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Research Article

Differential Protein Expression in the Nucleus Accumbens and Amygdala of Lewis and Fischer 344 Rats, and its Relevance in Drug Addiction

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Abstract The Lewis (LEW) and Fischer 344 (F344) rat strains are widely used as models of genetic vulnerability to drug addiction, particularly since *these two strains show different patterns of drug self-administration.* We previously performed an unbiased and genome-wide microarray analysis to define the differences in gene expression in the *nucleus accumbens (NAcc)* and prefrontal cortex between LEW and F344 rats. In this study, we set out to determine the strain differences in the NAcc and amygdala at the protein level using bidimensional gel electrophoresis. As such, *11 proteins* were found to be differentially expressed between these two strains, the activity of which was mainly related to vesicular trafficking (STXBP1 and GDI1), *energy metabolism (ATP5B, ENO2, and ACOT7)*, and cytoskeletal formation. This information might be useful to search for new molecular targets to screen individuals at high risk of developing an addictive disorder.

Keywords Lewis rats; Fischer 344 rats; proteomics; nucleus accumbens; amygdala; addiction

1. Introduction

Drug addiction is a chronic-relapsing disorder with negative personal and social consequences, which poses a heavy burden on today's society. Although factors such as drug availability, price, and lifestyle can modulate drug consumption [1], there are other important biological elements that play a significant role in this phenomenon. Among these biological factors, genetic determinants are regarded as major intermediates that influence drug addiction [22]. One strategy to study the genetic elements involved in drug addiction is to use rat strains with different responses to drugs of abuse [20]. Lewis (LEW) rats are more sensitive to the reinforcing properties of several drugs of abuse, such as morphine or ethanol, and they acquire self-administration behavior faster than *Fischer 344 (F344)* rats [21,31,32,35]. In addition, LEW rats also engage in cocaine self-administration escalation, which seems not to occur in F344 rats [25].

These behavioral differences may derive from the differential regulation of dopaminergic, opioidergic or endocannabinoid transmission in these strains [28,31,32], although other signaling cascades and neurotransmitter systems are likely to be involved. In order to gain a deeper understanding of the differences between the *LEW and F344* strains in terms of gene expression, we originally adopted a transcriptomic approach to analyze the differences in gene expression in the nucleus accumbens and prefrontal cortex in microarrays [14]. We found that only a very limited group of genes were differentially expressed in LEW rats when compared with the Fischer 344 strain. The genes that were induced in the LEW strain were related to oxygen transport, neurotransmitter processing, and fatty acid metabolism, while genes whose expression was repressed in this strain were involved in physiological functions, such as drug and proton transport, oligodendrocyte survival, and lipid catabolism.

Since translation is a highly regulated process in the cell, gene expression does not always mirror protein expression. Moreover, in brain tissue the nucleus of a cell may lie far from the site of a synapse, which may accentuate this disparity. For this reason, we used two dimensional polyacrylamide gel electrophoresis (2D-PAGE) to search for strain differences in protein expression between *LEW and F344 rats*. *Due to the implication of the extended amygdala circuitry in addiction, we analyzed both the nucleus accumbens (NAcc) and the amygdala (Amg) in this study* [19]. We detected a differential expression of highly abundant proteins like beta-actin, gamma-enolase, and peroxiredoxin 6 [24]. Moreover, Syntaxin-binding protein 1 (also known as Munc18-1), a key regulator of neurosecretion, was expressed in the *Amg* of F344 rats at higher levels than in LEW animals. We also found a similar pattern in the *NAcc* for the Rab-GDP dissociation inhibitor alpha, which was also overexpressed in F344 rats. As these two proteins are related to vesicular trafficking

and neurotransmitter secretion [8,15,17], it is tempting to speculate that these differences may contribute to some of the distinct neurochemical aspects of homeostasis found between both these strains.

2. Materials and methods

2.1. Animals

Male F344 ($n = 9$) and LEW ($n = 9$) rats weighing 300–320 g at the beginning of the experiments were used in this study. All animals were maintained at a constant temperature (20 ± 2 °C) on a 12 hour light-dark cycle (lights on at 08:00 hours), with free access to food and water (commercial diet for rodents A04/A03; Panlab, Barcelona, Spain). All animals were maintained and handled according to European Union Laboratory Animal Care Rules (2010/63/EU Directive).

2.2. Protein extraction

Two weeks after arrival, the rats were lightly anesthetized with isoflurane and sacrificed by decapitation. Their brain was removed quickly, coronal 1 mm slices were obtained with the aid of a brain matrix, and the NAcc (Bregma 1.60 mm) and Amg (Bregma −3.60 mm) were dissected out on ice. The tissue obtained was frozen on dry ice and stored at −80 °C. Each structure was homogenized with a motorized pellet pestle (Sigma-Aldrich, Z359971-1EA) in homogenization buffer (sucrose 320 mM; HEPES 50 mM [pH 7.8]; protease inhibitor cocktail—Complete EDTA-free, Roche, 11-873-580-001; phosphatase inhibitor cocktail—PhosSTOP, Roche, 04-906-837-001), and subsequently the samples were *centrifuged at 1000 g* and at 4 °C for 10 min. An aliquot of the supernatants obtained was used to measure the protein concentration by the Bradford method, while the remainder was stored at −80 °C. *Deionized water* (18.2 MΩ.cm) was used throughout the entire experiment.

2.3. Isoelectrofocusing and SDS-PAGE

The protein in the samples collected was precipitated and isolated with ReadyPrep 2D Cleanup kit (163-2130, Bio-Rad, CA, USA) according to the manufacturer's suggestions. Briefly, 3 pools of protein were gathered per structure and strain, resulting in 12 different pools of protein (80 mg of protein per pool): 3 for the NAcc from LEW rats; 3 for the Amg from LEW rats; 3 for the NAcc from F344 rats; 3 for the Amg from F344 rats. For each strain, the protein isolated from 9 rats was used.

Isoelectric focusing was carried out on 24 cm immobilized pH gradient strips (3–10 nonlinear pH gradient; Bio-Rad), which had been rehydrated for 16 h with the precipitated protein resuspended in rehydration buffer: 40 mM Tris [pH 8.5]; 7 M urea; 2 M thiourea; 2% w/v CHAPS; ampholytes (Bio-Lyte 3–10 buffer; Bio-Rad, 163-2094); hydroxyethyl disulfide (DeStreak Reagent; 17-6003-18, GE Healthcare, Buckinghamshire, UK); bromophenol blue. Focusing was carried out for 60,000 Vh in a Protean IEF Cell (Bio-Rad) at room temperature (RT) at a maximum of 10,000 V after a 30 min rapid ramping up to 250 V followed by a 2 h gradual increase up to 10,000 V. The strips were then stored at −80 °C until the second dimension was run.

To run the second dimension, the strips were thawed and incubated for 20 min at RT with gentle shaking in a dithiothreitol solution (DTT; SIGMA, D0632) containing 6 M urea, 2% w/v SDS, 375 mM Tris-HCl [pH 8.8], 20% v/v glycerol, and 2% w/v DTT. The strips were then equilibrated for 20 min at RT in an iodoacetamide solution (IAA; GE Healthcare, RPN6302), containing 6 M urea, 2% w/v SDS, 375 mM Tris-HCl pH 8.8, 20% v/v glycerol, and 2.5% w/v IAA. Subsequently, they were placed in running buffer (15 mM Tris, 192 mM glycine), laid on top of 12% w/v polyacrylamide gels, and covered with 0.5% w/v agarose prepared in running buffer and stained with bromophenol blue. An aliquot of Precision molecular mass marker (Precision Plus Protein All Blue Standards; Bio-Rad, 161-0373) was loaded in parallel in each gel, and the gels were resolved overnight at 84 V and RT in the Protean plus Dodeca Cell apparatus (Bio-Rad).

2.4. Silver staining and Coomassie staining

The 12 gels were silver stained in a Dodeca Stainer apparatus (Bio-Rad) and after overnight fixation in a methanol:glacial acetic:water solution (50:10:40) containing 0.04% formalin, the gels were washed three times for a total of 60 min in ethanol 35% v/v. The gels were then sensitized in 0.02% w/v $Na_2S_2O_3$, washed three times in water (5 min each), and then subsequently stained in 0.2% w/v $AgNO_3$ and 0.04% formalin for 20 min. After washing twice in water (1 min each), the gels were revealed in 6% w/v $NaCO_3$ with 0.04% formalin and 0.004% w/v $Na_2S_2O_3$, and the reaction was stopped by rinsing twice for 3 min each in methanol:glacial acetic:water solution (50:10:40), and by washing in water three times (5 min each).

To excise selected spots, two gels loaded with 800 mg of protein were Coomassie stained (one per structure) for 1 h at RT at constant shaking using a filtered methanol:glacial acetic:water solution (50:10:40) containing 0.1 w/v Coomassie blue (PhastGel Blue R; GE Healthcare, 17-0518-01). The gels were then washed three times in 40% methanol over 3.5 h and incubated overnight in water. The individual spots were cut out of the gel using a cut pipette tip, they were stored in 100 mL water and then analyzed at the Proteomics Facility UCM-FPCM, a member of ProteoRed network.

2.5. Image analysis

The gels were scanned in B/W at 600 ppp on a calibrated densitometer (Epson V750-M Pro) and the images were analyzed with the PDQuest v8 software (Bio-Rad). Gels were

Table 1: Proteins successfully identified by mass spectrometry peptide fingerprinting.

SSP	Protein name	**Official symbol**	Accession number	Mr (Da)	**pI**	MASCOT score	MASCOT expect	No. of peptides	Sequence coverage (%)	LEW/F344	t-Test
Proteins differing significantly in amygdala											
1405	ATP synthase F1 subunit beta, mitochondrial	Atp5b	P10719	56,318	5.19	118	1.2e-008	19	41	3.3	0.0085
3102	Gamma-enolase	Eno2	P07323	47,510	5.03	134	3.1e-010	17	47	2.1	0.0148
5305	Alcohol dehydrogenase [$NADP^+$]	AKR1A1	P51635	36,711	6.84	88	1.3e-005	11	40	3.3	0.0208
7602	Syntaxin-binding protein 1	STXBP1	P61765	67,925	6.49	57	0.016	14	17	0.5	0.0135
5308	Cytosolic acyl coenzyme A thioester hydrolase	ACOT7	Q64559	43,164	8.80	65	0.0025	10	30	8.2	0.0132
6504	**Adenylyl cyclase-associated protein 1**	**CAP1**	**Q08163**	**51,899**	**7.16**	**70**	**0.051***	**9**	**26**	**2.7**	**0.0095**
Proteins differing significantly in the nucleus accumbens											
1519	Actin, cytoplasmic	ACTB	P60711	42,052	5.29	181	6.2e-015	23	67	0.4	0.0160
1709	Rab-GDP dissociation inhibitor alpha	GDI1	P50398	51,074	5.00	71	0.00057	13	31	0.5	0.0345
1904	Creatine kinase B	CKB	P07335	42,983	5.39	305	2.5e-027	32	70	0.5	0.0240
2202	Peroxiredoxin 6	PRDX6	O35244	24,860	5.64	54	0.033	7	37	2.1	0.0340
2606	Ubiquitin thioesterase 1	OTUB1	B2RYG6	31,478	4.85	59	0.0092	8	32	2.0	0.0363
5801	Aconitate hydratase, mitochondrial	ACO2	Q9ER34	86,121	7.87	95	2.7e-006	17	22	0.5	0.0384

*Protein with $P > .05$ (spot 6504).

warped prior to spot detection and the spot quantities were normalized using a local regression model. The data were analyzed with a Student's t-test (using the statistics toolbox included in the PDQuest software). The spots that gave a significant result ($P < .05$) and that displayed a two-fold or higher change in their ratio were verified visually to exclude artifacts, and then they were excised for mass spectrometry.

2.6. Mass spectrometry

The proteins selected for analysis were reduced, alkylated, and digested with trypsin in-gel, according to Sechi and Chait [33]. Briefly, the isolated spots were washed twice with water, shrunk for 15 min with 100% acetonitrile, and dried in a Savant SpeedVac for 30 min. The samples were then reduced with 10 mM dithiothreitol in 25 mM ammonium bicarbonate for 30 min at 56 °C and subsequently, they were alkylated for 15 min in the dark with 55 mM iodoacetamide in 25 mM ammonium bicarbonate. Finally, the samples were *digested* overnight at 37 °C *with 12.5 ng/mL sequencing grade trypsin* (Roche Molecular Biochemicals) prepared in 25 mM ammonium bicarbonate (pH 8.5).

After digestion, the sample (1 μL) was spotted onto a MALDI target plate and allowed to dry in air at RT. Subsequently, 0.4 μL of a 3 mg/mL of α-cyano-4-hydroxy-transcinnamic acid matrix (Sigma) in 50% acetonitrile was added to the dried digested peptide spots and again allowed to dry in air at RT. The MALDI-TOF MS analyses were then performed in a 4800 Plus Proteomics Analyzer MALDI-TOF/TOF mass spectrometer (Applied Biosystems, MDS Sciex, Toronto, Canada) at the UCM-FPCM Proteomics Unit (Complutense University of Madrid), with the Maldi-TOF/TOF apparatus operated in positive reflector mode with an accelerating *voltage of 20,000 V*. All mass spectra were calibrated internally using peptides from the auto digestion of trypsin.

The MALDI-TOF/TOF mass spectrometry analysis produces peptide mass fingerprints and the peptides observed with a Signal to Noise greater than 10 can be collated and represented as a list of monoisotopic molecular weights. For protein identification, the UniProt SwissProt database (date 2012/11/12; 538,588 sequences; 191,241,550 residues) was searched *with the taxonomic restriction to Rattus* (7,844 sequences) and using a local license of MASCOT engine v2.3 (Matrix Science, London; http://www.matrixscience.com) through the Global Protein Server v3.6 software from ABSciex. The search parameters applied were as follows:

- carbamidomethyl cysteine as fixed modification and oxidized methionine as variable modification;
- peptide mass tolerance 50 ppm;
- 1 missed trypsin cleavage site allowed;
- peptide charge state +1.

For all protein identifications, the *significant* probability scores were greater than the score fixed *by MASCOT when* $P < .05$.

3. Results

After filtering all the matched points in function of their statistical significance and the difference between strains,

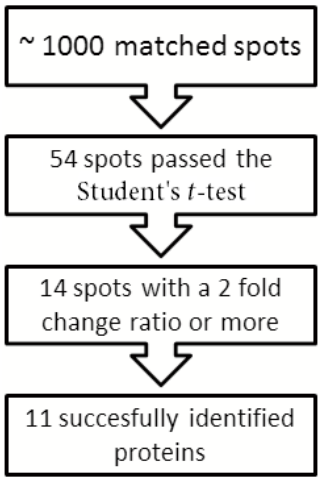

Figure 1: Flow chart of the selection of significantly different spots.

fourteen protein spots were found to be significantly different in LEW and F344 rats (Figure 1). Of these 14 spots, we submitted only 12 for MS peptide fingerprinting as the other two spots were not visible in the Coomassie gels. *One spot appeared to be contaminated during mass spectrometry, making it impossible to reliably identify the corresponding protein (spot 6504). However, the most likely identity of this protein spot is reflected, even though the differential expression between LEW and F344 rats was at the threshold of significance. In the light of these concerns, we will not discuss this protein spot further and* as such, we consider 11 proteins to have been successfully identified.

3.1. Protein differences observed in the NAcc

Proteins that were seen to accumulate distinctly in the NAcc were as follows: beta-actin (ACTB), involved in cytoskeleton formation and axonogenesis; Rab-GDP dissociation inhibitor alpha (GDI1), involved in GTPase activation, vesicular traffic regulation, and the inhibition of axonogenesis; peroxiredoxin 6 (PRDX6) that participates in hydrogen peroxide catabolic processes, lipid metabolism, and redox homeostasis; aconitate hydratase (ACO2) involved in citrate metabolism, energy production, and iron homeostasis; creatine kinase B (CKB), a regulator of ATP homeostasis, cellular chloride ion homeostasis, and brain development; ubiquitin thioesterase 1 (OTUB1) that is active in DNA repair, immunity, and proteolysis. Four of these genes were expressed more weakly in LEW than in F344 rats (ACTB, GDI1, ACO2, and CKB), while PRDX6 and OTUB1 were more strongly expressed in LEW rats.

3.2. Protein differences observed in Amg

The relative amounts and changes in Amg proteins were also assessed and compared in F344 and LEW rats (Figure 4).

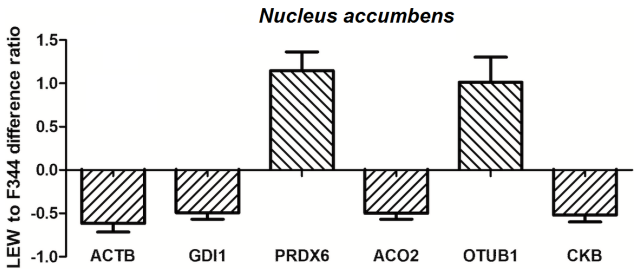

Figure 2: Differential protein expression in the NAcc between LEW and F344 rats. The data are expressed as the mean ratio of the difference, where 0% reflects equivalent protein values in both strains (±SEM).

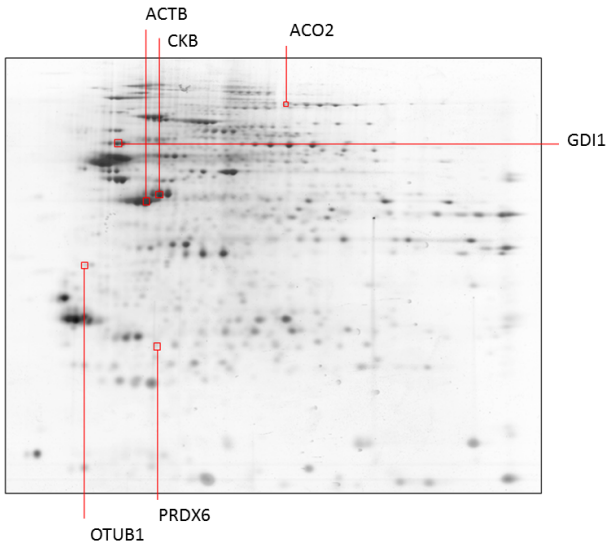

Figure 3: Representative gel showing the proteins that were expressed at significantly different levels in the NAcc of LEW and F344 rats.

The proteins that were found in significantly different amounts between strains were as follows: ATP synthase F1 subunit beta, mitochondrial (ATP5B), involved in ATP synthesis and ion transport; alcohol dehydrogenase [NADP$^+$] (AKR1A1), implicated in aldehyde catabolism and L-ascorbic acid biosynthesis; cytosolic acyl-CoA thioester hydrolase (ACOT7), which participates in acyl CoA and lipid metabolism; gamma-enolase (ENO2), active in gluconeogenesis and glycolysis, and as a promoter of cell survival; syntaxin-binding protein 1 (STXBP1), involved in neurotransmitter secretion, vesicle docking during exocytosis, and long-term synaptic depression. All these proteins accumulated more strongly in LEW rats, except for STXBP1 that was less prominent *in this strain than in* F344 animals. There was also a spot (6504) that we could not successfully characterize due to contamination during the peptide fingerprinting process, although we show it here for future reference.

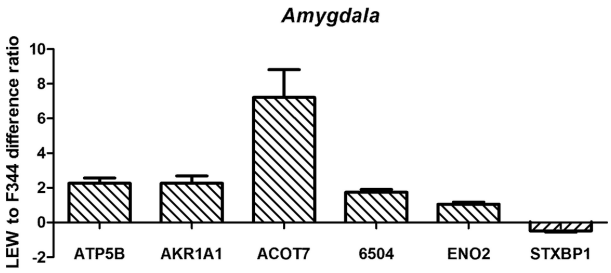

Figure 4: Differential protein expression in the Amg between LEW and F344 rats.

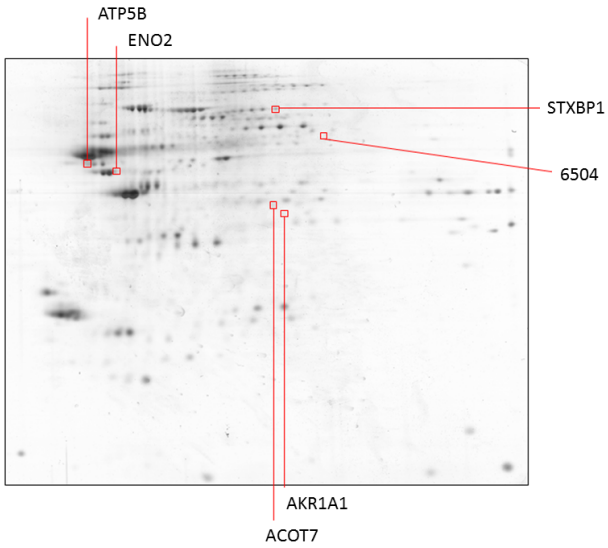

Figure 5: Representative gel showing the proteins that were expressed at significantly different levels in the Amg of LEW and F344 rats.

4. Discussion

The aim of this work was to analyze the difference in the proteins expressed in the LEW and F344 rat strains, an animal model of genetic susceptibility to the reinforcing effects of drugs of abuse. By resolving NAcc and Amg protein extracts by 2D-PAGE/MS, we found a limited set of proteins that differed between these two strains. Significantly, there was no one single protein that was differentially expressed in both brain structures. For the sake of clarity, we will discuss the results according to each brain region.

4.1. Protein differences observed in the NAcc

While 6 proteins were seen to be differentially expressed between the two strains analyzed, none of these coincided with those reported in our earlier microarray study [14]. This may be due to the fact that translation can be tightly regulated in different situations, that protein location may differ from mRNA location, and that 2D-PAGE underestimates membrane proteins.

We found higher levels *of PRDX6 and OTUB1 in the NAcc of LEW rats* than in that of the F344 strain. PRDX6 belongs to the group of peroxiredoxin proteins, a ubiquitous family of antioxidant enzymes that control hydrogen peroxide levels and that regulate signal transduction in mammalian cells [27]. This protein seems to regulate some addiction-related phenomena and it has been shown that extinction of cocaine-induced conditioned place preference (CPP) by the growth factor midkine is correlated to the differential phosphorylation of *PRDX6* in the dorsal striatum [13]. Interestingly, another member of the peroxiredoxin family, peroxiredoxin 2, was seen to be differentially regulated in the NAcc in response to a cocaine challenge when animals that do not extinguish cocaine-induced CPP were compared with those that do extinguish this behavior [7]. In addition, peroxiredoxin 2 has also been found to be decreased in the NAcc of rhesus monkeys following *cocaine self-administration* [37], *in the NAcc of cocaine overdose victims* [36] and in the striatum of rats after acute methamphetamine challenge [16]. Together these data may suggest that endogenous antioxidant activity might indeed affect the individual susceptibility to drug abuse. In fact, cocaine-induced oxidative stress contributes to the long-lasting behavioral alterations associated to cocaine addiction [39].

OTUB1 is an enzyme that can specifically remove conjugated ubiquitin from proteins and it plays an important regulatory role in protein turnover by preventing degradation. OTUB1 also inhibits cytokine gene transcription through its interaction with a ubiquitin protease and E3 ubiquitin ligase [34]. After a careful search in the literature, we failed to find any direct link between this protein and drug addiction or any other relevant behavioral process. Future research should assess the role of this enzyme in animal models of addiction and also, in tissue from patients with addictions.

ACO2 catalyzes the isomerization of citrate to isocitrate via *cis*-aconitate in the Krebs cycle [38]. In a proteomic study similar to ours, the ACO2 activator fumarate hydratase was one of the few proteins whose expression in the NAcc of rats that extinguish cocaine-induced CPP differed from that in the NAcc of rats that do not [7]. Indeed, it has been shown that a greater magnitude of CPP extinction correlates with increased tyrosine phosphorylation of ACO2 in the mouse prefrontal cortex [13], whereas lower levels of phosphorylation in the prefrontal cortex were evident after extinction of morphine-induced CPP [40]. From these data, it could be suggested that the activation of ACO2 might be a useful biomarker for persistent cocaine or morphine use, as also supported by the differential regulation of this protein in the NAcc of LEW and F344 rats.

There was less ACTB in the NAcc of LEW rats and although ACTB is strongly expressed in the brain and it fulfills many functions, the lower levels of ACTB in LEW

rats might have some functional relevance to the differences reported in learning, memory, and synaptic plasticity between these strains. Indeed, LEW rats have fewer dendritic spines in the hippocampus than F344 rats [11] and given that actin is the main structural protein of dendritic spines [29], it is tempting to speculate that the differences that we found in ACTB *might be related*, at least in part, to the fewer hippocampal dendritic spines found in LEW rats.

GDP dissociation inhibitors are important regulators of Rho GTPase function, which in turn *plays a pivotal role in the regulation of cell signaling* [8]. Polymorphisms in the GDI1 gene in humans have been associated with moderate mental retardation [6] and in rodent models, this protein has been seen to be involved in memory deficits, synaptic plasticity, and brain connectivity [3,4,15]. In addition, the phosphorylated form of this protein is diminished in the NAcc of rhesus monkeys after cocaine self-administration [37]. The differential regulation of this protein in LEW and F344 animals also suggests that it fulfills a possible role in phenomena associated with addiction, or in the regulation of the hypothalamic-pituitary-adrenal axis [20].

Creatinine kinases play a major role in the energy balance in tissues with large energy demands, such as the brain. These enzymes catalyze the transformation of creatine and they consume adenosine triphosphate in order to generate phosphocreatine and adenosine diphosphate [2]. Yet to our knowledge no direct relationship between this enzyme and addiction has yet to be established, or with any other behavioral process. However, an increase in the activity of creatine kinase was induced in certain brain regions, including the striatum, following a subchronic ketamine treatment (an animal model of some schizophrenia symptoms) [5].

4.2. Protein differences in the Amg

Of all the proteins expressed at *different levels in the Amg of LEW and F344* rats, they generally accumulated more *intensely in LEW animals*. The only protein seen to be expressed more weakly in LEW rats than in F344 rats was STXBP1. *The other four proteins (ATP5B, AKR1A1, ACOT7, and ENO2) are involved in energy balance and metabolism, such that their accumulation in the amygdala of LEW rats could be related with the excess activity needed to learn emotionally significant experiences* [30].

STXBP1 (also known as munc 18-homolog 1) participates in the regulation of synaptic vesicle docking and fusion, possibly through its interaction with GTP-binding proteins. The deletion of this protein completely inhibits exocytosis (see [17] for a review) and it is known to be modulated in some behavioral paradigms of addiction. Indeed, STXBP1 levels decrease in the NAcc after cocaine self-administration and extinction [26], and they increase in the prefrontal cortex after morphine CPP [40]. This protein also seems to mediate alcohol sensitivity in *C. Elegans* [12] and it has been proposed to be a candidate as an ethanol preference drinking *locus* on mouse chromosome 2 [10]. Using a similar proteomics approach in a monkey self-administration study, another member of the syntaxin binding protein family, SBP3, was enhanced following cocaine self-administration [37], *suggesting a general role* for this type of protein in addiction-like phenomena.

The mitochondrial ATP synthase, or complex V, mediates the phosphorylation of ADP to ATP using the energy created during oxidative phosphorylation by the proton electrochemical gradient. The beta subunit of this enzyme is located in the F1 fraction (in the mitochondrial matrix) [18]. We have shown previously that the expression of the *ATP synthase F0 subunit 8* gene also differed between LEW and F344 in the NAcc [14]. In addition, the expression of subunit A, another subunit of this enzyme, also differs between rats depending on their ability to extinguish a previously established cocaine-induced CPP [7]. Another component of this complex, the mitochondrial precursor of the ATP synthase beta chain, was expressed more weakly in the NAcc after cocaine self-administration in monkeys [37]. Together, all these data suggest that ATP synthase is involved in addiction-like behavior and that it could be a useful biomarker of addiction vulnerability.

AKR1A1 catalyzes the NADPH-dependent reduction of a variety of aromatic and aliphatic aldehydes to their corresponding alcohols. There appear to be no studies showing any kind of relationship between this enzyme and any behavioral process or addiction-like behavior. Thus, further studies are warranted to ascertain the role of this protein in addiction and in behavior in general.

ACOT7 levels were higher in LEW than in F344 rats. Acyl-CoA thioesterases are a group of enzymes that catalyze the hydrolysis of acyl-CoAs to the free fatty acid and coenzyme A (CoASH), providing the potential to regulate intracellular levels of acyl-CoAs, free fatty acids, and CoASH. Neurons have particularly high cytoplasmic long-chain acyl-CoA hydrolase activity, which is thought to be mediated mainly by the isoform that was *elevated in LEW animals, acyl-CoA thioesterase 7* [9]. In addition, another isoform of this family of enzyme, Acyl-CoA thioesterase 2, was found to be elevated after extinction of morphine-induced CPP [40].

Enolases belong to a family of three enzymes that catalyze the conversion of 2-phosphoglycerate to phosphoenolpyruvate in one of the final (energy-yielding) steps of glycolysis [23]. Gamma enolase, also known as enolase-2 or neuron-specific enolase (ENO2) accumulates more intensely in LEW than in F344 rats. In addition, the cytosolic levels of this protein were upregulated in the NAcc of cocaine overdose victims [36] and it was downregulated in the NAcc of rhesus monkeys after cocaine self-administration [37]. Together, this evidence suggests

that disturbances in energy balance might somehow be disrupted in addictive-like states, which may also be indicative of an increased vulnerability to the reinforcing actions of drugs of abuse.

In this study, we have identified some proteins that are found at different levels *in the NAcc and Amg of LEW and F344 rats*, which are two strains that show distinct susceptibility to the reinforcing effects of drugs of abuse. Most of these proteins have been previously found to be modulated after cocaine self-administration, cocaine overdose, or after morphine or methamphetamine exposure, and they might therefore be potential biomarkers to detect individuals at risk of developing addictive disorders. However, we would like to highlight that some of these proteins are often altered in proteomic studies. Indeed, in a metanalysis of 169 articles focusing on rodent tissues, peroxiredoxins, enolases, and units of the ATP synthase protein were among the "Top 15" most common differentially expressed proteins in 2D gel-based proteomic studies [24]. Nevertheless, as we found strain differences in these proteins, further studies must now be performed to validate these results and determine their significance in relation to addiction and neuroscience.

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