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Innate differences in protein expression in the nucleus accumbens and hippocampus of inbred alcohol-preferring and -nonpreferring rats

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Abstract

Two-dimensional gel electrophoresis (2-DE) was used to separate protein samples solubilized from the nucleus accumbens and hippocampus of alcohol-naïve, adult, male inbred alcohol-preferring (iP) and alcohol-nonpreferring (iNP) rats. Several protein spots were excised from the gel, destained, digested with trypsin, and analyzed by mass spectrometry. In the hippocampus, 1629 protein spots were matched to the reference pattern, and in the nucleus accumbens, 1390 protein spots were matched. Approximately 70 proteins were identified in both regions. In the hippocampus, only 8 of the 1629 matched protein spots differed in abundance between the iP and iNP rats. In the nucleus accumbens, 32 of the 1390 matched protein spots differed in abundance between the iP and iNP rats. In the hippocampus, the abundances of all 8 proteins were higher in the iNP than iP rat. In the nucleus accumbens, the abundances of 31 of 32 proteins were higher in the iNP than iP rat. In the hippocampus, only 2 of the 8 proteins that differed could be identified, whereas in the nucleus accumbens 21 of the 32 proteins that differed were identified. Higher abundances of cellular retinoic acid-binding protein 1 and a calmodulin-dependent protein kinase (both of which are involved in cellular signaling pathways) were found in both regions of the iNP than iP rat. In the nucleus accumbens, additional differences in the abundances of proteins involved in (i) metabolism (*e.g.*, calpain, parkin, glucokinase, apolipoprotein E, sorbitol dehydrogenase), (ii) cyto-skeletal and intracellular protein transport (*e.g.*, β -actin), (iii) molecular chaperoning (*e.g.*, grp 78, hsc70, hsc 60, grp75, prohibitin), (iv) cellular signaling pathways (*e.g.*, protein kinase C-binding protein), (v) synaptic function (*e.g.*, complexin I, γ -enolase, syndapin IIb), (vi) reduction of oxidative stress (thioredoxin peroxidase), and (vii) growth and differentiation (hippocampal cholinergic neurostimulating peptide) were found. The results of this study indicate that selective breeding for disparate alcohol drinking behaviors produced innate alterations in the expression of several proteins that could influence neuronal function within the nucleus accumbens and hippocampus.

Keywords

Alcohol-preferring P rats; Hippocampus; Nucleus accumbens; Two-dimensional gel electrophoresis

1 Introduction

The alcohol-preferring (P) and -nonpreferring (NP) lines of rats were originally derived from a randomly bred, closed colony of Wistar rats (Wm:WRC(WI)BR) that was maintained at the Walter Reed Army Institute of Research [1]. Ethanol intakes of the P rat are $\geq$ than 5 g/kg body wt/day, whereas ethanol intakes of the NP line are 1 g/kg/day [1]. The P line of rats meets all the criteria proposed [2] for an animal model of alcoholism (reviewed in [3]). Innate differences have been reported in several neurotransmitters and receptors between the P and NP rat within the nucleus accumbens and hippocampus (reviewed in [3]). Compared to the NP line, the P line has lower dopamine (DA) and serotonin (5-HT) contents [4,5] and reduced 5-HT innervation [6,7] in several central nervous system (CNS) regions, including the nucleus accumbens and hippocampus. Reduced DA innervation [8], lower densities of DA D2 [9] and 5-HT2 [10] receptors, and higher densities of mu-opioid [11] receptors were reported in the nucleus accumbens of P than NP rats. Higher densities of 5-HT1A receptors have been found in the hippocampus of P than NP rats [12], whereas lower densities of mu-opioid [11] and delta [13] receptors were found in the hippocampus of P than NP rats. In addition, differences in γ -amino-*n*-butyric acid (GABA)-stimulated flunitrazepam binding were also observed in the hippocampus between the lines [14]. Because of the involvement of the nucleus accumbens in regulating alcohol intake [15], some of differences in the nucleus accumbens may be associated with the disparate alcohol drinking behaviors of these lines. Furthermore, the hippocampus is involved in the development of alcohol tolerance [16], and there are differences between the P and NP lines in the development of acute tolerance [17] and in the persistence of tolerance to a single dose of ethanol [18].

Many factors can influence gene and protein expression. To reduce genetic variance, inbred strains were used in the present study. Inbred strains are isogenic, single lines and are useful whenever genetic uniformity is desired. By removing genetic variants, other variances are limited to the effects of environmental or experimental manipulations, and the precision of the assay. Although a strain is frequently designated as inbred when it has been mated by brother-sister inbreeding for at least 20 generations, the coefficient of inbreeding still increases beyond the 20th generation [19]. For example, after the 20th generation, 1.4% of the total number of genes will still be heterozygous, whereas after the 40th generation, only 0.02% of the total number will be heterozygous. With such low residual heterozygosity, the inbred strain offers a nearly genetically homozygous animal pool that can be used to reduce innate variances and provide a nearly isogenic constant source of rats for integrative studies over a prolonged period.

Therefore, because of the innate differences already observed between the P and NP lines, and the involvement of the nucleus accumbens and hippocampus in mediating ethanol drinking and the development of tolerance, respectively, these structures were selected as regions to examine using proteomics technology. Relative abundances of proteins were determined to test the hypothesis that innate differences in the expression of proteins associated with synaptic function and cellular signaling pathways would be found in the nucleus accumbens and hippocampus of the inbred P and NP rats.

2 Materials and methods

2.1 Materials

Acrylamide for the slab gels was purchased from National Diagnostics (Atlanta, GA, USA) and for the IEF tube gels from Bio-Rad (Richmond, CA, USA). Other ultrapure electrophoretic reagents were obtained from Bio-Rad, Sigma Chemical (St. Louis, MO, USA), or BDH (Poole, UK). Sequence-grade trypsin was obtained from Promega (Madison, WI, USA). Dithiothreitol was obtained from Calbiochem (La Jolla, CA, USA) and *N*-isopropyl iodoacetamide from Molecular Probes (Eugene, OR, USA). All other chemicals used were reagent grade.

2.2 Animals

At this time, two of the iP strains (iP5c and iP10a) and two of the iNP strains (iNP1 and iNP4) have been genotypically characterized by genome screen and by creation of congenic strains [20,21]. Inbreeding by brother-sister mating was initiated after the S30th generation of mass selection and was carried out with 10 pairs of sibmates each from the P and NP lines. Inbreeding of the P/NP lines has progressed to their F37th generation. Adult male iP and iNP rats ($n = 5$ /strain), 90-95 days of age, were used in these studies. The iP rats were from strain 5c and the iNP rats were from strain 1. Rats were single housed in standard animal colony rooms under normal 12 h light cycle conditions (lights on at 700 h). Rats were handled each morning and habituated to the guillotine daily for at least 4 days prior to killing. Rats were killed by decapitation, and the brain rapidly dissected on a glass plate in a cold box maintained at -15°C . Dissections were done between 900-1000 h. The nucleus accumbens and hippocampus were stored at -70°C until assayed. Animals used in this study were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All experimental procedures were approved by the Institutional Animal Care and Use Committee and are in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, and the Guide for the Care and Use of Laboratory Animals of the National Research Council, 1996.

2.3 Sample preparation and protein separation

Frozen (-70°C) samples of iP and iNP rat brain tissue from the hippocampal and nucleus accumbens regions ($n = 5$) were weighed and then minced in a 50 mL beaker along with 8 volumes of a solution containing 9 M urea, 4% Igepal CA-630 ((octylphenoxy) polyethoxyethanol), 1% DTT, and 2% carrier ampholytes (pH 8-10.5) and thoroughly minced with surgical scissors. The minced samples were then placed in 3 mL DUALLR® ground-glass tissue grinders and manually homogenized. After complete solubilization at room temperature for 120 min, samples were centrifuged at $100\,000 \times g$ for 30 min using a Beckman TL-100 ultracentrifuge to remove nucleic acid and insoluble materials, and the supernatants stored at -45°C until 2-DE separation.

2.4 Two-dimensional electrophoresis and image analysis

Two-dimensional electrophoresis was performed essentially as described previously [22]. Twenty microliters of each of the 20 solubilized test samples were placed on each of 20 IEF tube gels (23×0.15 cm) containing 3.3% acrylamide (diacrylyl-piperazine as a cross-linker), 9 M urea, 2% Igepal CA-630, and 2% ampholyte (BDH pH 4-8). The IEF gels were run simultaneously using progressively increasing voltage (500 V for 1 h, 750 V for 1 h, 1000 V for 1 h, and 1400 V for the balance of the run) for a total of 27 500 Vh. A computer-controlled gradient casting system was used to prepare 20 second-dimensional SDS gradient slab gels in which the acrylamide concentration varied linearly from 11% to 17%T. First-dimensional IEF tube gels were loaded directly onto the slab gels without equilibration. Second-dimensional slab gels were run in parallel in a 20-gel slab electrophoresis tank at 8°C for 18 h at 160 V.

Slab gels were stained using a colloidal Coomassie Brilliant Blue G-250 procedure in covered plastic boxes, 10 gels per box. Gels were fixed in 1.5 L of 50% ethanol/2% phosphoric acid overnight followed by three 30 min washes in 2 L of deionized water. Gels were transferred to 1.5 L of 30% methanol/17% ammonium sulfate/3% phosphoric acid for 1 h followed by addition of 1 g of powdered Coomassie Brilliant Blue G-250 stain. After 96 h, the 20 gels were washed several times with water and scanned at 95.3 $\mu\text{m}/\text{pixel}$ resolution using a GS-800 Calibrated Imaging Densitometer (Bio-Rad). The resulting 12 bit images were analyzed using PDQuestTM software (Bio-Rad, V.6.2) running under Windows 2000 on a PC-workstation. Background was subtracted and peaks for the protein spots located and counted. Because total spot counts and the total optical density are directly related to the total protein concentration, individual protein quantities were thus expressed as parts-permillion (ppm) of the total integrated optical density. A reference pattern was constructed and each of the 20 gels in the matchset was matched to the reference gel. Numerous proteins that were uniformly expressed in all patterns were used as landmarks to facilitate rapid gel matching. Statistical comparisons between individual protein abundances were conducted within the PDQuestTM analysis by calculation of Student *t*-test assuming equal variances, and after data export to Excel.

2.5 Peptide mass fingerprinting

Protein spots that differed in abundance between iNP and iP groups along with other spots from the stained and image-analyzed 2-DE gels were cut from the gel robotically using the Protean[®] 2D Spot Cutter (Bio-Rad), placed in each of 94 wells of a 96-well plate, along with one gel blank and serum albumin in acrylamide, and processed using the MassPREP Station (MicroMass). In this automated system, the excised protein spots were destained with 100 mM ammonium bicarbonate-50% acetonitrile followed by 100% acetonitrile, reduced with 10 mM dithiothreitol in 100 mM ammonium bicarbonate, alkylated with 55 mM iodoacetamide in 100 mM ammonium bicarbonate, and tryptically digested using Promega sequence grade, modified trypsin at a final concentration of 13 ng/ μL in 100 mM ammonium bicarbonate for 14 h (overnight). The resulting peptides were extracted by addition of 10 μL trypsin solution to the wells and refrigerating the plate for 30 min. Four microliters of peptide extract was then placed onto each corresponding position (A1-H12) on the MALDI target plate, air-dried, and the application repeated until all extract buffer was used up. When the peptide sample targets were dry, each was overlaid with 1 μL of matrix (10 mg/mL α -cyano-4-hydroxycinnamic acid-0.05% trifluoroacetic acid) and analyzed by MALDI-TOF-MS using a MicroMass M@LDI System (MicroMass). Prior to data collection, the instrument was calibrated using peptide standards and internal standards based on tryptic autolysis peaks (842.5099 and 2211.1045 Da were used for calibration). Proteins were identified by manual ProFoundTM (Proteometrics LLC) database searches using the mass lists obtained from MALDI spectra of 188 spots cut from the gels. A z-score of 1.30, corresponding to the 90th percentile, was the threshold for what was considered a positive identification. This means that for a given peptide mass search with a z-score of at least 1.30, no more than 10% of random matches could yield higher z-scores. More than half of the proteins reported in Table 1 exceeded the 95th percentile.

3 Results

The 2-DE protein patterns obtained from hippocampus and nucleus accumbens are shown in Figs. 1a and b. The patterns for these two regions were similar. In the hippocampus, 1629 protein spots were matched to the reference pattern, and in the nucleus accumbens, 1390 protein spots were matched to the reference pattern. Approximately 70 proteins were identified in both the hippocampus and nucleus accumbens (Table 1). Of the 1629 protein spots that were matched in the hippocampus, only 8 proteins were significantly different ($P < 0.05$) between the inbred strains in abundance, representing 0.5% of the proteins analyzed. Based on mean integrated densities determined by image analysis, the expression of all 8 proteins was higher

in the iNP than iP group. Unfortunately, only 2 of these were identified by peptide mass fingerprinting, *i.e.*, cellular retinoic acid-binding protein 1, and a calmodulin-dependent protein kinase. Cellular retinoic acid-binding protein was in very low abundance in the hippocampus of the iP rat, and was nearly 12-fold higher in the hippocampus of the iNP rat. However, even in the hippocampus of the iNP rat, this protein was present in relatively low abundance compared to the levels of the other identified proteins (Table 1). Calmodulin-dependent protein kinase was 3-fold higher in abundance in the hippocampus of the iNP than iP rats. However, the abundance of this kinase was relatively low in the hippocampus of both inbred strains. Moreover, this protein had a low z-score and only reached the 64th percentile, suggesting that there is some doubt concerning the identification of this protein.

Fold differences of 1.3 and higher were found in another 27 identified proteins in the hippocampus, but these differences did not reach statistical significance (Table 1). Of these 27 proteins, 24 were in greater abundance in the hippocampus of the iNP than iP rats. These 24 proteins included syndapin IIbb, BM259, 2 forms of the calpain small subunit, acetylcholine receptor epsilon, voltage-gated K channel protein KV1.4, mitochondrial import stimulation factor S1 chain, β -actin, glial fibrillary acidic protein α , grp78 (and/or synaptic vesicle glycoprotein 2b), hsc70, prohibitin, neutral sphingomyelinase II, grp75, protein tyrosine phosphatase (fragment), HIP70, hexokinase, sorbitol dehydrogenase, parkin, matricin, R8f DNA-binding protein, and TIC. Protein spots 2606 HIP and 2634 NA were identified as grp78 and synaptic vesicle glycoprotein 2b. ProfoundTM z-scores were 0.67 and 0.60, respectively. It is likely that these proteins occupy similar x, y-coordinate positions on the 2-D gel and were cut and digested together. The 3 proteins that tended to be higher in the hippocampus of the iP than iNP were the yotiao protein, glial fibrillary acidic protein α , and RP58 protein (and/or syndapin IIaa). With the exception of the R8f DNA-binding protein, all the proteins that tended to show a difference in the hippocampus between the iP and iNP strains were in much greater abundance than the 2 proteins that were statistically different between the strains.

In the nucleus accumbens, 1390 protein spots were matched to the reference pattern and 32 proteins differed significantly ($P < 0.05$) in abundance, which represents 2.6% of the analyzed proteins. Among these differences, 31 of 32 protein abundances were higher in the iNP than iP group. Of these proteins, 21 were identified. Higher abundances were found in the iNP compared to iP rats for syndapin IIbb, BM259, cellular retinoic acid-binding protein 1, complexin 1, thioredoxin peroxidase, hippocampal cholinergic neurostimulating peptide (HCNP), calpain small subunit 1, apolipoprotein-E (Apo-E), γ -enolase, grp78 (and/or synaptic vesicle glycoprotein 2b), Hsc70, prohibitin, calmodulin-dependent protein kinase, β -actin, protein kinase C-binding protein, hsp60, glucokinase, grp75, sorbitol dehydrogenase, and parkin. Only the abundance of Hsc70 (charge variant) was statistically higher in the iP than iNP rats. Furthermore, complexin 1, grp78, HCNP, calpain small subunit 1, calmodulin-dependent protein kinase and BM259 had low z-scores and ranked well below the 90th percentile, suggesting that there is some doubt regarding the identification of these proteins.

As was the case in the hippocampus, the largest fold differences in abundances between the iP and iNP rats were found for cellular retinoic acid-binding protein 1 (5.5-fold) and a calmodulin-dependent protein kinase (2.4-fold). The abundance of cellular retinoic acid-binding protein was relatively low in the nucleus accumbens. For the most part, the other proteins, which differed between the iP and iNP rats, were present in relative high abundances, with the highest values being found for γ -enolase, hsc70, and parkin (Table 1). Differences in abundances between the strains of 1.7- and 1.8-fold were found for syndapin IIbb, BM259, protein kinase C-binding protein, and grp75.

There were trends toward higher abundances of several proteins in the nucleus accumbens of iNP than iP rats that were not statistically significant. Trends for differences between the iP

and iNP strains in protein abundances of 1.3-fold and higher were found for both charge forms of calpain small unit, voltage-gated K channel protein KV1.4, mitochondrial import stimulation factor S1 chain, neutral sphingomyelinase II, protein tyrosine phosphatase (frag), and p60 protein (Table 1). In addition, there was a 1.7-fold higher abundance of RP58 protein (and/or syndapin IIaa) in the nucleus accumbens of the iP than iNP rats, which was also not statistically significant.

4 Discussion

The results of the present study support our hypothesis that there are innate differences in the expression of proteins involved in synaptic function and cellular signaling pathways in two key limbic structures of rat lines selectively bred for disparate alcohol drinking behaviors. The largest number of differences was found in the nucleus accumbens, a structure that plays a key role in regulating alcohol intake [3,15]. The data suggest that there may be some basic differences in the mechanisms underlying synaptic transmission in the nucleus accumbens between the iP and iNP rats. Differences in the abundances of (i) complexin 1, which plays a key role in Ca²⁺-dependent transmitter release [23]; (ii) γ -enolase, which plays a role in the fusion of the synaptic vesicle with the synaptic plasma membrane [24]; (iii) Hsc70, which is part of a protein complex isolated from purified synaptic plasma membranes and synaptic vesicles, and may be involved in endocytosis [24]; (iv) syndapin IIbb, a member of a family of cytoplasmic phosphoproteins that appears to be involved in synaptic vesicle recycling (endocytosis) and actin cytoskeletal organization [25]; and (v) synaptic vesicle glycoprotein 2b (grp78), which has a role in calcium homeostasis [26], were found between the iNP and iP rats. The higher abundances of these five proteins in the nucleus accumbens of the iNP *versus* the iP rat might suggest that the mechanisms mediating exocytotic transmitter release and vesicle recycling are operating at a lower capacity in the nucleus accumbens of the iP than iNP rat. Alternatively, the lower abundances of these five proteins in the nucleus accumbens of the iP than iNP rat might reflect lower 5-HT and DA innervation to the nucleus accumbens of the P rat compared to the NP rat [6-8], and, therefore, fewer nerve terminals and synaptic vesicles in the iP rats.

The lower abundances of (i) protein kinase C-binding protein, which can affect the interactions of protein kinase C with its substrates as well as its cellular location [27]; (ii) calmodulin-dependent protein kinase, which can phosphorylate several proteins involved in synaptic function [28-30]; and (iii) cellular retinoic acid binding protein 1, which can negatively regulate protein kinase C activity [31], in the nucleus accumbens of iP compared to iNP rats suggest that there may be significantly altered cellular signaling systems in the iP rat. Alternatively, the lower abundances of these three proteins may reflect the reduced 5-HT and DA innervation noted above.

The lower abundances of (i) β -actin, an intracellular protein transporter; (ii) the endoplasmic reticular molecular chaperones grp78, Hsc70 and Hsc60 [26,32]; and (iii) grp75 and prohibitin, which are membrane-bound mitochondrial chaperones [33,34], might also reflect the reduced 5-HT and DA innervation to the nucleus accumbens. These proteins would be involved in transporting proteins manufactured in the cell body to the nerve terminals and ensuring that the transported proteins are properly incorporated into the functional elements of the synaptic terminal.

The HCNP was present in relatively large abundance in the nucleus accumbens with 1.4-fold higher amounts in the iNP than iP. The HCNP is a undecapeptide involved in differentiation of presynaptic cholinergic neurons in the medial septum and can enhance the production of choline acetyl transferase [35]. The nucleus accumbens of the rat contains cholinergic interneurons, and this system has not been studied thus far in the P and NP rats. It is possible

that the lower abundance of HCNP in the nucleus accumbens of the iP rat may suggest that the cholinergic system within the nucleus accumbens of the iP rat has not developed to the same extent as that for the iNP rats.

Thioredoxin peroxidase is one of a family of peroxiredoxins that destroy peroxides [36]. These enzymes are cellular antioxidants and help fight oxidative stress and maintain normal cell function. The lower abundance of thioredoxin peroxidase in the nucleus accumbens of iP rats compared to iNP rats could indicate that iP rats are more vulnerable to oxidative stress. This may also indicate that the CNS of the P line of rats may be more vulnerable to the chronic effects of high dose ethanol through its oxidation to acetaldehyde by hydrogen peroxide *via* a catalase-mediated reaction.

The abundances of several proteins involved in protein degradation, glucose metabolism, and lipid metabolism were significantly different in the nucleus accumbens of iP *versus* iNP rats. Lower abundances of (i) calpain (small subunit 1), a Ca²⁺-activated neutral protease that has been implicated in playing a role in neuronal injury and excitotoxicity [37]; and (ii) parkin, a ubiquitin protein ligase, which tags proteins for degradation [38], were found in the iP compared to the iNP, suggesting that the degradation rate of certain proteins may be slower in the nucleus accumbens of the iP rat. The lower abundances of (i) glucokinase, an enzyme that modulates activity of ATP-sensitive K⁺ channel and can alter the rate of cell firing in specialized cells using glucose as a regulator of cell activity [39]; and (ii) sorbitol dehydrogenase, an enzyme along with aldose reductase that constitutes the polyol pathway [40] (and is an alternate route of glucose metabolism), in the iP than iNP rat suggest that there may be differences in the utilization of glucose between the two rat strains, and that this could result in differences in basal firing rates of glucose sensitive cells in the nucleus accumbens. Apo-E is involved in cell growth and maintenance, and lipid (cholesterol) metabolism. The lower abundance of Apo-E in the nucleus accumbens may indicate retarded cellular growth and/or maintenance in the iP than iNP rat.

Lower abundances ($p < 0.05$) of cellular retinoic acid-binding protein 1 and a calmodulin-dependent protein kinase were also found in the hippocampus of the iP than iNP rat. However, only trends for lower abundances of other proteins were found in the hippocampus of the iNP *versus* the iP rat. These trends were observed for syndapin IIbb, BM259, calpain small subunit 1, grp78, Hsc70, prohibitin, β -actin, grp75, sorbitol dehydrogenase, and parkin. The hippocampus of the P rat has reduced 5-HT innervation compared to that for NP rats [6,7]. However, there is very little if any DA input into the hippocampus of the rat. Therefore, the smaller number of differences in protein expression observed in the hippocampus may reflect a relatively smaller degree of reduced innervation. The nucleus accumbens receives major DA and 5-HT innervation and has relatively high densities of these monoamine nerve terminals [6-8]. Therefore, a reduction in these inputs would have a greater relative impact on this region than the reduction that 5-HT innervation has in the hippocampus, because 5-HT innervation contributes a relatively small proportion of the total nerve terminals in this region.

The nucleus accumbens has been implicated in regulating alcohol drinking behavior [3,15], and the hippocampus appears to be involved in the development of tolerance [16]. The P and NP rats differ in alcohol drinking behavior as well as in the development and persistence of tolerance [17,18]. If the lower abundances of proteins in the nucleus accumbens and hippocampus of the iP rat compared to the iNP rat reflect reduced monoamine innervation, then it is possible that the innately lower DA and 5-HT innervations in the P rats may be major factors contributing to their high alcohol drinking behavior and their more rapid development of acute ethanol tolerance and persistence of ethanol tolerance after it has developed.

Thus far, proteins associated with receptor function (*e.g.*, 5-HT_{1A}, delta and mu-opioid receptors in the hippocampus, and D₂ and 5-HT₂ receptors in the nucleus accumbens) or DA and 5-HT innervation (*e.g.*, DA or 5-HT transporter) have not been identified on the 2-DE gels. These proteins may be in relatively low abundance and may be present in levels below the sensitivity of the gel assay. Also, receptors and transporters are membrane bound proteins and may not readily solubilize with our current procedure. Thus, the combination of low abundance and poor extraction may have prevented their detection in the present study.

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Abbreviations

Apo-E, apolipoprotein-E; DA, dopamine; grp, glucose-regulated protein; HCNP, hippocampal cholinergic neurostimulating peptide; HIP, hippocampus; hsc, heat shock cognate; 5-HT, serotonin; iNP, inbred alcohol-nonpreferring; iP, inbred alcohol-preferring; NA, nucleus accumbens.

5 References

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Figure 1. 2-DE pattern of (A) rat hippocampus and (B) nucleus accumbens proteins, solubilized and separated on 23.5 cm IEF tube gels and 20 × 25 × 0.15 cm slab gels, and stained with colloidal Coomassie blue as described in Section 2.4. By convention, the horizontal dimension represents a pH gradient of approx. 4-7.5 and the vertical an acrylamide gradient of 11-17%. The protein spots were analyzed by peptide mass fingerprinting using MALDI-MS and those successfully identified are designated by their spot number (SSP) in the PDQuest™ reference pattern. The corresponding protein names and expression information appear in Table 1.

Table 1

Mean abundance and fold differences between iNP and iP rats for HIP and NA proteins identified by peptide mass fingerprinting

HIP SSP ^{a)}	NA SSP	Identification	GenBank Accession	Abundance		Fold ^{b)}	Abundance		ProFound™		Statistics
				HIP iNP	HIP iP		NA iNP	NA iP	Fold	z-score ^{c)}	
420	1407	Syndapin IIbb	AAF22214	1 881	1 467	1.3	2 754	1 532	1.8	1.83	96.6
1008	2103	BM259 (stroke prone associated)	AAAK64519	1 804	1 164	1.5	2 950	1 787	1.7	0.60	72.6
1027	2119	Retinoic acid-binding protein	P02695	405	34	11.8	575	104	5.5	1.78	96.3
1033	2012	Complexin I	NP_074055	2 006	1 646	1.2	3 858	2 824	1.4	0.51	69.5
1222	2204	Calpain small subunit (charge variant)	AAC53002	1 992	1 333	1.5	2 621	1 552	1.7	1.66	95.2
1227	2208	Calpain small subunit	AAC53002	1 360	988	1.4	1 754	1 368	1.3	0.58	71.9
1439	2433	ATP synthase	AAB02288	8 794	7 582	1.2	15 662	12 978	1.2	2.43	99.3
1441	2436	Yotiao protein	AAF27283	1 219	1 752	0.7	2 048	2 103	1.0	0.83	79.7
1526	2427	ATP synthase	A28701	1 685	1 753	1.0	2 530	3 015	0.8	2.43	99.3
1528	2431	RP58 protein	NP_073169	1 042	1 273	0.8	1 811	2 003	0.9	1.80	96.4
1531	2477	Ryanodine receptor type	AAB70013	2 513	2 248	1.1	1 435	1 739	0.8	0.99	83.9
1533	2420	Fibulin-3	O35568	3 164	2 977	1.1	5 865	5 181	1.1	1.66	95.2
1534	2474	Acetylcholine receptor	NP_058890	2 680	1 721	1.6	3 262	2 641	1.2	2.43	99.3
1618	2505	Voltage-gated potassium channel protein KV1.4	P15385	3 776	2 895	1.3	2 968	2 209	1.3	2.43	99.3
1812	21576	Tenascin-R	NP_037177	3 090	2 514	1.2	5 675	5 191	1.1	0.90	81.6
2105	2138	Thioredoxin peroxidase	NP_058865	1 359	1 125	1.2	2 830	1 940	1.5	1.37	91.5
2111	3107	Hippocampal cholinergic neurostimulating peptide	NP_058932	4 882	3 981	1.2	7 264	5 249	1.4	1.06	85.5
2209	2244	Mitochondrial import stimulation factor S1 chain	JC2502	1 977	1 282	1.5	3 664	2 773	1.3	2.43	99.3
2225	2256	Calpain small subunit	AAC53002	4 354	3 437	1.3	8 634	6 208	1.4	0.31	62.2
2338	3223	Apo-E	P02650	1 682	1 383	1.2	2 688	2 202	1.2	1.78	96.3
2405	2444	Enolase, γ	P07323	7 548	7 195	1.0	12 729	10 714	1.2	2.43	99.3
2435	3308	Actin,	NP_112406	5 914	4 470	1.3	11 029	9 095	1.2	1.71	95.6
2439	3315	Actin,	P02571	2 829	2 743	1.0	5 015	4 862	1.0	1.89	97.1
2444	3420	Glial fibrillary acidic protein	AAD01873	995	1 339	0.7	711	591	1.2	2.43	99.3
2451	3430	Glial fibrillary acidic protein	AAD01873	4 916	3 539	1.4	6 136	5 648	1.1	2.41	99.2
2511	2451	RP58 protein	NP_073169	749	1 473	0.5	1 686	2 728	0.6	1.51	93.5

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HIP SSP ^{d)}	NA SSP	Identification	GenBank Accession	Abundance			Abundance			ProFound™	Statistics	
				HIP iNP	HIP iP	Fold ^{b)}	NA iNP	NA iP	Fold		z-score ^{c)}	Percentile ^{d)}
2541	3510	Unknown (specific to rat suprachiasmatic nucleus)	AAD26207	994	1 093	0.9	1 627	1 738	0.9	1.16		87.7
2549	3512	Intermetxin,	NP_062001	3 176	2 604	1.2	5 115	4 684	1.1	2.43		99.3
2606	2634	Grp78, synaptic vesicle glycoprotein 2 b	P06761	1 147	854	1.3	2 009	1 599	1.3	0.67		74.9
2637	3514	Hsc70 (charge variant)	S31716	2 184	2 620	0.8	3 143	3 421	0.9	1.22		88.9
2643	3521	Hsc70	NP_077327	11 424	8 960	1.3	19 308	15 062	1.3	2.43		99.3
2728	3606	Pervin	NP_077377	801	672	1.2	1 335	1 361	1.0	1.24		89.2
3228	3257	Prohibitin	NP_114039	846	638	1.3	1 407	1 047	1.3	1.92		97.3
3305	3336	Calmodulin-dependent protein kinase	1A06	528	177	3.0	1 231	520	2.4	0.52		69.9
3336	4232	Lactate dehydrogenase B	NP_036727	713	823	0.9	1 086	890	1.2	2.30		98.9
3338	4325	Lactate dehydrogenase B	NP_036727	5 426	4 621	1.2	10 887	9 240	1.2	1.11		86.6
3408	3347	Actin, β	ATRTC	3 060	2 443	1.3	4 747	3 739	1.3	2.43		99.3
3415	3472	Protein kinase C-binding protein	AAK54603	1 051	932	1.1	2 471	1 462	1.7	1.36		91.3
3419	44307	Creatine kinase B	AAA40933	11 169	9 523	1.2	17 912	15 260	1.2	2.43		99.3
3506	3439	Hsp60	NP_071565	4 289	3 672	1.2	7 433	5 537	1.3	2.43		99.3
3527	4410	Glucokinase	NP_036697	1 059	930	1.1	1 797	1 354	1.3	2.43		99.3
3530	4419	Vacuolar ATP synthase subunit B, brain	P50517	1 041	856	1.2	1 466	1 394	1.1	1.11		86.6
3605	4532	Neutral sphingomyelinase II	NP_067466	5 621	3 899	1.4	7 260	4 308	1.7	1.80		96.4
3614	3538	Grp75	S31716	1 920	1 353	1.4	3 540	2 081	1.7	2.43		99.3
3641	4522	Integrin β -7 subunit	AAB61241	1 748	1 442	1.2	2 441	2 226	1.1	1.42		92.2
4010	5002	Protein tyrosine phosphatase (frag)	AAB32800	1 217	914	1.3	1 703	1 278	1.3	0.77		77.9
4017	5025	Dismutase	CAA29121	1 503	1 827	0.8	3 199	2 654	1.2	0.21		58.3
4505	4472	HIP-70	P11598	1 536	1 144	1.3	2 502	2 055	1.2	2.43		99.3
4508	4447	Glucokinase	AAA41239	905	724	1.3	495	404	1.2	2.43		99.3
4542	5546	Serotonin receptor 3B	NP_064670	110	101	1.1	92	89	1.0	0.47		68.1
4561	5551	Lamins C and C2	P11516	718	936	0.8	1 629	1 377	1.2	1.41		92.1
4639	5545	Serum albumin	P02770	1 672	2 216	0.8	3 122	3 934	0.8	1.44		92.5
4815	4838	Kalirin	P97924	844	735	1.1	1 556	1 869	0.8	0.54		70.5
5303	6202	Phosphatidylinositol transfer protein beta	P53812	2 082	1 899	1.1	3 635	4 517	0.8	0.41		65.9

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HIP SSP ^{a)}	NA SSP	Identification	GenBank Accession	Abundance			Abundance			ProFound™	Statistics	
				HIP iNP	HIP iP	Fold ^{b)}	NA iNP	NA iP	Fold		z-score ^{c)}	Percentile ^{d)}
5337	6348	Sorbitol dehydrogenase	NP_058748	1 196	770	1.6	1 459	1 082	1.3		2.29	98.9
5402	6401	Protein phosphatase 2A	P56932	1 911	2 264	0.8	3 590	3 172	1.1		0.26	60.3
5428	6427	Parkin	AAF68666	14 233	10 859	1.3	21 982	17 066	1.3		1.93	97.3
5522	6413	c-Jun N-terminal kinase 3	P49187	1 062	1 202	0.9	1 404	1 256	1.1		1.89	97.1
5530	6528	Pyruvate dehydrogenase phosphatase isoenzyme 1	NP_0622345	5 916	5 127	1.2	9 606	8 593	1.1		1.07	85.8
5546	6444	Matricin	S42723	828	617	1.3	1 252	1 218	1.0		2.43	99.3
6078	7030	Insulin-like growth factor-binding protein	2207299A	785	714	1.1	813	810	1.0		0.73	76.7
6201	7204	Phosphoglycerate mutase 1	NP_445742	760	732	1.0	1 090	1 020	1.1		2.30	98.9
6319	7331	Zinc transporter 2	NP_037022	1 251	1 196	1.0	2 005	1 934	1.0		0.56	71.2
6417	7323	Aflatoxin B1 aldehyde reductase	P38918	1 159	1 006	1.2	1 417	1 800	0.8		2.43	99.3
6517	7525	Proprotein convertase subtilisin/kexin type 2	NP_036878	418	275	1.5	463	419	1.1		2.43	99.3
6532	7539	TTC	AAC21449	1 234	982	1.3	1 773	1 752	1.0		2.04	97.9
7033	8009	p75NTR-associated cell death executor	NP_445853	974	849	1.1	1 580	1 412	1.1		0.76	77.6
7329	8313	Glutamine synthetase	1717354A	5 163	4 896	1.1	8 216	7 807	1.1		2.41	99.2
7561	8424	p60 protein	CAA75351	857	717	1.2	1 019	793	1.3		1.57	94.2
8102	8226	Type 3 5'detodinase	P49897	853	722	1.2	1 140	1 381	0.8		2.11	98.3

^{a)}Protein spot number in PDQuest reference pattern

^{b)}iNP protein abundance/iP protein abundance

^{c)}The z-score is used as an indicator of the quality of the search result and is estimated when the search result is compared against an estimated random match population

^{d)}Probability that the candidate in the ProFound database search is the protein whose peptide masses were submitted; bold print signifies $P < 0.05$, iNP vs. iP.