based on animal models. Freeman et al. [33] analyzed the proteomics profiles of amphetamine self-administration transitional states in the hippocampus of rats. Actin- and cytoskeletal-related proteins were over-represented among those changing during abstinence. Iwazaki et al. [34] profiled protein expression in the striatum of rats subjected to acute methamphetamine administration. Proteins involved in energy metabolism, oxidative stress response, or the ubiquitin-proteasome pathway were significantly altered. In addition, Iwazaki et al. analyzed the protein expression profile in the amygdala and striatum of rats with methamphetamine-induced behavioral sensitization [35, 36]. Their results indicate that the amygdala reacts differently at the protein level to acute vs. repeated administration of methamphetamine. A number of proteins in the categories of synaptic, cytoskeletal, oxidative stress, apoptosis, and mitochondria-related were differentially expressed in the amygdala of sensitized animals. For the striatum, 31 differentially expressed proteins were identified for methamphetamine-sensitized rats compared with control animals, including synapsin II, synaptosomal-associated protein 25 (SNAP25), and adenylyl cyclase-associated protein 1. A proteomics analysis of rat cortex following acute methamphetamine exposure identified 82 differentially expressed proteins, 40 of which showed reduced expression and the other 42 induced expression [37]. Proteins that decreased in abundance included collapsin response mediator protein-2, superoxide dismutase 1 (SOD 1), phosphatidylethanolamine-binding protein-1, and mitogen activated kinase kinase-1. Proteins that increased in abundance included authophagy-linked microtubule-associated protein light chain 3, synapsin-1,

and Parkinsonism-linked ubiquitin carboxy-terminal hydroxylase-L1 (UCHL1). Faure et al. [38] examined protein expression profiles in the rat frontal cortex after exposure to eight methamphetamine injections, and 47 altered cytosolic and 42 altered membrane proteins were identified, which were associated with protein degradation, redox regulation, energy metabolism, cellular growth, cytoskeletal modifications, and synaptic function. Li et al. [39] used proteomics to identify global protein profiles associated with methamphetamine-induced neurotoxicity in the striatum, hippocampus, and frontal cortex. Of the proteins identified, alpha-synuclein had induced expression, and mitochondrial ATP synthase D chain had reduced expression in all brain regions. Two proteins, Cu/Zn SOD 1 and subunit of mitochondrial H-ATP synthase, also had reduced expression. The parallel expression patterns of these proteins suggested that the pathogenesis of methamphetamine neurotoxicity involves the same pathways in different brain regions. In a study intended to explore the effect of methamphetamine in the mesolimbic dopamine system, Yang et al. [40] found that a conditioned place preference was induced by methamphetamine exposure in rats. Protein profiles in the NAc, striatum, PFC, cingulate cortex, and hippocampus were compared. Further screening identified 27 differentially expressed proteins, including some related to the cytoskeleton, transport/endocytosis or exocytosis, and signal transduction.

From eight proteomics studies, we collected 207 proteins reported to be differentially expressed in response to amphetamine or methamphetamine (for a detailed list, please see Supplemental Table 1). Besides the pathways involved in energy generation-related biological processes, some of the representative pathways enriched in these proteins are 14-3-3-mediated signaling, Huntington's disease signaling, p70S6K signaling, VEGF signaling, protein kinase A signaling, and CREB signaling in neurons.

Cocaine

Because some comprehensive reviews of the application of proteomics and other high-throughput approaches to the study of cocaine addiction are already available [10, 41], only a brief review is provided here. Hemby and colleagues [14, 42] analyzed the protein profile in the NAc of cocaine overdose victims. Among the proteins differentially expressed in the overdose and control groups were beta-tubulin, liprin-alpha3, and neuronal enolase, with decreases in parvalbumin, ATP synthase beta-chain, and peroxiredoxin 2. Tannu et al. [43] further examined the proteome and phosphoproteome of the NAc following chronic cocaine self-administration in rhesus monkeys. Proteins involved in cytoskeleton structure, mitochondrial function, energy metabolism, and cell signaling were differentially expressed in this brain region. In another study, by examining the proteome in the NAc of Sprague Dawley rats, del Castillo et al. [44] identified five proteins responsible for the different rate of extinction of cocaine-induced conditioned place preference. They also found that a single administration of cocaine could lead to an alteration of the expression of multiple proteins in this brain region. It was also found that cocaine can enhance the replication of HIV-1 in normal human astrocytes; multiple proteins involved in different pathways are modulated in this process [45].

From five proteomics studies, we collected 68 proteins reported to be associated with cocaine exposure (Supplemental Table 1). Besides the pathways related to energy generation-related biological processes, some of the representative pathways enriched were 14-3-3-mediated signaling, Huntington's disease signaling, clathrin-mediated endocytosis signaling, and glutamate receptor signaling.

Marijuana

Cannabinoids, the main psychoactive component of marijuana, dysregulate the CNS and immune responses via interaction with cannabinoid receptors. Bindukumar et al. [46] investigated the global molecular effects of cannabinoids on normal human astrocytes using a combination of genomic and proteomics analyses. Among the differentially expressed proteins are those involved in cell structure (e.g., chromobox homolog 1, RAN binding protein 1, tubulin alpha 6), energy metabolism (e.g., aldolase fructose-bisphosphate [ALDOA], creatine kinase B chain, enolase 1 alpha [ENO1], M2 pyruvate kinase [PKM2]), signal transduction (e.g., prohibitin, rho GDP-dissociation inhibitor beta), protein synthesis or modification (e.g., cathepsin D, heat shock 60-kDa protein 1 [HSPD1], ribosomal protein S4), as well as transport (e.g., chloride channel and heterogeneous nuclear ribonucleoprotein F). There are a few studies focusing on the protein expression patterns in brain tissues of animals exposed to a notable cannabinoid, delta-9- tetrahydrocannabinol (THC), determined with a proteomics approach. By comparing the protein expression profiles in the hippocampus of adolescent and adult rats subjected to repeated THC injections, Quinn et al. [47] uncovered 17 unique differentially expressed proteins in the adolescent rats and nine in the adult rats, with many of the affected proteins being involved in oxidative stress/mitochondrial function and cytoarchitecture. In another study, Rubino et al. [48] identified 11 proteins differentially expressed in the PFC synaptosomes of THC-treated female rats. Of these proteins, ten were suppressed by THC and only one was induced, which might indicate a reduced synaptic efficiency in the PFC caused by THC treatment. Via proteomics analysis, Colombo et al. [49] identified 24

unique differentially expressed proteins in the cerebellum of chronically THC-treated mice. Among these proteins, several alpha and beta subunits of guanine nucleotide-binding proteins, calcium-binding proteins (calretinin and hippocalcin-like protein 1), and dynamin-1 had induced expression.

From the four proteomics studies discussed above, we collected 80 proteins reported to be associated with exposure to cannabinoids (provided in Supplemental Table 1). Besides the pathways involved in energy generation-related biological processes, some representative enriched pathways are Huntington's disease signaling, axonal guidance signaling, protein kinase A signaling, 14-3-3-mediated signaling, CREB signaling in neurons, and alpha-adrenergic signaling.

Morphine/Heroin/Butorphanol

The diverse pharmacologic and physiological actions of morphine and similar drugs (e.g., butorphanol, heroin) are attributed mainly to their interaction with opioid receptors to trigger multiple signal transduction pathways. Applying proteomics techniques to morphine addiction has attracted much attention. Most of the published investigations are based on animal models or cell lines. Because of their importance in the development and maintenance of drug dependence, a few brain regions, such as the NAc [50], frontal cortex [12, 51], hippocampus [12], and striatum [12, 52], are the primary targets of proteomics analysis in the study of morphine, heroin, or butorphanol addiction. Whole brain [53] or components of the nervous system, such as the spinal cord [54], dorsal root ganglia [55], or cultured neuronal cells [56, 57], also have been investigated. In some studies, proteins expressed in the synapse were extracted and analyzed directly via proteomics approaches [58–61].

Because a few reviews have provided comprehensive summaries of the proteins associated with exposure to morphine [62, 63], here, we provide only a brief overview. Most of the proteins identified from proteomics analyses fall into several major functional categories, such as protein modification, energy metabolism, cell cytoskeleton, signal transduction, and vesicular trafficking; many of these proteins are involved in biological processes such as synaptic plasticity, learning, and memory. Similar patterns are also observed in analyses focused on phosphotyrosyl proteins in the frontal cortex of morphine- or butorphanol-dependent rats [51, 64].

From 14 proteomics studies focused on the effect of morphine/heroin/butorphanol on brain tissues or neurons, we collected 145 proteins reported to be associated with drug exposure (provided in Supplemental Table 1). Besides the pathways related to energy generation-related processes, some of the representative signaling pathways enriched in these proteins include Huntington's disease signaling, CREB signaling in neurons, protein kinase A signaling, GNRH signaling, alpha-adrenergic signaling, and RhoA signaling.

Nicotine

Although only a few proteomics studies on nicotine addiction have been reported, much information on the biochemistry and biological processes underlying nicotine dependence has been obtained. Yeom et al. [65] reported that chronic nicotine administration (7 days) significantly regulated the expression of seven proteins in the striatum of male Holtzman rats. Of these proteins, three were induced by nicotine, i.e., zinc-finger binding protein-89, cyclic nucleotide phosphodiesterase, and deoxyribonuclease 1-like 3; the other four proteins, i.e., tandem pore domain halothane-inhibited potassium channel 2, brain-specific hyaluronan-binding protein, death effecter domain-containing protein, and brain-derived neurotrophic factor, were suppressed. The expression patterns of most proteins were coincident with their mRNAs. This work demonstrated the potential of the proteomics approach as a powerful method to identify the novel markers related to nicotine addiction. In a more comprehensive study [11], protein expression profiles were analyzed for five brain regions—the amygdala, NAc, PFC, striatum, and ventral tegmental area (VTA)—of rats that received nicotine for 7 days. Region-specific expression patterns were observed. Several proteins (e.g., dynamin1, laminin receptors, aldolase A, SNAP-beta, and *N*-ethylmaleimide-sensitive fusion protein) were differentially expressed in multiple brain regions. These differentially expressed proteins can be grouped into several biological process categories, such as energy metabolism, synaptic transmission, and oxidative stress response. Although nicotine addiction is caused primarily by neural adaptations to nicotine in the CNS, alterations related to nicotine exposure also happen in other tissues [66]. Because of their being readily available, peripheral tissues have been used in proteomics studies to infer the status of the CNS. In one study, 15 proteins were identified in serum and polymorphonuclear and mononuclear leukocytes from rats receiving chronic nicotine (14 days) [67]. The regulation of these proteins was suggested to be related to activities of nicotine in the central nervous system. In another study, serum proteomics analysis was conducted in rats subjected to nicotine self-administration, extinction, and relapse [68], which revealed specific protein patterns in the serum.

From the two proteomics studies focused on the effect of nicotine on brain tissues of animals [11, 65], 67 proteins appearing to be associated with nicotine treatment were

collected (provided in Supplemental Table 1). Besides the pathways related to energy generation-related biological processes, some of the representative pathways enriched in these proteins include Huntington's disease signaling, gamma-aminobutyric acid (GABA) receptor signaling, clathrin-mediated endocytosis signaling, and glucocorticoid receptor signaling.

Proteins Associated with the Exposure of Multiple Addictive Drugs

Because of the diversity of addictive substances, it would be difficult to cover all the proteins for all the drugs. Thus, here, we concentrate on a few relatively common drugs, i.e., alcohol, amphetamine/methamphetamine, cocaine, morphine, heroin, butorphanol, marijuana, and nicotine. Because morphine, butorphanol, and heroin have similar mechanisms of action, they are considered in one category. Altogether, there are 497 proteins reported to be directly or indirectly related to the biological response to addictive drugs (as of March 2011; Supplemental Table 1). In the present study, we defined a protein as being of interest when it was identified for at least two addictive drugs. After applying this rule, we compiled a list of 160 proteins (Table 1). These proteins may be among the most important molecules related to exposure to drugs of abuse and thus be of great interest. At the same time, because they have been identified for different drugs by various research laboratories, this information is considered to be more reliable.

Similar to other high-throughput approaches such as microarray technology, the true power of the proteomics approach is its ability to provide a comprehensive perspective on the protein family and pathways related to a specific condition such as exposure to a substance of abuse. Proteomics provides an efficient way to map the expression of complex biological pathways and identifies proteins associated with an experimental condition of interest. Furthermore, it enables comparison of the expression of the pathways or function-related proteins under different but related experimental conditions. This is especially valuable for the study of drug addiction because such a comparison not only helps us understand the specificity of the physiological effects of different drugs but also provides insight into the similarity between the molecular and cellular mechanisms related to addiction to these drugs.

The proteins were grouped into multiple categories according to their functional relevance to biological processes in neurons (Table 1; Fig. 1). The category assignment of each protein is based on the Gene Ontology (GO) information, but it is not identical to GO category. For each protein, the corresponding GO categories were retrieved. Because the proteins were assigned to different GO categories at different levels, the GO information was compared with the function description of the protein given in NCBI OMIM (http://www.ncbi.nlm.nih.gov/omim) or SOURCE (http://source.stanford.edu), and the most relevant one was selected. Of the proteins, more than one third (56/160) are related to energy metabolism, some of which play important roles in cellular energy generation and are identified for multiple addictive drugs; e.g., ALDOA, PKM2, ATP synthase ATP5G1, ENO1, and triosephosphate isomerase 1. Oxidative stress response proteins (14; 8.75%) that function in the detoxification of hydrogen peroxide and other reactive oxidative species also have been identified as being affected multiple addictive drugs, the examples being glutathione peroxidase 1, glutathione *S*-transferase pi 1, peroxiredoxin 2, and SOD 1. A number of proteins involved in protein modification and degradation (15; 9.38%) are associated with the addictive drugs, among which are several chaperones such as heat shock 70kD protein 8 (HSPA8), chaperonin 60, chaperonin containing t-complex polypeptide 1 subunit 6a, peptidylprolyl isomerase A, as well as proteins of the ubiquitin pathway, i.e., UCHL1 and ubiquitin-conjugating enzyme E2N (UBE2N). The identification of a large number of proteins involved in energy metabolism, oxidative stress response, and protein modification and degradation for each additive drug clearly demonstrates the importance of these biological processes in the development and maintenance of drug dependence. In an earlier review, we presented a detailed summary of proteins involved in these biological processes and discussed their potential roles in the physiological conditions of drug dependence [17]. The correlation of these biological processes with each addictive drug also has been discussed in some proteomics studies [11, 28, 34, 43, 46, 52].

Besides those involved in energy metabolism, oxidative stress response, and protein modification and degradation, proteomics analyses have identified proteins associated with other biological processes, including synaptic function, signal transduction, neuronal function (other than synaptic transmission), cell structure and organization, and transport. Although all these biological processes are related to the function of the nervous system, considering their potential roles in drug dependence, we will focus on synaptic function and signal transduction in this section.

Synaptic Transmission

Most of the proteins related to synaptic function are involved in synaptic vesicle trafficking. Trafficking of vesicles is one of the primary tools for the exchange of macromolecules between different compartments of the endomembrane system [69–71], as well as between the cell and the extracellular environment [71]. For neurons, besides trafficking cargo necessary for cell survival and

Table 1 Proteins found to be modified by at least two drugs of abuse

Gene symbol	Protein name	Alcohol	Amphetamine/ methamphetamine	Cocaine	Marijuana	Morphine/ heroin/ butorphanol	Nicotine
Amino acid and protein metabolism							
EEF1A1	Eukaryotic translation elongation factor 1, alpha-1	[20]	[37]				
EEF1G	Elongation factor 1-gamma	[27]	[37]	[45]			
EEF2	Elongation factor 2	[23]	[37]				
EEFTU	Elongation factor Tu				[46]	[52]	
GLN1	Gln synthetase					[60]	[11]
GOT1	Glutamate oxaloacetate transaminase 1					[60]	[11]
LAP3	Leucine aminopeptidase 3	[20]	[35]				
PPP3R1	Protein phosphatase 3, catalytic subunit, alpha isoform			[42]	[47]		[11]
Apoptosis							
CSTB	Cystatin-B		[34, 39]		[46]		
GLO1	Glyoxalase 1	[29, 30]	[36, 38]				
Cell structure and organization							
ACTB	Beta actin	[19, 23, 27]	[36, 38]	[14, 43]	[48]	[50, 52, 57, 64]	
ACTG1	Gamma-actin		[34, 38]	[43]			
ACTR2	Actin-related protein 2	[20]				[61]	
CAPZB	F-actin-capping protein subunit beta		[37]			[12]	
FSCN1	Fascin	[32]	[38]				
PACSIN1	Protein kinase C and casein kinase substrate in neurons protein 1		[38]			[12]	
TPM1	Tropomyosin 1	[19]				[55]	
TPM3	Tropomyosin 3	[19, 31]	[35, 36]				
TUBA1	Tubulin A	[30]		[14, 43]		[51, 64]	
TUBA1A	Tubulin, alpha-1a	[20]	[37]			[52]	
TUBA2	Tubulin, alpha-2				[47]	[52]	
TUBB3	Tubulin b-3 chain				[47]	[52]	
Energy metabolism							
ACO2	Aconitase 2, mitochondrial	[27]	[36, 38]		[47]	[54, 60, 61]	[11]
AKR1B4	Aldo-keto reductase family 1, member B4			[42]			[11]
ALDH5A1	Succinate-semialdehyde dehydrogenase, mitochondrial		[35, 36]				[11]
ALDH6A1	Aldehyde dehydrogenase 6 family, member a1	[117]	[36]				
ALDOA	Aldolase, fructose-bisphosphate	[19, 22]	[37]	[45]	[46]	[50, 64]	[11]
ALDOC	Aldolase C, fructose-bisphosphate	[27, 32]	[36, 38]	[43, 45]		[55]	
ATP1A1	ATPase, Na^+/K^+ transporting, alpha-1 polypeptide	[20]	[37]				
ATP1A3	ATPase, Na^+/K^+ transporting, alpha-3 polypeptide	[20]				[58]	
ATP5A1	ATP synthase subunit alpha, mitochondrial	[19, 23, 27]		[44]	[48]	[12]	
ATP5B	Mitochondrial ATP synthase, beta subunit		[34, 37, 38]	[43]	[48]	[12, 55]	
ATP5G1	ATP synthase, H^+ transporting, mitochondrial F0 complex, alpha subunit, isoform 1	[18]	[33, 39]	[14]		[60]	[11]
ATP6V1A1	Similar to ATPase, H^+ transporting, V1 subunit A, isoform 1	[21]				[12, 64]	[11]
ATP6V1B2	Similar to ATPase, H^+ transporting, V1		[38]	[43]		[61]	

Table 1 (continued)

Gene symbol	Protein name	Alcohol	Amphetamine/ methamphetamine	Cocaine	Marijuana	Morphine/ heroin/ butorphanol	Nicotine
	subunit B, isoform 2						
ATP6V1H	ATPase, H^+ transporting, lysosomal, 50/57-kd, v1 subunit H	[20]	[37]				
CKB	Creatine kinase B chain	[19, 27, 30, 32]	[34, 37, 38, 40]	[43]	[46]	[55, 61]	
CKMT1	Creatine kinase, mitochondrial 1, ubiquitous		[36]			[58]	
CNDP2	Cytosolic non-specific dipeptidase	[30]				[52]	
COX5B	Cytochrome c oxidase polypeptide Vb	[20]				[12]	
CS	Citrate synthase, mitochondrial	[117]	[37]				
DDAH1	Dimethylarginine dimethylaminohydrolase 1		[39]	[43]	[49]		
DDAH2	Dimethylarginine dimethylaminohydrolase 2		[35]	[43]			
DLAT	Dihydrolipoamide acetyltransferase		[38]			[60, 61]	[11]
DLD	Dihydrolipoamide dehydrogenase	[27]	[34, 38]			[52]	[11]
ENO1	Enolase 1, alpha	[24, 117]	[33–35, 38]	[14, 43, 45]	[46]	[64]	[11]
ENO2	Enolase 2, gamma	[19, 20, 30]	[38]	[14, 43]		[50, 51, 64]	
ENOA	Alpha-enolase		[37]			[52]	
FABP7	Fatty acid-binding protein 7	[117]	[39]			[57]	
GAPDH	Glyceraldehyde 3-phosphate-dehydrogenase	[19, 25, 117]	[33, 36, 37]		[46]	[52, 64]	
GLUD1	Glutamate dehydrogenase 1	[117]	[36]			[52, 58, 61]	[11]
GPI	Glucose-6-phosphate isomerase	[20]	[37]				
HK1	Hexokinase 1	[20]	[37]				
IDH3A	Isocitrate dehydrogenase 3 (NAD^+) alpha	[30]	[36, 38]	[14]		[60]	[11]
LDHA	L-lactate dehydrogenase A chain	[20, 31]	[37]				
LDHB	L-lactate dehydrogenase B chain	[27, 30]	[34, 36]	[43]	[49]	[12]	[11]
MDH1	Malate dehydrogenase, mitochondrial	[19, 23]	[36, 37]	[45]		[12]	
MOR1	Malate dehydrogenase, mitochondrial	[15]				[60, 64]	[11]
NDPKB	Nucleoside diphosphate kinase B			[44]	[47]		
NDUFA10	NADH dehydrogenase 1 alpha subcomplex 10-like protein	[20]	[40]	[44]		[52]	[11]
NDUFA5	NADH-ubiquinone oxidoreductase 13-kDa B subunit	[20, 29]				[59]	
NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2	[19, 25]	[33]			[58]	[11]
NDUFV2	NADH-ubiquinone oxidoreductase 24-kDa subunit		[38]	[44]		[59, 61]	
PDHA	Pyruvate dehydrogenase (lipoamide) alpha		[38]			[12]	
PDHB	Pyruvate dehydrogenase (lipoamide) beta	[20, 24, 27]	[38]				[11]
PFKP	Phosphofructokinase, platelet type	[20]	[37]				
PGAM1	Phosphoglycerate mutase 1		[36, 37]		[47]		[11]
PGK1	Phosphoglycerate kinase 1	[117]	[34, 36, 38]	[45]	[46]		
PGM1	Phosphoglucomutase 1	[20]	[37]				
PHGDH	D-3-Phosphoglycerate dehydrogenase	[32]	[36, 38]		[49]		
PKM2	M2 pyruvate kinase	[21]	[34, 37, 38]		[46]	[52, 58, 61, 64]	[11]
SDHA	Succinate dehydrogenase complex,				[47]		[11]

Table 1 (continued)

Gene symbol	Protein name	Alcohol	Amphetamine/ methamphetamine	Cocaine	Marijuana	Morphine/ heroin/ butorphanol	Nicotine
	subunit A, flavoprotein variant						
SIRT2	NAD-dependent deacetylase sirtuin-2	[31]			[47]		[11]
TKT	Transketolase	[25, 27]		[44]		[51]	[11]
TPI1	Triosephosphate isomerase 1	[19, 27, 29, 32, 117]	[35–38]	[42]	[47]	[12, 64]	[11]
UQCRC1	Ubiquinol-cytochrome c reductase core protein I	[23]	[34, 36, 37]	[14]	[48]	[53]	[11]
UQCRC21	Ubiquinol-cytochrome c reductase core protein III	[23]	[34, 36, 37]	[14]	[48]	[53]	[11]
Neuronal function							
DPYSL1	Dihydropyrimidinase-related protein 1		[37]		[49]	[52]	
DPYSL2	Dihydropyrimidinase-related protein 2	[30, 32]	[35–38]	[43, 44]		[52]	
DPYSL5	Dihydropyrimidinase-related protein 5		[36, 38]			[52]	
DRP2	Dystrophin-related protein 2	[25, 31]				[56, 64]	
GDA	Guanine deaminase		[36–38]	[43]			
GMFB	Glia maturation factor, beta		[35, 39]				
NEFL	Neurofilament light polypeptide	[31]	[36, 40]	[43]		[55]	
SEPT5	Septin 5		[38]			[56]	[11]
STIP1	Stress-induced-phosphoprotein 1	[32]	[36, 38]			[52]	
STMN1	Stathmin	[29]	[36, 39]				
TAGLN3	Transgelin 3		[38]		[47]	[54]	
TMOD2	Tropomodulin 2		[34]			[61]	
Oxidative stress response							
GPX1	Glutathione peroxidase 1	[31]			[46]		
GSTM1	Glutathione *S*-transferase Mu 1	[29]	[36]				
GSTM3	Glutathione *S*-transferase Mu3		[36]		[47]		
GSTO1	Glutathione *S*-transferase omega 1			[42, 44]	[47]	[60]	[11]
GSTP1	Glutathione *S*-transferase, pi 1	[30]	[36]			[53]	[11]
PARK7	Parkinson disease protein 7	[32]			[47]		
PDIA1	Protein disulfide isomerase	[29]	[36]				
PDIA3	Protein disulfide isomerase 3		[36, 38]			[52, 57]	
PEBP	Phosphatidylethanolamine-binding protein 1	[20, 32]	[35–38]		[48]	[12]	
PRDX2	Peroxiredoxin 2	[15, 18, 24, 29]	[33, 34, 38]	[14, 43, 44]	[49]		[11]
PRDX5	Peroxiredoxin 5	[20]	[34, 36]	[44]			
PRDX6	Peroxiredoxin 6	[27]	[36, 37]		[47]		
SOD1	Superoxide dismutase	[29, 32] [20]	[35, 37, 39]			[12]	
SOD2	Superoxide dismutase [Mn], mitochondrial		[37]			[12]	
Protein modification and degradation							
CCT2	Chaperonin containing tcp1, subunit 2	[23]				[57]	
CCT3	Chaperonin containing tcp1, subunit 3	[19]	[35, 38]			[52]	
CCT5	Chaperonin containing tcp1, subunit 5	[117]	[38]	[45]			
CTSD	Cathepsin D	[29]			[46]	[51, 64]	[11]
HSC70-ps1	Heat shock 70-kDa protein 8, pseudogene 1	[19]	[40]				[11]
HSP60	Heat-shock 60-kD protein 1			[43, 45]	[47]		[11]
HSP7C	Heat-shock 71-kD protein 1				[47]	[12]	

Table 1 (continued)

Gene symbol	Protein name	Alcohol	Amphetamine/ methamphetamine	Cocaine	Marijuana	Morphine/ heroin/ butorphanol	Nicotine
HSP90AB1	Heat shock protein HSP 90-beta		[37]			[52]	
HSPA2	Heat shock 70-kDa protein 2	[19, 24, 30]	[37]	[42]		[59]	[11]
HSPA8	Heat shock 70-kDa protein 8	[19, 24, 30]	[37, 38, 40]	[42, 43]	[48, 49]	[59]	[11]
HSPA9	Heat shock 70-kDa protein 9		[37]		[47]	[54]	
HSPD1	Heat shock 60-kDa protein 1		[33, 37, 40]	[14, 44]	[46]	[60]	[11]
PPIA	Peptidylprolyl isomerase A					[60, 64]	[11]
UBE2N	Ubiquitin-conjugating enzyme E2N	[19]	[36, 39]				
UCHL1	Ubiquitin carboxy-terminal hydrolase L1	[24, 25, 32, 117]	[34, 37, 38]		[48]	[50, 55]	[11]
Response to stimulus							
CRYM	Crystallin, mu	[29]	[38]	[43]		[51, 64]	[11]
GFAP	Glial fibrillary acidic protein	[31]	[36, 40]	[43]			
WDR1	WD repeat-containing protein 1	[20]				[12]	
Signal transduction							
ANXA3	Annexin A3	[19]	[38]		[47]		
ANXA5	Annexin A5	[19, 30]			[47]	[56]	[11]
ARF1	ADP-ribosylation factor 1	[29]	[36, 37]				
CALM	Calmodulin (CaM)	[20, 23]	[38]				
CAMK2A	Calcium/calmodulin-dependent protein kinase II alpha	[20]				[61]	
CAMK2B	Calcium/calmodulin-dependent protein kinase II beta	[20]	[37]				
GDI1	Rab GDP dissociation inhibitor alpha	[30]	[38, 40]	[43]			
GNAI1	Guanine nucleotide-binding protein Gi, alpha-1 subunit	[20]				[59]	
GNAO	GTP-binding protein alpha o	[20]			[49]	[51, 64]	[11]
GNB1	Guanine nucleotide-binding protein, beta-1 subunit	[32]	[36, 38, 40]			[55, 56]	[11]
GNB2	Guanine nucleotide-binding protein, beta-2 subunit		[38]		[49]	[61]	
GNB42	Guanine nucleotide-binding protein, beta-24 subunit		[38]		[49]	[52, 61]	
MAP2K1	ERK activator kinase 1	[20]	[37, 38]				
PHB	Prohibitin	[23]	[38]		[46]		[11]
RAB1	Ras-related protein Rab-1A	[20, 27]			[47]	[59]	
YWHAE	14-3-3 protein eps	[30]	[36, 40]		[47]		
YWHAZ	14-3-3 protein isoform zeta	[19, 23, 117]	[35]	[14, 43]	[47]	[53, 58, 64]	
Synaptic function							
ABAT	4-Aminobutyrate aminotransferase		[36]			[12]	
CALB1	Calbindin 1	[20]	[37]				
CALB2	Calbindin 2	[20]	[37]		[49]		
CLTC	Clathrin, heavy polypeptide	[20]				[58]	
CNP	Cyclic nucleotide phosphodiesterase					[58, 61]	[65]
DNM1	Dynamin 1				[49]	[58]	[11]
DNM1L	Dynamin-1-like protein		[34]			[12]	
GLUL	Glutamate-ammonia ligase	[32]	[36]	[43]		[58]	
GRIP1	Glutamate receptor interacting protein			[43]		[54]	
NAPA	*N*-Ethylmaleimide sensitive fusion					[51, 56]	[11]

Table 1 (continued)

Gene symbol	Protein name	Alcohol	Amphetamine/ methamphetamine	Cocaine	Marijuana	Morphine/ heroin/ butorphanol	Nicotine
	protein attachment protein alpha						
NAPB	Similar to beta-soluble NSF attachment protein (SNAP-beta)			[43]		[64]	[11]
NSF	*N*-Ethylmaleimide sensitive fusion protein	[22]	[36]			[59, 60]	[11]
SNAP25	Synaptosomal-associated protein 25 kDa	[19]	[35]				
SNCA	Alpha synuclein (phosphoneuroprotein 14)	[20]	[37, 39]	[44]			
SNCB	Beta synuclein (phosphoneuroprotein 14)	[19]	[36–38]				
STXBP1	Syntaxin binding protein 1	[20, 117]	[37, 40]			[58, 60, 61]	[11]
SYN2	Synapsin IIb	[117]	[35, 36, 38]			[50, 59, 61]	[11]
Transport							
ERP29	Endoplasmic reticulum protein, 29-kDa		[36, 37]			[12]	
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1		[37]			[12]	
HNRPH2	Heterogeneous nuclear ribonucleoprotein H2			[45]		[57]	
VDAC1	Voltage-dependent anion channel 1	[19, 21, 24]	[34, 36, 38]			[58, 60]	[11]
VDAC2	Voltage-dependent anion channel 2	[19, 20]	[36, 38]				

development, vesicle-mediated trafficking plays a special role in neuronal function, as synaptic transmission relies on neurotransmitter release via the exocytotic pathway and its recycling via the endocytotic pathway [72–75]. Vesicle trafficking involves the generation of a vesicle from the membrane of the donor compartment, the transport of the vesicle to its destination, and the fusion of the vesicle with the target compartment [69, 70]. Vesicle formation requires uptake of coat proteins binding to the cargo and induction of membrane curvature and targeting and fusion factors, including soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) [70, 71]. The specificity

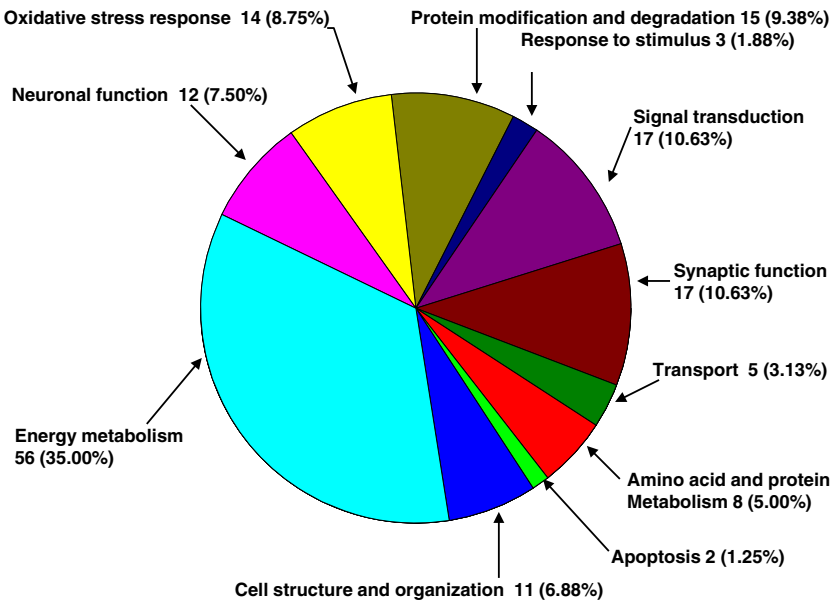

Fig. 1 Functional categories of proteins found to be associated with exposure to at least two addictive drugs. *Numbers* following the category names indicate the number of proteins in the category, and those in parentheses are the corresponding percentages

of vesicle targeting and docking is achieved through mutual recognition of SNAREs located on the membrane of the vesicle and target apparatus [70]. Once the two classes of SNAREs bind to each other, other proteins, such as soluble *N*-ethylmaleimide-sensitive factor (NSF) and NSF attachment protein (NAP), are recruited to form a 20S complex that drives membrane fusion [69, 70]. During synaptic transmission, synaptic vesicles collapse and fuse with the cell membrane at the presynaptic terminals of the neuron to release the neurotransmitters and retrieve the vesicles via clathrin-mediated endocytosis.

Multiple proteins involved in vesicle budding, docking, and fusion in cellular vesicular transport have been found to be associated with addictive substances in proteomics studies (Table 1; Supplemental Table 1), e.g., dynamin 1 (DNM1), NSF, NSF attachment proteins (NAPA and NAPB), syntaxin binding protein 1 (STXBP1), and synapsin IIb (SYN2). These proteins are critical for the regulation of highly specific and coupled intracellular vesicle trafficking, as well as the cellular endocytotic and exocytotic pathways.

Dynamin 1, a brain-specific protein GTPase critical for membrane retrieval at the presynaptic terminal following fusion of neurotransmitter vesicles [73], is differentially expressed at the protein level in response to treatment with either nicotine or morphine. Chronic nicotine exposure reduces the concentration of dynamin 1 in the amygdala and VTA of rat but induces the protein in the NAc and PFC [11]. A similar pattern was observed for this gene at both the mRNA and protein levels in an independent study on animals and the SH-SY5Y cell line [76]. It is suggested that this gene is regulated by nicotine via the miRNA pathway [77]. Chronic morphine treatment increased the expression of dynamin 1 at the hippocampal postsynaptic density [58]. At the same time, dynamin 1-like protein, dynamin 2, as well as the coat protein closely related to dynamin 1, clathrin, are regulated by morphine [12, 54, 58]. An earlier study showed that both heroin and morphine induce the expression of dynamin 1 in the cerebral cortex of rats [78]. On the other hand, microarray analysis showed that chronic ethanol treatment decreased the dynamin 1 mRNA content of the rat hippocampus [79]. Dynamin 1 is involved in clathrin-mediated endocytosis and other vesicular trafficking processes [80]. In neurons, it plays a key role in synaptic vesicle recycling [72, 73, 81–83]. Also, dynamin 1 interacts with mGluR5, one of the major glutamate receptors that modulate cell excitability and synaptic transmission [84]. Moreover, it may be able to regulate signal transduction pathways such as the MAPK cascade [85, 86].

Several other proteins associated with exposure to addictive drugs are connected with the SNARE complex. For example, NSF, NAPA, NAPB, STXBP1, and SYN2 are associated with nicotine treatment (Table 1), whereas NSF, NAPA, STXBP1, and SYN2 are associated with morphine exposure (Table 1; Supplemental Table 1) and NSF, STXBP1, SYN2, NSFL1 cofactor (NSFL1C or p47), synaptosomal-associated proteins (SNAP25 and SNAP29), and syntaxin 1B2 (STX1B) are associated with alcohol exposure (Table 1; Supplemental Table 1). Syntaxins are target membrane SNAREs [69, 70, 87] and are directly involved in calcium ion-dependent synaptic transmission [88]. Syntaxin-binding proteins (e.g., STXBP1) are neural-specific proteins that participate in the regulation of synaptic vesicle docking and fusion [89]. NSF, SNAPs (e.g., NAPA, NAPB, and NAPG), and synapsins are involved in the formation of the SNARE complex and play important roles in the regulation of cellular exocytosis [69, 70, 90, 91]. Consistently, the clathrin-mediated endocytosis signaling pathway is enriched among the proteins we collected (Table 2).

Although vesicle trafficking is a ubiquitous process, operating in many cell types, it plays a critical role in neurons [73]. On one hand, it is the major tool for synaptic vesicle recycling within the presynaptic compartment. It is also involved in regulating the trafficking of postsynaptic receptors, thus allowing fine-tuning of signal strength during neurotransmission. On the other hand, vesicle trafficking is fundamental in the cellular physiological activity of neurons. The size and architecture of mature neurons remain relatively stable in their lifetimes even though the proteins and lipids making up the cells are continually being degraded and replenished. The morphologic stability of neurons requires an overall balance between endocytosis and exocytosis of lipids, proteins, and other metabolites to avoid marked changes in cell size and shape [73, 91]. Thus, the stable and controllable expression of the vesicle trafficking-related proteins is extremely important for proper function [73]. The modification of the expression of proteins involved in the vesicle-mediated trafficking system implies a disturbance of this balance, which may influence the efficiency of synaptic transmission and cause modification of neuron size and architecture. Repeated exposure to addictive substances not only results in the alteration of vesicle-trafficking machinery but also leads to a persistent change in synaptic plasticity in certain neurons.

The mechanisms underlying the modification of vesicle-mediated trafficking by addictive substances are not clear. As shown in Table 1 and discussed earlier [17], exposure to addictive drugs alters the expression of a large number of proteins involved in fundamental cellular processes such as energy supply, oxidative stress, or the protein synthesis and turnover rate. The modulation of these processes implies an altered environment in the CNS in response to the drug. The alteration in the concentration of vesicle-trafficking-

Table 2 Signaling pathways enriched in genes associated with exposure to multiple drugs

Pathway[a]	*P* value	FDR	Proteins included
Huntington's disease signaling	1.26×10^{-9}	8.22×10^{-7}	ATP5B, CLTC, CTSD, DNM1, GNB1, GNB2, GNB4, HSPA2, HSPA8, HSPA9, NAPA, NAPB, NSF, PACSIN1, SDHA, SNAP25, SNCA
14-3-3-mediated signaling	9.33×10^{-7}	2.80×10^{-5}	GFAP, MAP2K1, PDIA3, SNCA, TUBA1A, TUBA4A, TUBB3, YWHAE, YWHAZ
Axonal guidance signaling	5.37×10^{-5}	5.78×10^{-4}	CAMK2A, CAMK2B, GNAI1, GNB1, GNB2, GNB4, MAP2K1, PDIA3, PPP3R1, YWHAE, YWHAZ
Protein kinase A signaling	1.07×10^{-4}	9.38×10^{-4}	CAMK2A, CAMK2B, GNAI1, GNB1, GNB2, GNB4, MAP2K1, PDIA3, PPP3R1, YWHAE, YWHAZ
p70S6K signaling	7.59×10^{-4}	4.30×10^{-3}	EEF2, GNAI1, MAP2K1, PDIA3, YWHAE, YWHAZ
CREB signaling in neurons	1.66×10^{-4}	1.39×10^{-3}	CAMK2A, CAMK2B, GNAI1, GNB1, GNB2, GNB4, MAP2K1, PDIA3
P2Y purigenic receptor signaling	6.31×10^{-4}	3.79×10^{-3}	GNAI1, GNB1, GNB2, GNB4, MAP2K1, PDIA3
GABA receptor signaling	9.12×10^{-4}	5.04×10^{-3}	ALDH5A1, ABAT, DNM1, NSF
Clathrin-mediated endocytosis signaling	1.82×10^{-3}	0.0118	ACTB, ACTG1, ACTR2, CLTC, DNM1, HSPA8, PPP3R1
GNRH signaling	5.13×10^{-3}	0.0189	CAMK2A, CAMK2B, DNM1, GNAI1, MAP2K1
VEGF signaling	7.94×10^{-3}	0.0278	ACTB, ACTG1, MAP2K1, YWHAE
Synaptic long-term potentiation	0.0144	0.0428	CAMK2A, CAMK2B, MAP2K1, PPP3R1
Glutamate receptor signaling	0.0148	0.0431	GLUL, GNB1, GRIP1
RhoA signaling	0.0170	0.0489	ACTB, ACTG1, ACTR2, SEPT5
Calcium signaling	0.0204	0.0494	CAMK2A, CAMK2B, PPP3R1, TPM1, TPM3

[a] These pathways are significantly enriched in the proteins listed in Table 1. A significantly enriched pathway is defined as the one with $p<0.05$ and a false discovery (FDR)<0.05. The FDR is calculated by the method of Benjamini and Hochberg [118]

related proteins is one possible approach for the neurons in some brain regions to adapt to the altered extracellular and intracellular environment. It may also be a result of the regulation of signaling pathways by addictive drugs. Exposure to drug disturbs the synaptic transmission frequency and strength at the synapse, which can change the distribution of receptors (e.g., nAChRs, opioid receptors, etc.), as well as the regulation of downstream signaling cascades involved in various physiological processes. As feedback, these events may alter the efficiency and function of the vesicle-trafficking machinery, as evidenced by alterations in the expression of related proteins observed in proteomics studies.

Signal Transduction Pathways Associated with the Exposure of Multiple Addictive Drugs

A few other proteins involved in synaptic transmission are closely related to signal transduction. 4-Aminobutyrate aminotransferase (ABAT), the enzyme responsible for catabolism of GABA into succinic semialdehyde, is induced by methamphetamine in the amygdala of rats [36] and is suppressed by morphine in the rat cerebral cortex [12]. This protein is expressed in multiple tissues, including brain [92, 93]. This gene and *GRIN2A* are strongly co-associated with autism in humans [94]. The expression of glutamate-ammonia ligase (GLUL; also known as L-glutamine synthetase) is reduced in the hippocampus of human alcoholics [32]. It is also suppressed by acute methamphetamine exposure in the rat amygdala [36], by cocaine in the NAc of rhesus monkeys [43], and by morphine in the hippocampal postsynaptic density of mice [58]. The GLUL protein is expressed ubiquitously throughout the body and catalyzes the use of L-glutamate and ammonia to create glutamine. Within the CNS, GLUL clears L-glutamate, the primary excitatory amino acid neurotransmitter. At the same time, glutamine is the precursor for the synthesis of the neurotransmitters L-aspartate and GABA. Thus, GLUL has an important role in CNS neurotransmission [95]. Regulation of these proteins indicates the modulation of multiple signal transduction cascades in the neuron, such as the GABA and glutamate receptor signaling pathways.

Proteomics analyses have also identified other proteins involved in signal transduction. For example, chronic nicotine treatment reduces the abundance GTP-binding protein alpha o (GNAO), guanine nucleotide-binding protein beta-1 subunit (GNB1), and protein phosphatase 3 catalytic subunit alpha isoform (PPP3CA) in multiple brain regions of the rat [11]. The G proteins transduce extracel-

lular signals received by transmembrane receptors to intracellular effector proteins and are involved in numerous signal transduction pathways. The PPP3CA protein (known also as calcineurin A), the catalytic subunit of Ca^{2+}/calmodulin-regulated protein serine-threonine phosphatase, plays an important role in the induction of long-term potentiation and long-term depression and in the establishment of learning and memory [96–98]. Proteomics analyses showed that members of the 14-3-3 family (e.g., YWHAE and YWHAZ) are associated with exposure to multiple addictive drugs such as alcohol, butorphanol, cocaine, and morphine (Table 1). Previous study demonstrated that nicotine can regulate the interaction between bcl2 antagonist of cell death and 14-3-3 [99]. This protein can also affect the relative abundance of high and low agonist-sensitivity α4β2 nACh receptors [100, 101]. The 14-3-3 proteins are involved in the activation of RAF, a central component of signal transduction, by their participation in the protein kinase C signaling pathway [102, 103]; they are also crucial in a wide variety of cellular responses, including cell cycle progression, DNA damage checkpoints, and apoptosis [104].

Because of the diversity of the function of the proteins involved in signal transduction, we used an analysis tool called Ingenuity Pathway Analysis (www.ingenuity.com) to uncover the major biological pathways enriched among the 160 proteins. Some of the signal transduction pathways related to neuronal functions are shown in Table 2, among which are CREB signaling in neurons, GABA receptor signaling, glutamate receptor signaling, synaptic long-term potentiation, and calcium signaling. These pathways are involved in diverse neuronal functions. They are also among the central hubs of the molecular mechanisms underlying addiction to different drugs [2, 105, 106]. The correlation of these pathways and the addiction to individual drugs has been investigated extensively [10, 107–109]. Whereas earlier studies usually focused on one or a few individual targets in these pathways, the proteomics approach, as shown in this study, has the power to analyze multiple molecular targets, even multiple pathways, simultaneously, thus providing more direct evidence on the interaction between the addictive drugs and brain signaling pathways and between different signaling pathways.

Although the proteins identified in proteomics studies are involved in a number of biological processes, in this work, we mainly focus on those related to synaptic transmission and signaling pathways related to neuronal function and drug addiction. The mechanism underlying the correlation between the drug-addiction-related signaling pathways and synaptic transmission, especially vesicle trafficking, is an interesting topic. One approach is that vesicle trafficking is responsible for receptor transport between surface and intracellular pools or between synaptic and extrasynaptic pools on the cell surface. For example,

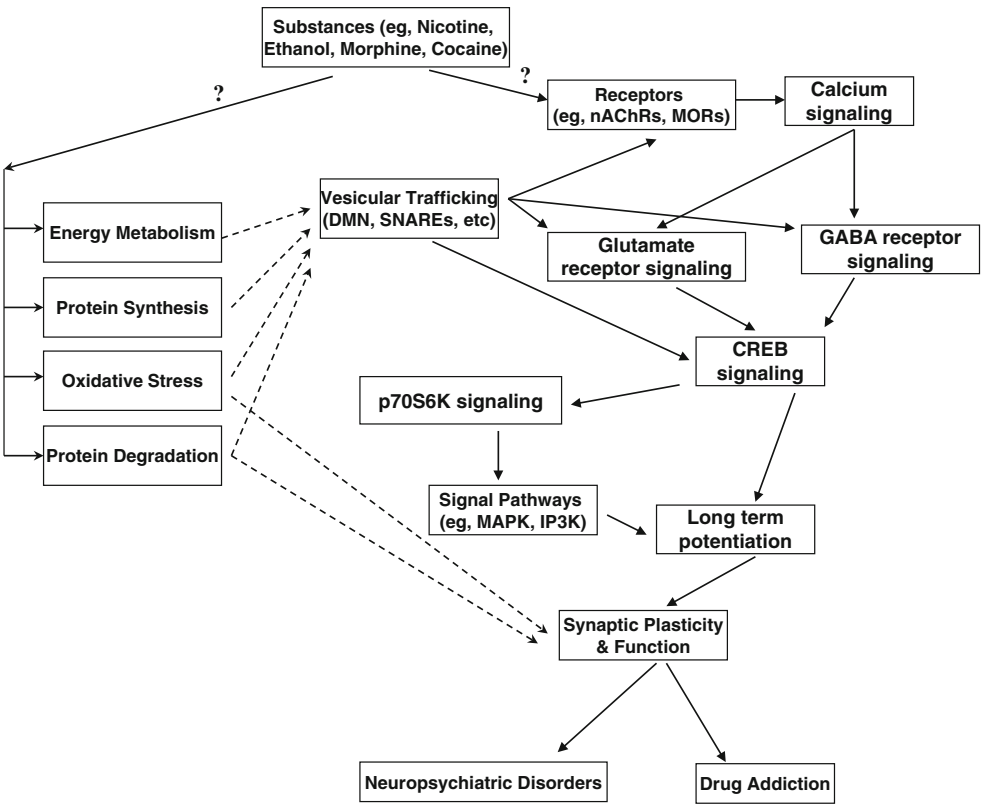

Fig. 2 Schematic model for the biological effects of addictive drugs on neurons. By interacting with certain receptors, substances of abuse modulate multiple signaling pathways. At the same time, some other biological processes, such as energy supply, oxidative stress, and protein synthesis, are also disturbed in neurons, which may evoke an alteration in the intracellular environment, forcing the neuron to adapt. These events may result in regulation of the vesicular trafficking system that is closely related to synaptic transmission and modulation of signaling pathways related to cell fate and neuron plasticity. The regulation of these pathways can evoke changes in neuron viability and structure and, ultimately, drug dependence and other neuronal disease

rapid trafficking of glutamate receptors in the postsynaptic neuron is important to synaptic plasticity. Receptor trafficking can generate long-term potentiation by increasing the number of postsynaptic AMPA receptors and induce long-term depression by decreasing AMPA receptors [110]. In somatic spines, α7-nAChRs are subject to nicotine-induced SNARE-dependent trafficking [110]. In this procedure, the receptors can either be removed from the cell surface or be recruited from intracellular pools to repopulate the surface. Such trafficking appears essential for maintaining coupling between receptor activation and downstream signaling pathways, including CREB signaling. Vesicle trafficking is also directly involved in the regulation of the signaling pathway. It is reported that dynamin-regulated endocytosis of MAP kinase kinase is required for the activation of the MAPK signaling cascade [85]. It is also suggested that endocytosis is involved in the regulation of Ras signaling [111]. Much detailed information about the interaction between vesicle trafficking and signaling pathways is still missing, but it is clear that elucidating such interaction will greatly improve our understanding of the development and maintenance of drug dependence.

Summary and Challenges

By comparing the protein expression profiles in response to treatment with alcohol, amphetamine, cocaine, marijuana, morphine, and nicotine, we compiled a list of 160 proteins that have been reported to be associated with exposure to multiple addictive drugs. We also identified a few biological processes that are enriched in these proteins, among them are energy metabolism, oxidative stress response, protein degradation and modification, synapse function, and signal transduction. Such similarity indicates that despite the obvious differences among their chemical properties and the receptors they interact with, different drugs may cause similar changes in cellular activities and biological processes in neurons. Earlier, we discussed the potential involvement of energy metabolism, oxidative stress response, and protein modification and degradation in the development and maintenance of drug dependence [17]. We further demonstrated that substances of abuse may directly or indirectly disturb the vesicle trafficking machinery and thus influence synaptic transmission in the neuron, which may be related to the regulation of multiple downstream signal transduction cascades and is a possible mechanism underlying the alteration in neuronal synaptic plasticity in the response to drug exposure (Fig. 2).

Even at its early stage, proteomics has become a powerful tool for examining and identifying the dynamics of proteins and the biological processes underlying drug dependence. However, because of the high complexity of the proteome and the limitations of current technology, not all the proteins associated with exposure to addictive drugs can be detected by proteomics approaches. As shown in Table 1 and Supplemental Table 1, in most proteomics studies on drug addiction, the majority of identified proteins fall into a relatively small number of categories, such as energy metabolism, cytoskeletal proteins, and protein modification and degradation, whereas proteins critical for drug dependence, such as neurotransmitter receptors, GTPases, kinases, and phosphatases, are underrepresented. Taking the receptors as an example, some receptors, such as nAChRs and opioid and cannabinoid receptors serve as the gateways in the interactions between drugs and the neurons [112–114], whereas receptors such as those for dopamine, glutamate, and GABA are important to the control of neurotransmitter release and thus play a fundamental role in the development of drug dependence. However, no such receptor has been identified in proteomics study so far, although these genes are known to be associated with drug exposure at both the mRNA and protein levels. The major cause underlying such observation is that the most commonly detected proteins belong to abundant and easily separable species, whereas the underrepresented proteins usually are expressed in low abundance and may also be difficult to separate by classical 2D-PAGE and mass spectrometry, often being obscured by more abundant proteins in proteomics studies. As suggested earlier, advances in technology, appropriate experimental model selection, and improvement in sample preparation will enhance the sensitivity and power of proteomics analysis and help us maximize the insights gained from proteomics studies of drug addiction [16, 17, 115, 116].

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References

1. Potenza MN, Sofuoglu M, Carroll KM, Rounsaville BJ (2011) Neuroscience of behavioral and pharmacological treatments for addictions. Neuron 69:695–712
2. Nestler EJ (2004) Molecular mechanisms of drug addiction. Neuropharmacology 47(Suppl 1):24–32
3. Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ (2010) The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. Trends Neurosci 33:267–276
4. Tan KR, Rudolph U, Luscher C (2011) Hooked on benzodiazepines: GABAA receptor subtypes and addiction. Trends Neurosci 34:188–197
5. Luscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69:650–663

6. Wong CC, Mill J, Fernandes C (2011) Drugs and addiction: an introduction to epigenetics. Addiction 106:480–489
7. Renthal W, Nestler EJ (2009) Chromatin regulation in drug addiction and depression. Dialogues Clin Neurosci 11:257–268
8. Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128
9. Larsson A, Engel JA (2004) Neurochemical and behavioral studies on ethanol and nicotine interactions. Neurosci Biobehav Rev 27:713–720
10. Hemby SE (2010) Cocainomics: new insights into the molecular basis of cocaine addiction. J Neuroimmune Pharmacol 5:70–82
11. Hwang YY, Li MD (2006) Proteins differentially expressed in response to nicotine in five rat brain regions: identification using a 2-DE/MS-based proteomics approach. Proteomics 6:3138–3153
12. Bierczynska-Krzysik A, Pradeep John JP, Silberring J, Kotlinska J, Dylag T, Cabatic M, Lubec G (2006) Proteomic analysis of rat cerebral cortex, hippocampus and striatum after exposure to morphine. Int J Mol Med 18:775–784
13. Freeman WM, Hemby SE (2004) Proteomics for protein expression profiling in neuroscience. Neurochem Res 29:1065–1081
14. Tannu N, Mash DC, Hemby SE (2007) Cytosolic proteomic alterations in the nucleus accumbens of cocaine overdose victims. Mol Psychiatry 12:55–73
15. Witzmann FA, Strother WN (2004) Proteomics and alcoholism. Int Rev Neurobiol 61:189–214
16. Lull ME, Freeman WM, VanGuilder HD, Vrana KE (2010) The use of neuroproteomics in drug abuse research. Drug Alcohol Depend 107:11–22
17. Li MD, Wang J (2007) Neuroproteomics and its applications in research on nicotine and other drugs of abuse. Proteomics Clin Appl 1:1406–1427
18. Witzmann FA, Li J, Strother WN, McBride WJ, Hunter L, Crabb DW, Lumeng L, Li TK (2003) Innate differences in protein expression in the nucleus accumbens and hippocampus of inbred alcohol-preferring and -nonpreferring rats. Proteomics 3:1335–1344
19. Bell RL, Kimpel MW, Rodd ZA, Strother WN, Bai F, Peper CL, Mayfield RD, Lumeng L, Crabb DW, McBride WJ et al (2006) Protein expression changes in the nucleus accumbens and amygdala of inbred alcohol-preferring rats given either continuous or scheduled access to ethanol. Alcohol 40:3–17
20. McBride WJ, Schultz JA, Kimpel MW, McClintick JN, Wang M, You J, Rodd ZA (2009) Differential effects of ethanol in the nucleus accumbens shell of alcohol-preferring (P), alcohol-non-preferring (NP) and Wistar rats: a proteomics study. Pharmacol Biochem Behav 92:304–313
21. Damodaran S, Dlugos CA, Wood TD, Rabin RA (2006) Effects of chronic ethanol administration on brain protein levels: a proteomic investigation using 2-D DIGE system.Eur J Pharmacol 547:75–82
22. Park B, Jeong SK, Lee WS, Seong JK, Paik YK (2004) A simple pattern classification method for alcohol-responsive proteins that are differentially expressed in mouse brain. Proteomics 4:3369–3375
23. Sari Y, Zhang M, Mechref Y (2010) Differential expression of proteins in fetal brains of alcohol-treated prenatally C57BL/6 mice: a proteomic investigation. Electrophoresis 31:483–496
24. Lewohl JM, Van Dyk DD, Craft GE, Innes DJ, Mayfield RD, Cobon G, Harris RA, Dodd PR (2004) The application of proteomics to the human alcoholic brain. Ann N Y Acad Sci 1025:14–26
25. Alexander-Kaufman K, James G, Sheedy D, Harper C, Matsumoto I (2006) Differential protein expression in the prefrontal white matter of human alcoholics: a proteomics study. Mol Psychiatry 11:56–65
26. Alexander-Kaufman K, Cordwell S, Harper C, Matsumoto I (2007) A proteome analysis of the dorsolateral prefrontal cortex in human alcoholic patients. Proteomics Clin Appl 1:62–72
27. Alexander-Kaufman K, Harper C, Wilce P, Matsumoto I (2007) Cerebellar vermis proteome of chronic alcoholic individuals. Alcohol Clin Exp Res 31:1286–1296
28. Matsumoto I (2009) Proteomics approach in the study of the pathophysiology of alcohol-related brain damage. Alcohol Alcohol 44:171–176
29. Kashem MA, Etages HD, Kopitar-Jerala N, McGregor IS, Matsumoto I (2009) Differential protein expression in the corpus callosum (body) of human alcoholic brain. J Neurochem 110:486–495
30. Kashem MA, Harper C, Matsumoto I (2008) Differential protein expression in the corpus callosum (genu) of human alcoholics. Neurochem Int 53:1–11
31. Kashem MA, James G, Harper C, Wilce P, Matsumoto I (2007) Differential protein expression in the corpus callosum (splenium) of human alcoholics: a proteomics study. Neurochem Int 50:450–459
32. Matsuda-Matsumoto H, Iwazaki T, Kashem MA, Harper C, Matsumoto I (2007) Differential protein expression profiles in the hippocampus of human alcoholics. Neurochem Int 51:370–376
33. Freeman WM, Brebner K, Amara SG, Reed MS, Pohl J, Phillips AG (2005) Distinct proteomic profiles of amphetamine self-administration transitional states. Pharmacogenomics J 5:203–214
34. Iwazaki T, McGregor IS, Matsumoto I (2006) Protein expression profile in the striatum of acute methamphetamine-treated rats. Brain Res 1097:19–25
35. Iwazaki T, McGregor IS, Matsumoto I (2007) Protein expression profile in the striatum of rats with methamphetamine-induced behavioral sensitization. Proteomics 7:1131–1139
36. Iwazaki T, McGregor IS, Matsumoto I (2008) Protein expression profile in the amygdala of rats with methamphetamine-induced behavioral sensitization. Neurosci Lett 435:113–119
37. Kobeissy FH, Warren MW, Ottens AK, Sadasivan S, Zhang Z, Gold MS, Wang KK (2008) Psychoproteomic analysis of rat cortex following acute methamphetamine exposure. J Proteome Res 7:1971–1983
38. Faure JJ, Hattingh SM, Stein DJ, Daniels WM (2009) Proteomic analysis reveals differentially expressed proteins in the rat frontal cortex after methamphetamine treatment. Metab Brain Dis 24:685–700
39. Li X, Wang H, Qiu P, Luo H (2008) Proteomic profiling of proteins associated with methamphetamine-induced neurotoxicity in different regions of rat brain. Neurochem Int 52:256–264
40. Yang MH, Kim S, Jung MS, Shim JH, Ryu NK, Yook YJ, Jang CG, Bahk YY, Kim KW, Park JH (2008) Proteomic analysis of methamphetamine-induced reinforcement processes within the mesolimbic dopamine system. Addict Biol 13:287–294
41. Lull ME, Freeman WM, Vrana KE, Mash DC (2008) Correlating human and animal studies of cocaine abuse and gene expression. Ann N Y Acad Sci 1141:58–75
42. Hemby SE (2006) Assessment of genome and proteome profiles in cocaine abuse. Prog Brain Res 158:173–195
43. Tannu NS, Howell LL, Hemby SE (2010) Integrative proteomic analysis of the nucleus accumbens in rhesus monkeys following cocaine self-administration. Mol Psychiatry 15:185–203
44. del Castillo C, Morales L, Alguacil LF, Salas E, Garrido E, Alonso E, Perez-Garcia C (2009) Proteomic analysis of the nucleus accumbens of rats with different vulnerability to cocaine addiction. Neuropharmacology 57:41–48
45. Reynolds JL, Mahajan SD, Bindukumar B, Sykes D, Schwartz SA, Nair MP (2006) Proteomic analysis of the effects of cocaine

on the enhancement of HIV-1 replication in normal human astrocytes (NHA). Brain Res 1123:226–236
46. Bindukumar B, Mahajan SD, Reynolds JL, Hu Z, Sykes DE, Aalinkeel R, Schwartz SA (2008) Genomic and proteomic analysis of the effects of cannabinoids on normal human astrocytes. Brain Res 1191:1–11
47. Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, Thompson MR, Dawson B, Mallet PE, Kashem MA et al (2008) Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology 33:1113–1126
48. Rubino T, Realini N, Braida D, Alberio T, Capurro V, Vigano D, Guidali C, Sala M, Fasano M, Parolaro D (2009) The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. Neurotox Res 15:291–302
49. Colombo G, Rusconi F, Rubino T, Cattaneo A, Martegani E, Parolaro D, Bachi A, Zippel R (2009) Transcriptomic and proteomic analyses of mouse cerebellum reveals alterations in RasGRF1 expression following in vivo chronic treatment with delta 9-tetrahydrocannabinol. J Mol Neurosci 37:111–122
50. Li KW, Jimenez CR, van der Schors RC, Hornshaw MP, Schoffelmeer AN, Smit AB (2006) Intermittent administration of morphine alters protein expression in rat nucleus accumbens. Proteomics 6:2003–2008
51. Kim SY, Chudapongse N, Lee SM, Levin MC, Oh JT, Park HJ, Ho IK (2005) Proteomic analysis of phosphotyrosyl proteins in morphine-dependent rat brains. Brain Res Mol Brain Res 133:58–70
52. Bodzon-Kulakowska A, Suder P, Mak P, Bierczynska-Krzysik A, Lubec G, Walczak B, Kotlinska J, Silberring J (2009) Proteomic analysis of striatal neuronal cell cultures after morphine administration. J Sep Sci 32:1200–1210
53. Bierczynska-Krzysik A, Bonar E, Drabik A, Noga M, Suder P, Dylag T, Dubin A, Kotlinska J, Silberring J (2006) Rat brain proteome in morphine dependence. Neurochem Int 49:401–406
54. Shui HA, Ho ST, Wang JJ, Wu CC, Lin CH, Tao YX, Liaw WJ (2007) Proteomic analysis of spinal protein expression in rats exposed to repeated intrathecal morphine injection. Proteomics 7:796–803
55. Li Q, Zhao X, Zhong LJ, Yang HY, Wang Q, Pu XP (2009) Effects of chronic morphine treatment on protein expression in rat dorsal root ganglia. Eur J Pharmacol 612:21–28
56. Neasta J, Uttenweiler-Joseph S, Chaoui K, Monsarrat B, Meunier JC, Mouledous L (2006) Effect of long-term exposure of SH-SY5Y cells to morphine: a whole cell proteomic analysis. Proteome Sci 4:23
57. Suder P, Bodzon-Kulakowska A, Mak P, Bierczynska-Krzysik A, Daszykowski M, Walczak B, Lubec G, Kotlinska JH, Silberring J (2009) The proteomic analysis of primary cortical astrocyte cell culture after morphine administration. J Proteome Res 8:4633–4640
58. Moron JA, Abul-Husn NS, Rozenfeld R, Dolios G, Wang R, Devi LA (2007) Morphine administration alters the profile of hippocampal postsynaptic density-associated proteins: a proteomics study focusing on endocytic proteins. Mol Cell Proteomics 6:29–42
59. Van den Oever MC, Lubbers BR, Goriounova NA, Li KW, Van der Schors RC, Loos M, Riga D, Wiskerke J, Binnekade R, Stegeman M et al (2010) Extracellular matrix plasticity and GABAergic inhibition of prefrontal cortex pyramidal cells facilitates relapse to heroin seeking. Neuropsychopharmacology 35:2120–2133
60. Prokai L, Zharikova AD, Stevens SM Jr (2005) Effect of chronic morphine exposure on the synaptic plasma-membrane subproteome of rats: a quantitative protein profiling study based on isotope-coded affinity tags and liquid chromatography/mass spectrometry. J Mass Spectrom 40:169–175
61. Yang L, Sun ZS, Zhu YP (2007) Proteomic analysis of rat prefrontal cortex in three phases of morphine-induced conditioned place preference. J Proteome Res 6:2239–2247
62. Bodzon-Kulakowska A, Kulakowski K, Drabik A, Moszczynski A, Silberring J, Suder P (2011) Morphinome—a meta-analysis applied to proteomics studies in morphine dependence. Proteomics 11:5–21
63. Bodzon-Kulakowska A, Bierczynska-Krzysik A, Drabik A, Noga M, Kraj A, Suder P, Silberring J (2005) Morphinome—proteome of the nervous system after morphine treatment. Amino Acids 28:13–19
64. Kim SY, Chudapongse N, Lee SM, Levin MC, Oh JT, Park HJ, Ho IK (2004) Proteomic analysis of phosphotyrosyl proteins in the rat brain: effect of butorphanol dependence. J Neurosci Res 77:867–877
65. Yeom M, Shim I, Lee HJ, Hahm DH (2005) Proteomic analysis of nicotine-associated protein expression in the striatum of repeated nicotine-treated rats. Biochem Biophys Res Commun 326:321–328
66. Benowitz NL (1996) Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol 36:597–613
67. Piubelli C, Cecconi D, Astner H, Caldara F, Tessari M, Carboni L, Hamdan M, Righetti PG, Domenici E (2005) Proteomic changes in rat serum, polymorphonuclear and mononuclear leukocytes after chronic nicotine administration. Proteomics 5:1382–1394
68. Cecconi D, Tessari M, Wille DR, Zoli M, Domenici E, Righetti PG, Carboni L (2008) Serum proteomic analysis during nicotine self-administration, extinction nd relapse in rats. Electrophoresis 29:1525–1533
69. Jahn R, Scheller RH (2006) SNAREs—engines for membrane fusion. Nat Rev Mol Cell Biol 7:631–643
70. Ungermann C, Langosch D (2005) Functions of SNAREs in intracellular membrane fusion and lipid bilayer mixing. J Cell Sci 118:3819–3828
71. Bonifacino JS, Glick BS (2004) The mechanisms of vesicle budding and fusion. Cell 116:153–166
72. Schweizer FE, Ryan TA (2006) The synaptic vesicle: cycle of exocytosis and endocytosis. Curr Opin Neurobiol 16:298–304
73. Kennedy MJ, Ehlers MD (2006) Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci 29:325–362
74. Clayton EL, Cousin MA (2009) The molecular physiology of activity-dependent bulk endocytosis of synaptic vesicles. J Neurochem 111:901–914
75. Kennedy MJ, Ehlers MD (2011) Mechanisms and function of dendritic exocytosis. Neuron 69:856–875
76. Xu Q, Li MD (2011) Nicotine modulates expression of dynamin 1 in rat brain and SH-SY5Y cells. Neurosci Lett 489:168–171
77. Huang W, Li MD (2009) Nicotine modulates expression of miR-140*, which targets the 3′-untranslated region of dynamin 1 gene (Dnm1). Int J Neuropsychopharmacol 12:537–546
78. Garcia-Fuster MJ, Ferrer-Alcon M, Miralles A, Garcia-Sevilla JA (2003) Modulation of Fas receptor proteins and dynamin during opiate addiction and induction of opiate withdrawal in rat brain. Naunyn Schmiedebergs Arch Pharmacol 368:421–431
79. Saito M, Smiley J, Toth R, Vadasz C (2002) Microarray analysis of gene expression in rat hippocampus after chronic ethanol treatment. Neurochem Res 27:1221–1229
80. Sontag JM, Fykse EM, Ushkaryov Y, Liu JP, Robinson PJ, Sudhof TC (1994) Differential expression and regulation of multiple dynamins. J Biol Chem 269:4547–4554

81. Shupliakov O, Low P, Grabs D, Gad H, Chen H, David C, Takei K, De Camilli P, Brodin L (1997) Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. Science 276:259–263
82. Takei K, Mundigl O, Daniell L, De Camilli P (1996) The synaptic vesicle cycle: a single vesicle budding step involving clathrin and dynamin. J Cell Biol 133:1237–1250
83. Robinson PJ, Liu JP, Powell KA, Fykse EM, Sudhof TC (1994) Phosphorylation of dynamin I and synaptic-vesicle recycling. Trends Neurosci 17:348–353
84. Farr CD, Gafken PR, Norbeck AD, Doneanu CE, Stapels MD, Barofsky DF, Minami M, Saugstad JA (2004) Proteomic analysis of native metabotropic glutamate receptor 5 protein complexes reveals novel molecular constituents. J Neurochem 91:438–450
85. Kranenburg O, Verlaan I, Moolenaar WH (1999) Dynamin is required for the activation of mitogen-activated protein (MAP) kinase by MAP kinase kinase. J Biol Chem 274:35301–35304
86. Whistler JL, von Zastrow M (1999) Dissociation of functional roles of dynamin in receptor-mediated endocytosis and mitogenic signal transduction. J Biol Chem 274:24575–24578
87. Bennett MK, Calakos N, Scheller RH (1992) Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 257:255–259
88. Smirnova T, Stinnakre J, Mallet J (1993) Characterization of a presynaptic glutamate receptor. Science 262:430–433
89. Pevsner J, Hsu SC, Scheller RH (1994) n-Sec1: a neural-specific syntaxin-binding protein. Proc Natl Acad Sci U S A 91:1445–1449
90. Greengard P, Valtorta F, Czernik AJ, Benfenati F (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. Science 259:780–785
91. Wang Y, Tang BL (2006) SNAREs in neurons—beyond synaptic vesicle exocytosis (review). Mol Membr Biol 23:377–384
92. Osei YD, Churchich JE (1995) Screening and sequence determination of a cDNA encoding the human brain 4-aminobutyrate aminotransferase. Gene 155:185–187
93. De Biase D, Barra D, Simmaco M, John RA, Bossa F (1995) Primary structure and tissue distribution of human 4-aminobutyrate aminotransferase. Eur J Biochem 227:476–480
94. Barnby G, Abbott A, Sykes N, Morris A, Weeks DE, Mott R, Lamb J, Bailey AJ, Monaco AP (2005) Candidate-gene screening and association analysis at the autism-susceptibility locus on chromosome 16p: evidence of association at GRIN2A and ABAT. Am J Hum Genet 76:950–966
95. Clancy KP, Berger R, Cox M, Bleskan J, Walton KA, Hart I, Patterson D (1996) Localization of the L-glutamine synthetase gene to chromosome 1q23. Genomics 38:418–420
96. Malleret G, Haditsch U, Genoux D, Jones MW, Bliss TV, Vanhoose AM, Weitlauf C, Kandel ER, Winder DG, Mansuy IM (2001) Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell 104:675–686
97. Mansuy IM, Mayford M, Jacob B, Kandel ER, Bach ME (1998) Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. Cell 92:39–49
98. Mulkey RM, Endo S, Shenolikar S, Malenka RC (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486–488
99. Jin Z, Gao F, Flagg T, Deng X (2004) Nicotine induces multi-site phosphorylation of Bad in association with suppression of apoptosis. J Biol Chem 279:23837–23844
100. Exley R, Moroni M, Sasdelli F, Houlihan LM, Lukas RJ, Sher E, Zwart R, Bermudez I (2006) Chaperone protein 14-3-3 and protein kinase A increase the relative abundance of low agonist sensitivity human alpha 4 beta 2 nicotinic acetylcholine receptors in *Xenopus* oocytes. J Neurochem 98:876–885
101. Jeanclos EM, Lin L, Treuil MW, Rao J, DeCoster MA, Anand R (2001) The chaperone protein 14-3-3eta interacts with the nicotinic acetylcholine receptor alpha 4 subunit. Evidence for a dynamic role in subunit stabilization. J Biol Chem 276:28281–28290
102. Rajalingam K, Rudel T (2005) Ras–Raf signaling needs prohibitin. Cell Cycle 4:1503–1505
103. Fu H, Xia K, Pallas DC, Cui C, Conroy K, Narsimhan RP, Mamon H, Collier RJ, Roberts TM (1994) Interaction of the protein kinase Raf-1 with 14-3-3 proteins. Science 266:126–129
104. Hermeking H, Benzinger A (2006) 14-3-3 proteins in cell cycle regulation. Semin Cancer Biol 16:183–192
105. Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8:1445–1449
106. Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nat Rev Neurosci 8:844–858
107. Wang J, Li MD (2010) Common and unique biological pathways associated with smoking initiation/progression, nicotine dependence, and smoking cessation. Neuropsychopharmacology 35:702–719
108. Mukherjee S, Das SK, Vaidyanathan K, Vasudevan DM (2008) Consequences of alcohol consumption on neurotransmitters—an overview. Curr Neurovasc Res 5:266–272
109. Nestler EJ (1997) Molecular mechanisms of opiate and cocaine addiction. Curr Opin Neurobiol 7:713–719
110. Liu Z, Tearle AW, Nai Q, Berg DK (2005) Rapid activity-driven SNARE-dependent trafficking of nicotinic receptors on somatic spines. J Neurosci 25:1159–1168
111. Roy S, Wyse B, Hancock JF (2002) H-Ras signaling and K-Ras signaling are differentially dependent on endocytosis. Mol Cell Biol 22:5128–5140
112. Ferre S, Lluis C, Justinova Z, Quiroz C, Orru M, Navarro G, Canela EI, Franco R, Goldberg SR (2010) Adenosine-cannabinoid receptor interactions. Implications for striatal function. Br J Pharmacol 160:443–453
113. Contet C, Kieffer BL, Befort K (2004) Mu opioid receptor: a gateway to drug addiction. Curr Opin Neurobiol 14:370–378
114. Changeux JP (2010) Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. Nat Rev Neurosci 11:389–401
115. Huber LA (2003) Is proteomics heading in the wrong direction? Nat Rev Mol Cell Biol 4:74–80
116. Garbis S, Lubec G, Fountoulakis M (2005) Limitations of current proteomics technologies. J Chromatogr A 1077:1–18
117. Hargreaves GA, Quinn H, Kashem MA, Matsumoto I, McGregor IS (2009) Proteomic analysis demonstrates adolescent vulnerability to lasting hippocampal changes following chronic alcohol consumption. Alcohol Clin Exp Res 33:86–94
118. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc, Series B 57:289–300