Pharmacology, Biochemistry and Behavior 92 (2009) 304–313

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Differential effects of ethanol in the nucleus accumbens shell of alcohol-preferring (P), alcohol-non-preferring (NP) and Wistar rats: A proteomics study

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ARTICLE INFO

Article history:
Received 24 July 2008
Received in revised form 15 December 2008
Accepted 20 December 2008
Available online 6 January 2009

Keywords:
Alcohol-preferring rats
Alcohol-non-preferring rats
Nucleus accumbens shell
Protein expression
Proteomics
Alcohol

ABSTRACT

The objective of this study was to determine the effects of ethanol injections on protein expression in the nucleus accumbens shell (ACB-sh) of alcohol-preferring (P), alcohol-non-preferring (NP) and Wistar (W) rats. Rats were injected for 5 consecutive days with either saline or 1 g/kg ethanol; 24 h after the last injection, rats were killed and brains obtained. Micro-punch samples of the ACB-sh were homogenized; extracted proteins were subjected to trypsin digestion and analyzed with a liquid chromatography–mass spectrometer procedure. Ethanol changed expression levels (1.15-fold or higher) of 128 proteins in NP rats, 22 proteins in P, and 28 proteins in W rats. Few of the changes observed with ethanol treatment for NP rats were observed for P and W rats. Many of the changes occurred in calcium–calmodulin signaling systems, G-protein signaling systems, synaptic structure and histones. Approximately half the changes observed in the ACB-sh of P rats were also observed for W rats. Overall, the results indicate a unique response to ethanol of the ACB-sh of NP rats compared to P and W rats; this unique response may reflect changes in neuronal function in the ACB-sh that could contribute to the low alcohol drinking behavior of the NP line.

1. Introduction

The alcohol-preferring (P) and -non-preferring (NP) rats were selectively bred for high and low alcohol-drinking behavior, respectively (Lumeng et al., 1977). There are innate neurobiological differences within the nucleus accumbens (ACB) between P and NP rats (reviewed in Bell et al., 2005; McBride and Li, 1998; Murphy et al., 2002). In addition, the P and NP rats differ in a number of behavioral measures (reviewed in McBride and Li, 1998; Murphy et al., 2002). With regard to responses to ethanol, NP rats are more sensitive than P rats to the motor impairing effects of moderate to high dose ethanol (Lumeng et al., 1982; Rodd et al., 2004). In contrast, P rats are sensitive to the low-dose stimulating effects of ethanol, whereas NP rats are not (Waller et al., 1986; Rodd et al., 2004). P rats develop tolerance to the high dose motor impairing effects of ethanol more readily than NP rats (Waller et al., 1983), and tolerance persists longer in the P than NP rat (Gatto et al., 1987). In another study, repeated intraperitoneal (i.p.) injections of 1 g/kg ethanol produced differential changes in dopamine and serotonin neurotransmission in the ACB of P, NP and Wistar (W) rats (Smith and Weiss, 1999). In addition, using the oscillating bar task, NP rats were more sensitive than P rats to the motor impairing effects of 1 g/kg i.p. ethanol; with repeated ethanol injections, the performance of the NP rats on the oscillating bar task improved, suggesting the development of tolerance (Bell et al., 2001). Overall, these results suggested that ethanol exposure produced differential neuronal alterations in the CNS between P and NP rats.

The nucleus accumbens appears to be involved in mediating alcohol drinking behavior (reviewed in Koob et al., 1998; McBride and Li, 1998). The studies of Smith and Weiss (1999) and Thielen et al. (2004) suggested that repeated ethanol injections or chronic ethanol drinking could produce alterations in monoamine neurotransmission within the ACB.

The effects of ethanol in the CNS are very complex and likely to produce a number of alterations at the cellular level. In order to better understand the complex actions of ethanol at both the behavioral and cellular level, it is important to have multiple experimental approaches. One approach is to study one system at a time with a well-defined and focused hypothesis. An alternative is to take a much broader approach, using genomics and proteomics tools to obtain more information, which could better define the effects of ethanol at multiple cellular and biological systems levels. There have been several studies that applied genomics (mainly) and proteomics analyses to examining the effects of ethanol in rodent models and

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doi:10.1016/j.pbb.2008.12.019

post-mortem human tissue (Alexander-Kaufman et al., 2006, 2007; Bell et al., 2006; Flatscher-Bader et al., 2005; Kerns et al., 2005; Lewohl et al., 2000, 2004; Liu et al., 2004; Matsumoto et al., 2007; Mayfield et al., 2002; Rodd et al., 2008; Saito et al., 2002, 2004; Treadwell and Singh, 2004). The studies conducted on post-mortem human tissue measured the consequences of long-term alcohol consumption and were aimed at regions where the neurotoxic effects of alcohol have been reported (Alexander-Kaufman et al., 2006, 2007; Flatscher-Bader et al., 2005; Lewohl et al., 2000, 2004; Liu et al., 2004; Matsumoto et al., 2007; Mayfield et al., 2002). Several studies were conducted examining the effects of chronic forced ethanol drinking by mice and rats on changes in gene expression in whole brain or cerebral cortex (Saito et al., 2002, 2004; Treadwell and Singh, 2004). A more recent study (Bell et al., 2006) examined the effects of chronic alcohol drinking by P rats on protein levels in the ACB and amygdala, using a 2-dimensional gel (2-DG) electrophoresis technique with mass spectrometry (MS). Although differences in protein levels were observed, this technique is relatively insensitive, and only the most abundant proteins were detected.

The objective of the present study was to use a sensitive liquid chromatography (LC)–MS procedure (Higgs et al., 2005) to determine the effects of repeated ethanol administration on protein expression in the ACB shell of P and NP rats. The ACB shell was selected because this region of the ACB is involved in mediating reinforcement, whereas the core portion does not appear to be involved in reinforcement processes (Ikemoto et al., 1997). The repeated ethanol injection procedure was used because this protocol has been shown to produce differential effects on monoamine neurotransmission in the ACB of P, NP and W rats (Smith and Weiss, 1999). In addition, the present experimental approach permitted changes in protein levels to be detected in a key limbic region of rats that exhibit disparate alcohol drinking characteristics. In the present study, it was important to include W rats for comparison purposes to help interpret differences between P and NP rats as being mainly due to unique responses to ethanol in the P line, or unique responses to ethanol in the NP line. The hypothesis to be tested is that ethanol will differentially alter the levels of proteins involved in synaptic function in the ACB-shell between P and NP rats.

2. Methods

Adult male P, NP and W rats (n=20/strain), 90–100 days old at the time of the experiment, were used in this study. P and NP rats were from the 60th generation, and were obtained from breeding facilities on the Indiana University School of Medicine campus. P and NP rats were originally derived from an out-bred W stock at Walter Reed Army Institute of Research (Lumeng et al., 1977). W rats used in the present study were purchased from Harlan Industries (Indianapolis, IN). Animals were received in our facilities 3 weeks prior to the experiment. Rats were double housed on a reverse 12:12 light–dark cycle with lights off at 0900 h. Rats had water and rat chow *ad libitum*. Animals were habituated to handling and injection procedures for 5 days prior to initiating the experiment. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols 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, NIH, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

2.1. Experimental protocols

Using the protocol of Smith and Weiss (1999), 10 rats in each strain were injected i.p. with saline and 10 rats in each strain were injected with 1 g/kg ethanol once each day for 5 consecutive days; 24 h after the last injection, rats were killed by decapitation, the brains quickly removed and frozen in isopentane in dry ice. Brains were stored at −70 °C until sectioned. Two samples were lost during the sample analysis, i.e., one saline injected P rat and one ethanol injected W rat. Therefore, there were 10 samples in 4 of the 6 groups, and 9 samples in the remaining 2 groups.

On the day of preparation of micro-punch samples, brains were transferred to a cryostat set at −6 to −10 °C at least 2 h prior to sectioning. Sections (300 µm) were obtained and transferred to glass slides that had been pre-cooled in the cryostat. Micro-punch sampling was done on a frozen stage (−25 to −35 °C) with an anatomic microscope equipped with a cool microscope lamp. Micropunch samples (0.77 mm dia.) were obtained bi-laterally; usually samples of the ACB-shell could be obtained from 2–3 sections from each rat. The stereotaxic atlas of Paxinos and Watson (1998) was used to identify the ACB-shell. After withdrawing the micro-punch sample, a distinct demarcated hole remained; this hole was used to validate the micro-dissection method. Two trained individuals independently verified the dissections. The micro-punched samples from the same animal were pooled and stored at −70 °C until samples from all rats had been collected. Samples from one animal were not pooled with samples from another animal.

2.2. Proteomics procedure

A label-free LC/MS-based protein quantification method of Higgs et al. (2005) was used to determine relative changes in protein levels. A brief description of the procedure is given below; more detailed information can be obtained from the original article of Higgs et al. (2005).

Tissue samples from individual rats were homogenized in 1 M urea and 10 mM DTT. The resulting protein extracts were reduced and alkylated by volatile reagents triethyphosphine and iodoethanol, as previously described (Hale et al., 2004); the protein extract was then digested with trypsin. The volatile reduction and alkylation steps allow all sample preparation steps to be carried out in one tube, which minimizes variations in sample preparation. The total peptide concentration was determined before and after Trypsin digestion with the Bradford Protein Assay (Bradford, 1976). Values were similar for both assays. The peptide determination after digestion was done to ensure similar amounts of each sample were injected onto the column.

Digested individual samples from the 6 groups were randomized for analysis to minimize technical artifacts. For each sample, 20 µg of the tryptic peptides were injected onto a C18 microbore column (i.d.=1 mm; length=5 cm). Peptides were eluted with a linear gradient from 5 to 45% acetonitrile over 120 min at a flow rate of 50 µl/min; the effluent was electro-sprayed into a LTQ linear ion-trap mass spectrometer (MS; Thermo-Finnigan).

The MS data were collected in the "Triple-Play" mode (MS scan, Zoom scan, and MS/MS scan). Each sample was analyzed with this approach; samples were injected only once. The acquired data were then filtered and analyzed by licensed software developed and described by Higgs et al. (2005). Searches against the IPI (International Protein Index) and the Non-Redundant (NCBI) databases were carried out using both the SEQUEST and X!Tandem algorithms.

Protein quantification was carried out as described by Higgs et al. (2005). Briefly, after the raw files were acquired from the LTQ, all extracted ion chromatograms (X!C) were aligned by retention time, using the algorithm and procedure described by Higgs et al. (2005). Each aligned peak must match precursor ion, charge state, fragment ions (MS/MS data), and retention time (within a three-minute window). After alignment, the area-under-the-curve (AUC) for each individually aligned peak from each sample was measured, normalized, and compared for relative abundance. Peak intensities were transformed to a log2 scale before quantile normalization (Bolstad et al., 2003).

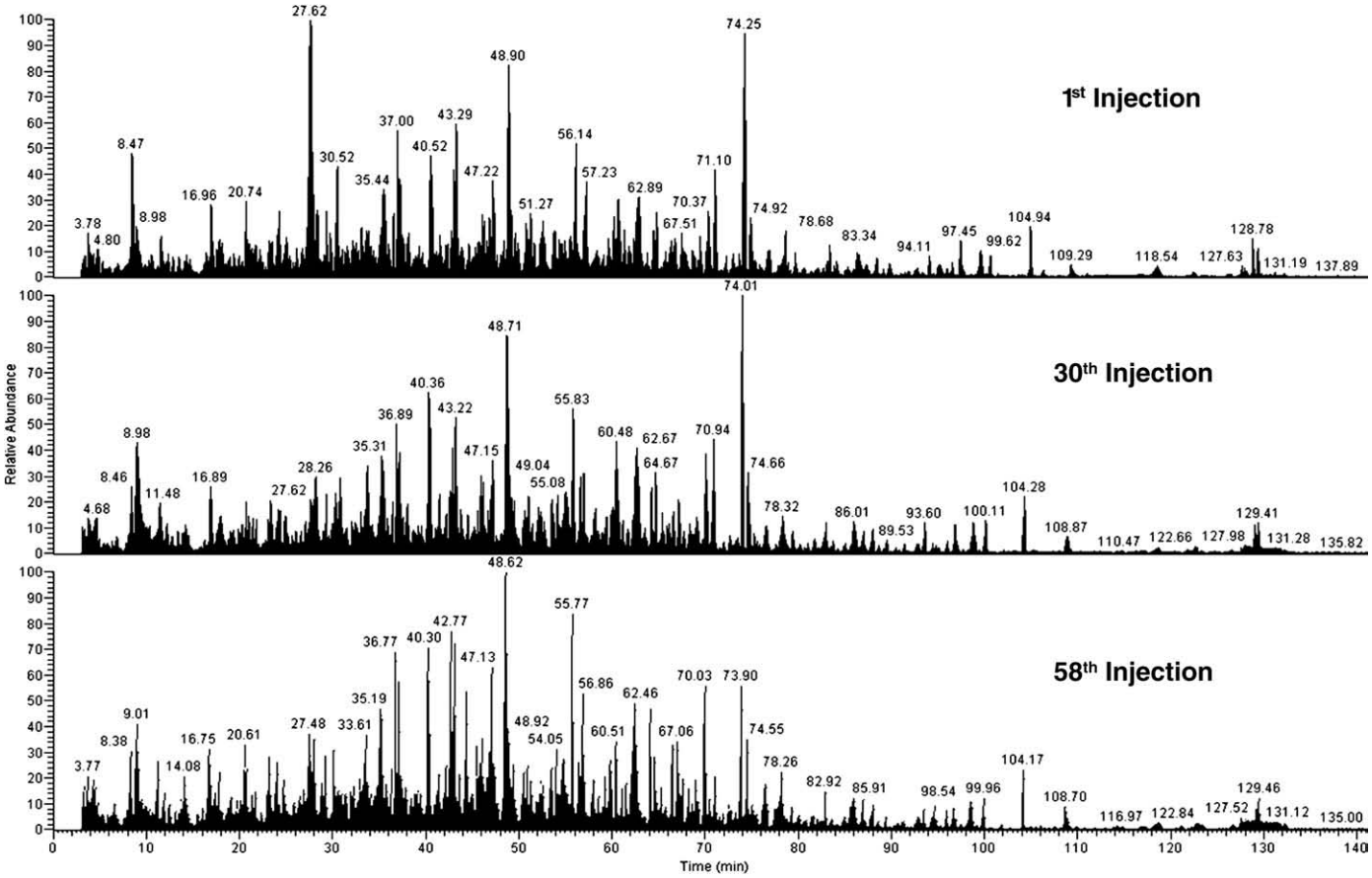

Fig. 1. Total ion chromatograms of the 1st, 30th and 58th injections illustrating that the high quality of the chromatograms did not diminish from the 1st to the 58th injection.

When multiple peptides had the same protein identification, their quantile normalized log2 intensities were averaged to obtain log2 protein intensities. The log2 protein intensity was used for the Linear Mixed Model statistical analysis for each protein. For each protein, estimates of individual *p*-values and *q*-values (measure of False Discovery Rate, FDR) were determined. Fold changes were computed as the ratio of mean treated/mean control.

Chicken lysozyme was added into every sample at a constant amount before tryptic digestion to serve as an internal standard for quality assurance and quality control (QA/QC) and assess stability of the LC column and MS instrument. After tryptic digestion, 9 chicken lysozyme peptides were quantified. The quantile normalized log2 intensities of the peptides were averaged to obtain the log2 protein intensity for chicken lysozyme in each sample. The log2 protein intensities for chicken lysozyme for each sample ($n=9, 10$/group) underwent a group comparison. There were no significant differences among the 6 groups in the intensity of the internal standard, suggesting that there was good procedural stability across samples and that no technical artifacts were contributing to differences between groups.

2.3. Protein identification

Proteins identified by SEQUEST and X!Tandem were categorized into priority groups based upon the quality of the protein identification. Proteins with a best peptide confidence level of 99% or higher were assigned to priority 1 (two or more unique peptide sequences) or 2 (a single unique peptide sequence). Proteins with a best peptide confidence level of 75–89% were assigned to the 'moderate' category. Peptides with a confidence level less than 75% were filtered out from further analysis. SEQUEST and X!Tandem database search algorithms were used for peptide sequence identification. Each algorithm compares the observed peptide MS/MS spectrum and theoretically derived spectra from the database to assign quality scores. These quality scores and other important predictors were combined in the algorithm that assigns an overall % ID confidence level for each peptide; the assignment was based on a random forest recursive partition supervised learning algorithm (Higgs et al., 2005, 2007). The priority system was based upon the quality of the amino acid sequence identification and whether one or more sequences were identified.

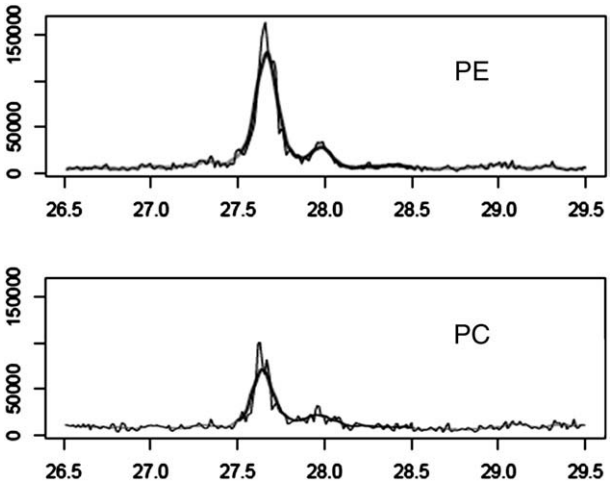

Fig. 2. Extracted ion chromatogram for peptide EIYTHFTCATDTK from protein IPI002311733.6 (guanine nucleotide-binding protein Gi, alpha-1 subunit) showing the retention time in min along the *X*-axis and the intensity along the *Y*-axis. The heavy solid line represents the AUC for this peptide.

Table 1
Normalized average area under the curve (AUC) values for each identified peptide for a single protein for the six experimental groups

Protein name	Peptides identified	ID confidence (%)	Normalized average AUC
Guanine	LLLLGAGESGK	>99	32137(NC); 44318(NE); 31662(PC); 32245(PE); 31665(WC); 30949(WE)
Nucleotide	EIYTHFTCATDTK	>99	16661(NC); 26018(NE); 15158(PC); 27247(PE); 20057(WC); 33736(WE)
Binding protein	MFDVGGQR	>99	11895(NC); 13008(NE); 10822(PC); 14345(PE); 10979(WC); 13449(WE)
Gi, alpha-1	IAQPNYIPTQQDVLR	97.67	22307(NC); 26098(NE); 23441(PC); 27436(PE); 22270(WC); 23027(WE)
Subunit (Gnai1)	DSGVQACFNR	>99	75407(NC); 78223(NE); 7152(PC); 7658(PE); 7035(WC); 7870(WE)

NC=saline treated NP rat; NE=ethanol treated NP rat; PC=saline treated P rat; PE=ethanol treated P rat; WC= saline treated Wistar rat; WE=ethanol treated Wistar rat.

Only proteins with priority 1 (confidence level>99% with 2 independent peptides) were included for further analysis in the present study.

2.4. Bioinformatics analyses

Protein ID numbers were used to obtain the corresponding Entrez-Gene identifier. Testing for over-representation of Gene Ontology (GO) (Harris et al., 2004; Ashburner et al., 2000) biologic process (BP) and molecular function (MF) categories was performed using the Bioconductor package GOstats (Gentleman 2004; Gentleman et al., 2004). Identification of over-represented GO categories was then accomplished within GOstats using the hypergeometric distribution. Categories, with 5 or more proteins, are listed. Categories were called significant for $p<0.05$. *Ingenuity*® Pathways Analyses (Ingenuity, Inc. www.ingenuity.com) were conducted on proteins that were statistically significant. Ingenuity builds networks based upon information extracted from the scientific literature that is deposited in Ingenuity proprietary database. Network Eligible Molecules are combined into networks that maximize their interconnectedness with each other relative to all molecules they are connected to in the database.

3. Results

Fig. 1 shows the total ion chromatogram (TIC) from the 1st, 30th and 58th injection to demonstrate that the overall quality of the chromatograms was maintained from the 1st to the 58th injection. Fig. 2 shows the extracted ion chromatogram (XIC) for a single peptide from guanine nucleotide-binding protein G(i), alpha-1 subunit (Gnai1) to illustrate how the area under the curve (AUC) was determined for two different samples. Table 1 shows the normalized average AUC values for the 5 peptides used to quantify levels of Gnai1 in the 6 experimental groups.

There were 875 proteins in category 1 (peptide ID confidence>99%; with 2 independent peptides) that were identified in the ACB-shell samples of P, NP and W rats (see Supplemental Table A for complete list of category 1 proteins; see Supplemental Table B for list of all peptides that were used to identify each priority 1 protein). There were no significant differences in protein expression levels in the ACB-shell between the saline treated (control) P and NP rats. Comparison of the saline treated P and W rats yielded significant differences ($q<0.25$; $p<0.015$; fold change≥1.15) in 2 proteins: fumarate hydratase mitochondrial precursor and fumarate hydratase-1. Both proteins had higher values in the ACB-shell of P than W rats. These same 2 proteins (and no others) also had higher expression levels in the ACB-shell of NP than W rats.

3.1. Effects of ethanol on protein levels in P rats

Repeated administration of ethanol significantly ($p<0.015$; q-values< 0.25; fold change≥1.15) altered the levels of 22 proteins in the ACB-shell of P rats (Table 2). Most of the changes were in the range of 1.15- to 1.25-fold, with approximately equal numbers of proteins with higher and lower levels in the ethanol-treated group. Neuronal guanine nucleotide exchange factor (Ngef) had the highest fold change (1.65×higher in the ethanol treated group). GO analysis did not yield any significant biological processes (BP) or molecular function (MF) categories, containing at least 5 proteins.

Table 2
Priority 1 (>99.9% confidence level) proteins that were significantly (p-values<0.015; q-values<0.25) different and changed by 15% or greater in the nucleus accumbens shell between the ethanol-injected (E) and control (C) alcohol-preferring (P) rats

Protein_ID	Gene symbol	Annotation	PE/PC
IPI00207891.2		31_kDa_protein	−1.17[a]
IPI00562632.1		33_kDa_protein	1.24[b]
15986733	Rab4b	AF408432_1_GTP-binding_protein_RAB4_[*Mus_musculus*]	−1.20[b]
IPI00231118.4	Calb1	Calbindin	−1.17[b]
IPI00189995.1	Calb2	Calretinin	−1.24
IPI00213015.1	Dctn2	Dynactin_2	1.18
IPI00231733.6	Gnai1	Guanine_nucleotide-binding_protein_G(i),_alpha-1_subunit	1.24[a,b]
57829	Nefh	heavy_neurofilament_polypeptide_(854_AA)_[Rattus_sp.]	−1.17
IPI00372709.3	Igsf8	immunoglobulin_superfamily,_member_8	−1.18
IPI00417225.1	Synpo	Isoform_1_of_Synaptopodin	1.16[b]
IPI00212566.3	Syngap1	Isoform_3_of_Ras_GTPase-activating_protein_SynGAP	−1.19
66857		KIRTPR_pyruvate_kinase_(EC_2.7.1.40),_erythrocyte_splice_form_R_-_rat	1.23
IPI00471530.1	Lap3	Leucine_aminopeptidase_3	1.20
IPI00231997.5	Ndufa5	NADH_dehydrogenase_[ubiquinone]_1_alpha_subcomplex_subunit_5	1.17
IPI00231641.4	Pgm1	Phosphoglucomutase-1	1.26[a,b]
IPI00369349.3	Atp6v1e2	PREDICTED:_similar_to_ATPase,_H+_transporting,_V1_subunit_E-like_2_isoform_2	−1.15[a]
IPI00371946.3	Marcks	PREDICTED:_similar_to_Myristoylated_alanine-rich_C-kinase_substrate	−1.37
IPI00464820.4	Ngef	PREDICTED:_similar_to_neuronal_guanine_nucleotide_exchange_factor	1.65[b]
IPI00369480.3	Otub1	PREDICTED:_similar_to_OTU_domain,_ubiquitin_aldehyde_binding_1	1.24
IPI00394488.2	LOC498174	Similar_to_NipSnap2_protein	1.26[b]
IPI00201969.1	Vat1	Vesicle_amine_transport_protein_1_homolog	−1.15
IPI00215349.3	Wdr1	WD_repeat_protein_1	1.18[a,b]

PE/PC = fold change ethanol over control value; minus sign indicates that PC value was greater than PE value; $n=10$ for PE, and $n=9$ for PC. There were no values for PE/PC that changed in the opposite direction in NP or W rats.

[a] Similar ratio change found for NP rats.

[b] Similar ratio change observed for W rats.

Table 3
Priority (>99.9% confidence level) proteins that were significantly (p-values<0.015; q-values<0.06) different and changed by 15% or greater in the nucleus accumbens shell between the ethanol-injected (E) and control (C) alcohol-non-preferring (NP) rats

Protein_ID	Gene symbol	Annotation	NPE/NPC
IPI00231340.8		11_kDa_protein	1.28
225775	rbcL	1313192A_calmodulin_dependent_protein_kinase_II	1.34
IPI00362291.1		16_kDa_protein	−1.23[a]
IPI00189519.1		19_kDa_protein	1.28[b]
IPI00188732.2		21_kDa_protein	1.25
1580888		2116232A_2-oxoglutarate_carrier_protein	1.16
IPI00207891.2		31_kDa_protein	1.26[c]
IPI00782342.1		44_kDa_protein	−1.16
IPI00361239.4		49_kDa_protein	1.33
IPI00200145.1	Rplp1	60S_acidic_ribosomal_protein_P1	−1.15
IPI00231954.5	Pfkp	6-phosphofructokinase_type_C	1.13
109966		A35244_hexokinase_(EC_2.7.1.1),_tumor_-_mouse	1.18
IPI00382300.3	Rab1	Ac2-048	1.21
IPI00362072.2	Actr2	Actin-like_protein_2	1.15
IPI00421909.3	Spg3a	Atlastin-like_protein	1.16
IPI00196107.1	Atp5f1	ATP_synthase_B_chain,_mitochondrial_precursor	1.20
IPI00198620.1	Atp5d	ATP_synthase_delta_chain,_mitochondrial_precursor	−1.21
IPI00214665.2		ATP-citrate_synthase	1.34
IPI00193173.4	Pcp4	Brain-specific_polypeptide_PEP-19	−1.35
IPI00192337.1	Camk2a	Calcium/calmodulin-dependent_protein_kinase_type_II_alpha_chain	1.33
IPI00231955.5	Calm1	Calmodulin	−1.28
IPI00209824.2	Camk2b	Calmodulin-dependent_protein_kinase_II_beta_M_isoform	1.21
IPI00215463.1	Calml3	Calmodulin-like_3	−1.37
38014843	Cd81	Cd81_protein_[*Rattus_norvegicus*]	1.21
IPI00199076.2	Pde2a	cGMP-dependent_3′,5′-cyclic_phosphodiesterase	1.21
230824	Grhl3	Chain_,_Calmodulin	−1.31
29747871	Csl	Citrate_synthase_like_[*Mus_musculus*]	1.15
IPI00193983.2	Cltc	Clathrin_heavy_chain	1.22
47605479	Slc25a12	CMC1_MOUSE_Calcium-binding_mitochondrial_carrier_protein_Aralar1_(mitochondrial_aspartate_glutamate_carrier_1	1.23
IPI00210978.1	Cplx1	Complexin-1	−1.21
117104	Cox5b	COX5B_MOUSE_Cytochrome_*c*_oxidase_polypeptide_Vb,_mitochondrial_precursor	−1.18
IPI00188313.1	Ckmt2	Creatine_kinase,_sarcomeric_mitochondrial_precursor	1.18
IPI00192246.1	Cox5a	Cytochrome_*c*_oxidase_polypeptide_Va,_mitochondrial_precursor	−1.18
IPI00193918.1	Cox5b	Cytochrome_*c*_oxidase_polypeptide_Vb,_mitochondrial_precursor	−1.17
IPI00734686.2	COII	Cytochrome_*c*_oxidase_subunit_II	1.31
IPI00421955.1	Ppp1r1b	Dopamine-and_cAMP-regulated_phosphoprotein_DARPP-32	−1.27
IPI00231407.4	Dbn1	Drebrin_1	−1.15
IPI00231247.8	Map2k1	Dual_specificity_mitogen-activated_protein_kinase_kinase_1	1.17
IPI00327630.1	Dync1h1	Dynein_heavy_chain,_cytosolic	1.21
IPI00195372.1	Eef1a1	Elongation_factor_1-alpha_1	1.15
119348	Eno2	ENOG_MOUSE_Gamma-enolase_(2-phospho-D-glycerate_hydro-lyase)_(Neural_enolase)_(Neuron-specific_enolase)_(NSE)_(Enolase_2)	−1.16
IPI00422053.1	Gna13	Galpha13	1.23
IPI00326412.3	Eno2	Gamma-enolase	−1.15
IPI00364311.1	Gpi	Glucose_phosphate_isomerase	1.16
IPI00191733.1	Gad2	Glutamate_decarboxylase_2	1.15
IPI00199465.1	Gls	Glutaminase_kidney_isoform,_mitochondrial_precursor	1.18
IPI00324020.5	Glul	Glutamine_synthetase_1	1.19
IPI00231150.4	GST	Glutathione_S-transferase_alpha_(Fragment)	1.28
IPI00287309.1	Gpm6a	Glycoprotein_m6a	1.17
IPI00213685.1	Rab3d	GTP-binding_protein_Rab-3D	1.20
IPI00230868.4	Gnaq	Guanine_nucleotide_binding_protein,_alpha_q_polypeptide	1.15
IPI00230866.6	Gna12	Guanine_nucleotide-binding_protein_alpha-12_subunit	1.23
IPI00231733.6	Gnai1	Guanine_nucleotide-binding_protein_G(i),_alpha-1_subunit	1.22[a,c]
IPI00231925.7	Gnai2	Guanine_nucleotide-binding_protein_G(i),_alpha-2_subunit	1.24
IPI00202543.1	Hk1	Hexokinase-1	1.18
IPI00231650.6	Hist1h1c	Histone_H1.2	1.19[a]
IPI00188688.1	H2a	Histone_H2a	1.36
IPI00231880.6	H2afz	Histone_H2A.Z	1.38
IPI00368293.4	Histh2a1	Histone_H2A_type_1	1.30
IPI00231475.6	H3f3b	Histone_H3.3	1.36
IPI00199273.1	St13	Hsc70-interacting_protein	−1.15
IPI00205372.3	Stxbp1	Isoform_1_of_Syntaxin-binding_protein_1	1.20
IPI00569103.3	Mbp	Isoform_3_of_Myelin_basic_protein_S	1.42
IPI00196730.1	Camk2g	Isoform_A_of_Calcium/calmodulin-dependent_protein_kinase_type_II_gamma_chain	1.34
IPI00212226.1	Camk2d	Isoform_Delta_1_of_Calcium/calmodulin-dependent_ protein_kinase_type_II_delta_chain	1.35
IPI00230956.1	Slc1a3	Isoform_GLAST-1A_of_Excitatory_amino_acid_transporter_1	1.24
IPI00213663.1	Slc1a2	Isoform_Glt1_of_Excitatory_amino_acid_transporter_2	1.21
IPI00188119.1	Atp12a	Isoform_Long_of_Potassium-transporting_ATPase_ alpha_chain_2	1.27
IPI00205493.1	Snca	Isoform_Syn1_of_Alpha-synuclein	−1.18

Table 3 (*continued*)

Protein_ID	Gene symbol	Annotation	NPE/NPC
IPI00197711.1	Ldha	L-lactate_dehydrogenase_A_chain	1.15
IPI00231261.6	Slc25a11	Mitochondrial_2-oxoglutarate/malate_carrier_protein	1.17
IPI00231819.5	Mbp1	Myelin_basic_protein_isoform_1	1.39
IPI00231265.2	Mbp4	Myelin_basic_protein_isoform_4	1.41
IPI00421549.3	Ndufa10	NADH_dehydrogenase_(Ubiquinone)_1_alpha_subcomplex_10	1.15
IPI00390362.1	Ndufv3	NADH_dehydrogenase_[ubiquinone]_flavoprotein_3,_ mitochondrial_precursor	−1.19
IPI00205396.1	Ncdn	NORBIN	1.15
IPI00214262.1	Nsfl1c	NSFL1_cofactor_p47	−1.26
IPI00230937.4	Pebp1	Phosphatidylethanolamine-binding_protein_1	−1.20
9790051	Pfkp	Phosphofructokinase,_platelet_[*Mus_musculus*]	1.15
IPI00231641.4	Pgm1	Phosphoglucomutase-1	1.21[a,c]
20141789	Prdx5	PRDX5_MOUSE_Peroxiredoxin-5,_mitochondrial_precursor_ (Prx-V)_(Peroxisomal_antioxidant_enzyme)_(PLP)_ (Thioredoxin_reductase)	−1.16
IPI00212258.1	Phpt	PREDICTED:_similar_to_14_kDa_phosphohistidine_Phosphatase	−1.21
IPI00369349.3	Atp6v1e2	PREDICTED:_similar_to_ATPase,_H+_transporting,_V1_subunit_ E-like_2_isoform_2	−1.15[c]
IPI00208551.3	6720456B0 7Rik	PREDICTED:_similar_to_chromosome_3_open_reading_frame_10	−1.15
IPI00200624.1	H2afj	PREDICTED:_similar_to_H2A_histone_family,_member_J	1.36
IPI00767428.1	H3f2	PREDICTED:_similar_to_H3_histone,_family_2_isoform_2	1.38
IPI00566481.2	Hist1h2ac	PREDICTED:_similar_to_histone_2,_H2ac	1.30
IPI00209163.1	Hist1h2be	PREDICTED:_similar_to_histone_2,_H2be	1.17
IPI00764762.1	Hist1h2a	PREDICTED:_similar_to_histone_2a	1.43
IPI00369397.5	H2afx	PREDICTED:_similar_to_Histone_H2A.x	1.31
IPI00365775.3	Me3	PREDICTED:_similar_to_malic_enzyme_3,_NADP(+)-dependent,_ mitochondrial	1.26
IPI00358441.1	Ndufa9	PREDICTED:_similar_to_NADH_dehydrogenase_(ubiquinone)_ 1_alpha_subcomplex,_9	1.20
IPI00569808.2	Nav3	PREDICTED:_similar_to_neuron_navigator_3	−1.30
IPI00191897.4	Nme2	PREDICTED:_similar_to_Nucleoside_diphosphate_kinase_B	−1.15
IPI00365705.6	Atp1a1	PREDICTED:_similar_to_Potassium-transporting_ATPase_alpha_ chain_1	1.45
IPI00207725.3	Pnp	PREDICTED:_similar_to_purine-nucleoside_phosphorylase	1.15
IPI00388687.3	Slc25a12	PREDICTED:_similar_to_solute_carrier_family_25_(mitochondrial_ carrier,_Aralar),_member_12	1.27
IPI00189416.2	Tpt1	PREDICTED:_similar_to_tumor_protein,_translationally-controlled_1	−1.17
IPI00199600.1		PREDICTED:_similar_to_vacuolar_H+_ATPase_G1	−1.24
50489	CD207	Pro-alpha-2(I)_collagen_[*Mus_musculus*]	−1.25
IPI00190557.2	Phb2	Prohibitin-2	1.20
IPI00201797.1	Prkcg	Protein_kinase_C_gamma_type	1.17
IPI00194324.1	Pdhb	Pyruvate_dehydrogenase_E1_component_subunit_beta,_ mitochondrial_precursor	−1.18
IPI00555185.1	Rab10	RAB10,_member_RAS_oncogene_family	1.21
6093900	Rab3b	RAB3B_RAT_Ras-related_protein_Rab-3B	1.19
IPI00325762.1	Rab3a	Ras-related_protein_Rab-3A	1.21
IPI00209150.1	Rab3c	Ras-related_protein_Rab-3C	1.16
IPI00187747.1	Rap1a	Ras-related_protein_Rap-1A_precursor	1.25
631898	Unc18	S39345_unc-18_protein_homolog,_67K_-_rat	1.20
IPI00326305.3	Atp1a1	Sodium/potassium-transporting_ATPase_alpha-1_chain_precursor	1.21
IPI00205693.1	Atp1a2	Sodium/potassium-transporting_ATPase_alpha-2_chain_precursor	1.23
IPI00231451.4	Atp1a3	Sodium/potassium-transporting_ATPase_alpha-3_chain	1.23
IPI00231643.4	Sod1	Superoxide_dismutase	−1.17
IPI00208115.4	Sv2a	Synaptic_vesicle_glycoprotein_2A	1.28
IPI00324381.5	Stx1a	Syntaxin-1A	1.23
IPI00188956.1	Thy1	Thy-1_membrane_glycoprotein_precursor	1.18
IPI00230925.4	Tmsb4x	Thymosin_beta-4	−1.21
IPI00362927.1	Tuba4a	Tubulin,_alpha_4	1.24
IPI00189795.1	Tuba1a	Tubulin_alpha-1_chain	1.23
IPI00339167.4	Tuba1b	Tubulin_alpha-2_chain	1.24
IPI00369093.1	Uqcrh	Ubiquinol-cytochrome_c_reductase_complex_11_kDa_protein,_ mitochondrial_precursor	−1.23
12847763	Hist1h4i	Unnamed_protein_product_[*Mus_musculus*]	1.31
26337455	Hdgfrp3	Unnamed_protein_product_[*Mus_musculus*]	−1.35
IPI00364780.2	Atp6v1h	Vacuolar_ATPase_subunit_H	1.16
IPI00198327.2	Vdac2	Voltage-dependent_anion-selective_channel_protein_2	1.28
IPI00213598.1	Wasf1	WAS_protein_family,_member_1	1.19
IPI00215349.3	Wdr1	WD_repeat_protein_1	1.19[a,c]
33468975	Zic5	Zinc_finger_protein_of_the_cerebellum_5_[*Mus_musculus*]	−1.27

NPE/NPC = fold change ethanol over control value; minus sign indicates that NPC value was greater than NPE value; $n = 10$ for NPE and $n = 10$ for NPC.

[a] Similar ratio change observed for W rats.

[b] Opposite ratio change found for W rats.

[c] Similar ratio change found for P rats.

Among the 22 proteins that were significantly different in the ACB-shell between the ethanol-treated and saline-treated P rats (Table 2), there were 5 proteins that were similarly changed in the ACB-shell of ethanol- and saline-treated NP rats (Table 3), and no proteins that changed in the opposite direction. There were 9 proteins that were similarly changed by ethanol treatment in the ACB-shell of P and W

Table 4
Significant GO categories containing 5 or more proteins that were different in the nucleus accumbens shell between the ethanol and control groups of NP rats

Function/process	Up-regulated	Down-regulated
GTP-binding	Hexokinase-1; alpha-tubulin; Ras-related protein Rab-3B; guanine nucleotide binding protein alpha q polypeptide; Ac2-048 (Rab1); Atlastin-like protein; Galpha 13	None
ATP-binding	Hexokinase-1; sodium–potassium transporting ATPase alpha 2 chain precursor; sodium–potassium transporting ATPase alpha 1 chain precursor; Vacuolar ATPase subunit H; Ac2-048 (Rab1); NADH dehydrogenase 1 alpha subcomplex 10	None
DNA-binding	Unnamed protein product; Histone H2a; Histone H1.2; Histone H2A.Z; Histone H2A type 1; Histone H3.3	Chain calmodulin; Zinc finger protein of the cerebellum 5
Protein binding	Dynein heavy chain; Unnamed protein product; Thy-1 membrane glycoprotein precursor; Prohibitin-2; Hexokinase-1; Syntaxin-binding protein-1; Sodium-potassium transporting ATPase alpha 2 chain precursor; Citrate synthase; Synaptic vesicle glycoprotein 2A; CD81 antigen; GTP-binding protein Rab-3D; Dual specificity mitogen-activated protein kinase kinase 1; Ras-related protein Rab-3A	Chain calmodulin; Prohibitin; Calmodulin; Nucleoside di- phosphate kinase B

rats (Tables 1 and 4), of which 3 were also in common with NP rats (i.e., guanine nucleotide-binding protein G(i) alpha-1 subunit, phosphoglucomutase-1 and WD repeat protein 1). Therefore, it appears that ethanol altered the levels of 11 of 22 proteins uniquely in the ACB-shell of P rats.

3.2. Effects of ethanol on protein levels in NP rats

Repeated administration of ethanol significantly (p-values <0.015; q-values <0.06; fold change ≥ 1.15) altered the levels of 128 proteins in the ACB-shell of NP rats (Table 3). Most of the changes were in the range of 1.15- to 1.35-fold, with 2.5 more proteins having higher than lower levels in the ethanol group. Two general classes of proteins (i.e., Histones and myelin basic proteins) had differences of approximately 1.3-fold between the control and ethanol groups, with all proteins in both classes having higher expression in the ethanol treated group (Table 3). In addition, there were several calcium–calmodulin proteins, guanine nucleotide binding proteins and Ras-related proteins that also had higher expression levels in the ACB-shell of the ethanol than saline group of NP rats.

Only 7 of the 128 proteins that were altered by ethanol treatment in the ACB-shell of the NP rats were similarly changed in the ACB-shell of P and W rats (Tables 2, 3 and 5); there was 1 protein (19 kDa protein) that was changed in the opposite direction in NP versus W rats. There were no proteins that were significantly changed in the opposite direction by ethanol in the ACB-shell of NP versus P rats. Therefore, ethanol appeared to produce unique changes in protein expression in the ACB-shell of NP rats that were not observed in P or W rats.

GO analysis yielded 4 significant MF categories, containing at least 5 proteins (Table 4). There were no significant BP categories containing at least 5 proteins. In all 4 MF categories, there were more proteins with higher expression levels in the ACB-shell of the ethanol than saline group, i.e., GTP binding (all 7 proteins were higher in the ethanol group), ATP-binding (all 6 proteins were higher in the ethanol group), DNA-binding (6 of 8 proteins were higher in the ethanol group), and protein binding (13 of 17 proteins were higher in the ethanol group).

In the 'GTP binding' category (Table 4), the 7 proteins with higher expression levels in the ACB-shell of ethanol included, hexokinase-1,

Table 5
Priority 1 (>99.9% confidence level) proteins that were significantly (p-values <0.015; q-values <0.2) different and changed by 15% or greater in the nucleus accumbens shell between the ethanol-injected (E) and control (C) Wistar (W) rats

Protein_ID	Gene symbol	Annotation	WE/WC
IPI00782125.1		13_kDa_protein	−1.28
IPI00362291.1		16_kDa_protein	−1.15[a]
IPI00189519.1		19_kDa_protein	−1.17[b]
IPI00568873.2		20_kDa_protein	−1.41
IPI00562632.1		33_kDa_protein	1.22[c]
IPI00366110.3		47_kDa_protein	−1.16
IPI00777829.1		54_kDa_protein	1.28
15986733	Rab4b	AF408432_1_GTP-binding_protein_RAB4_[*Mus_musculus*]	−1.19[c]
IPI00215523.1	Bcat1	Branched-chain-amino-acid_aminotransferase,_cytosolic	−1.17
IPI00231118.4	Calb1	Calbindin	−1.15[c]
IPI00566635.2		Discs_large_homolog_4	1.20
IPI00231733.6	Gnai1	Guanine_nucleotide-binding_protein_G(i),_alpha-1_subunit	1.19[a,c]
IPI00231650.6	Hist1h1c	Histone_H1.2	1.15[a]
IPI00417225.1	Synpo	Isoform_1_of_Synaptopodin	1.18[c]
IPI00202549.1	Pklr	Isoform_R-type_of_Pyruvate_kinase_isozymes_R/L	1.21
66857		KIRTPR_pyruvate_kinase_(EC_2.7.1.40),_erythrocyte_splice_form_R_-_rat	1.27
26006161		mKIAA0417_protein_[*Mus_musculus*]	1.15
IPI00191790.1	Efcbp2	Neuronal_calcium_binding_protein_NECAB2	−1.15
IPI00231641.4	Pgm1	Phosphoglucomutase-1	1.30[a,c]
IPI00199203.1	Gapdh	PREDICTED:_similar_to_glyceraldehyde-3-phosphate_dehydrogenase	1.24
IPI00358537.2	Hspa12a	PREDICTED:_similar_to_heat_shock_protein_12A	1.15
IPI00464820.4	Ngef	PREDICTED:_similar_to_neuronal_guanine_nucleotide_exchange_factor	1.53[c]
IPI00763565.1		PREDICTED:_similar_to_polyubiquitin	−1.41
IPI00190240.1	Rps27a	Ribosomal_protein_S27a	−1.38
IPI00471526.3	LOC298795	Similar_to_14-3-3_protein_sigma	−1.23
IPI00394488.2	LOC498174	Similar_to_NipSnap2_protein	1.30[c]
54038641	Tagln2	Transgelin_2_[*Rattus_norvegicus*]	−1.15
IPI00215349.3	Wdr1	WD_repeat_protein_1	1.22[a,c]

WE/WC = fold change ethanol over control value; minus sign indicates that WC value was greater than WE value; $n=10$ for WC, and $n=9$ for WE.
[a] Similar ratio change observed for NP rats.
[b] Opposite ratio change found for NP rats.
[c] Similar ratio change found for P rats.

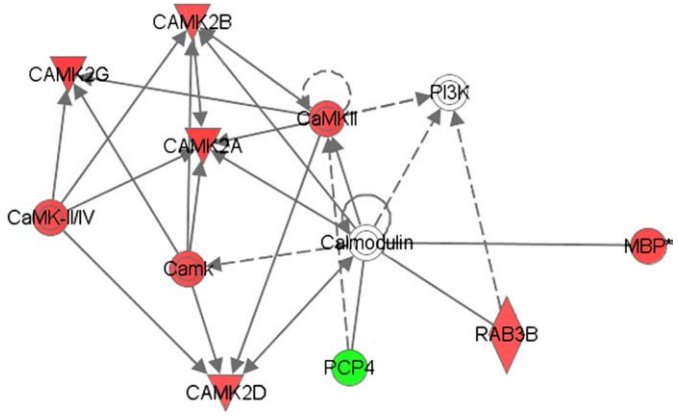

Fig. 3. Abridged *Ingenuity*® Pathways Analysis of effects of ethanol in the nucleus accumbens shell of NP rats showing up-regulation of calcium/calmodulin signaling pathways. Red indicates up-regulation, green indicates down-regulation, and clear symbol indicates proteins that were not identified as differentially expressed, but were linked to multiple proteins that had changed significantly. Solid lines indicate direct interactions and dashed lines indicate indirect interactions. Abbreviations: CAMK2A — calcium/calmodulin-dependent protein kinase II alpha; CAMK2B — calcium/calmodulin-dependent protein kinase II beta; CAMK2D — calcium/calmodulin-dependent protein kinase II delta; CAMK2G — calcium/calmodulin-dependent protein kinase II gamma; MBP — myelin basic protein; PCP4 — purkinje cell protein 4 or brain-specific polypeptide PEP-19; PI3K — phosphatidylinositol 3-kinase; RAB3B — ras related GTP-binding protein Rab-3B. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

alpha-tubulin, Ras-related protein Rab-3B, Ac2-048 (Rab1), Atlastin-like protein, Galpha 13, and guanine nucleotide binding protein alpha q polypeptide. None of these proteins were similarly changed by ethanol in the ACB-shell of P or W rats. In addition, none of the 6 proteins in the 'ATP binding' category (Table 4) were similarly changed by ethanol treatment in the P or W rats. In the 'DNA-binding' category, all 5 histones had higher levels in the ACB-shell of the ethanol-treated NP rats, with 4 of the 5 showing fold changes between 1.30 and 1.38 (Table 3). Histone H1.2 had the smallest increase (1.19-fold) and was the only histone of the 5 that was similarly changed in the W rat.

3.3. Effects of ethanol on protein levels in W rats

Repeated administration of ethanol significantly ($p<0.015$; q-values$<$ 0.2; fold change$\geq$1.15) altered the levels of 28 proteins in the ACB-shell of W rats (Table 5). Most of the changes were in the range of 1.15- to 1.25- fold, with approximately equal numbers of proteins having increased and decreased levels in the ethanol versus saline group. Similar to the findings with P rats, neuronal guanine nucleotide exchange factor (Ngef) had the highest fold difference with 1.5-fold higher levels in the ethanol group. GO analysis did not yield any significant BP or MF categories, containing at least 5 proteins.

Among the 28 proteins that were altered by ethanol in the ACB-shell of W rats (Table 5), there were 11 that were similarly changed in P or NP rats. Only one protein changed in the opposite direction with ethanol treatment in the W rats versus either P or NP rats, i.e., 19 kDa protein had lower expression levels in the ethanol-treated W rats and higher levels in the ethanol-treated NP line. Therefore, expression levels of 17 proteins were uniquely changed by ethanol in the ACB-shell of W rats (Table 5).

3.4. Common changes among P, NP and W rats

There were only 3 proteins that had expression levels similarly changed by ethanol treatment in the ACB-shell of P, NP and W rats. These proteins are guanine nucleotide-binding protein G(i) alpha-1 subunit, phosphoglucomutase-1, and WD repeat protein 1.

4. Discussion

The major findings of this study were that repeated i.p. injections of 1 g/kg ethanol differentially altered protein expression levels in the ACB-shell of P, NP and W rats (Tables 2–5), with ethanol uniquely altering expression levels of 11 of 22 proteins in the P rat, 121 of 128 proteins in the NP rat, and 17 of 28 proteins in the W rat. These differential alterations in protein expression levels among the 3 rat strains suggest a significant strain×ethanol interaction.

For NP rats, more proteins were increased than decreased by ethanol in the 'ATP-binding', 'GTP-binding', and 'DNA-binding' categories (Table 4). Similar changes were not observed for P and W rats. The ATP- and GTP-binding categories contain proteins that are involved in intracellular signaling pathways and membrane excitability, suggesting an enhancement of these processes in the NP rat following ethanol treatment. These changes are compatible with increased synaptic function. The higher expression levels of histones in the 'DNA-binding' category are consistent with enhanced protein expression levels in the other GO categories for the ACB-shell of NP rats. Overall, these changes in protein expression that occurred in the ACB-shell in NP rats, as a result of ethanol injections (Tables 3 and 4), could reflect alterations associated with initial exposures to ethanol that could contribute to the low alcohol drinking characteristics of this line.

Ingenuity® analysis of the proteins altered by ethanol in the ACB-sh of NP rats indicated a calmodulin network (Fig. 3) involved in calcium signaling and long-term potentiation (LTP) and a G-protein network (Fig. 4) involved in axonal guidance signaling and long-term depression (LTD). The calmodulin–calcium intracellular signaling pathway (Fig. 3) and the G-protein intracellular signaling network (Fig. 4) are composed of proteins that have been primarily up-regulated in the

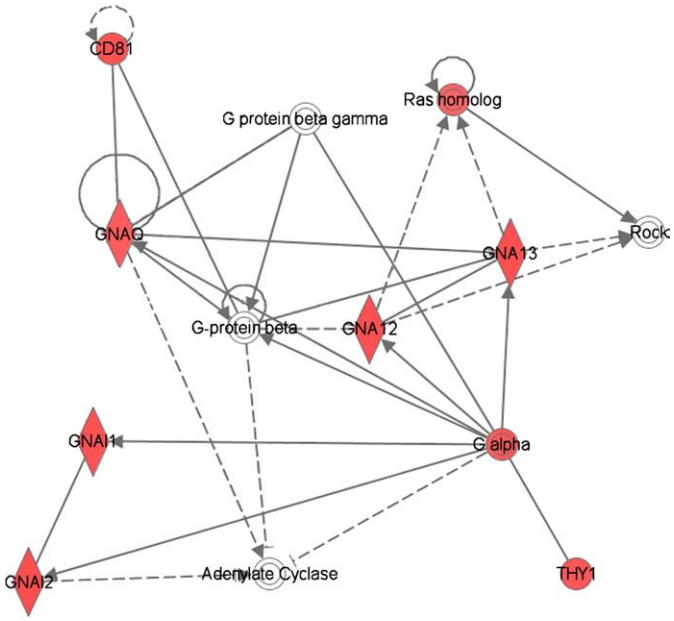

Fig. 4. Abridged *Ingenuity*® Pathways Analysis of effects of ethanol in the nucleus accumbens shell of NP rats showing up-regulation of G-protein signaling pathways. Red indicates up-regulation, green indicates down-regulation, and clear symbol indicates proteins that were not identified as differentially expressed, but were linked to multiple proteins that had changed significantly. Solid lines indicate direct interactions and dashed lines indicate indirect interactions. Abbreviations: CD81 — Cd 81 protein; GNA12 — guanine nucleotide binding protein alpha 12; GNA13 — guanine nucleotide binding protein alpha 13; GNAQ — guanine nucleotide binding protein alpha q polypeptide; GNAI1 — guanine nucleotide binding protein (Gi) alpha-1 subunit; GNAI2 — guanine nucleotide binding protein (Gi) alpha-2 subunit; ROCK — Roh-associated coiled-coil containing protein kinase; THY1 — thymus cell antigen 1 theta or Thy-1 membrane glycoprotein precursor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ACB-shell of the NP rat, suggesting ethanol-enhanced synaptic plasticity. The up-regulation of ARP2 actin-related protein (Actr2) and WAS protein family member 1 (Wasf1) are also consistent with an interpretation of ethanol-enhanced synaptic plasticity. Actr2 facilitates neuronal actin remodeling (Soderling et al., 2007), and Wasf1 is involved in linking actin dynamics and synaptic vesicle endocytosis (Shin et al., 2007). The higher protein expression levels of clathrin heavy chain (Cltc) also support ethanol-induced postsynaptic alterations.

Compared to the findings for NP rats, ethanol produced effects on fewer proteins in the ACB-shell of P rats (22 for P rats versus 128 for NP rats), with only 5 proteins in common between the two lines of rats (Tables 2 and 3). The altered expression of Synpo, Syngap1 and Ngef (Table 2) in the ethanol-treated group suggests that synaptic changes may have occurred in the ACB-shell of P rats. Ngef had the highest fold increase (1.65) in the ACB-shell of the P rats in the ethanol versus the saline group (Table 2). Ngef regulates ADP ribosylation factor 6, a small GTPase involved in forming a postsynaptic complex of PSD-95 and NMDA receptors at excitatory synapses (Sakagami et al., 2008; Inaba et al., 2004). The up-regulation of synaptopodin (Synpo), which is involved in maintaining activity-dependent enlargement of dendritic spines (Okubo-Suzuki et al., 2008), supports the idea that some form of ethanol-induced synaptic plasticity may have occurred within the ACB-shell of P rats. In contrast to the up-regulation of Ngef and Synpo, the protein expression level of the Ras GTPase-activating protein SynGap (Syngap1), which appears to have a role in LTP and is associated with NMDA receptors (Komiyama et al., 2002), was significantly reduced in the ACB-shell. However, in cultured neurons, overexpression of Syngap reduced AMPA receptor function and insertion of AMPA receptors into the plasma membrane (Rumbaugh et al., 2006). Therefore, in the present situation, it is possible that reduced levels of Syngap could results in enhanced AMPA receptor function. The findings that ethanol-induced synaptic plasticity may have occurred in the ACB-shell of both P and NP rats, but that different proteins are involved, suggest that different synaptic events have occurred, possibly involving alterations in different synaptic connections in the two rat lines.

The effects of the ethanol treatment on changes in protein levels in the ACB-shell of W rats (Table 5) indicated that few similar alterations were occurring in the W rats as were evident in NP rats (Tables 3 and 5). There were some changes in the levels of proteins that were observed for the W rats that suggested possible alterations in neuronal function; some of these changes were also observed for the P rat (Tables 2 and 5). For example, there were increased protein levels for NipSnap 2, involved in vesicular transport (Seroussi et al., 1998), and Synpo and Ngef, which are involved in postsynaptic function (Okubo-Suzuki et al., 2008; Sakagami et al., 2008). The overall results suggest that W rats may be undergoing ethanol-induced neuronal alterations in the ACB-shell, and that some of these alterations may be similar to changes observed for P, but different than changes observed for NP rats.

Differences in ethanol-induced changes in protein expression levels are not likely a result of differences in absorption or elimination of ethanol between the lines of rats. Previous studies indicated that similar time-course changes in blood ethanol levels following i.p. ethanol administration between P and NP rats (Lumeng et al., 1982; Strother et al., 2005).

The more widespread effects of repeated ethanol treatments on changes in protein expression levels in the NP than P rat, as exemplified by the greater number of proteins that were altered (Tables 2 and 3), are in agreement with the behavioral effects of repeated i.p. injections of 1 g/kg ethanol on performance on the oscillating bar task of P and NP rats, in which repeated injections produced significant changes in performance in the NP rats, but had little apparent effect on performance in the P rat (Bell et al., 2001).

Bell et al. (2006) reported protein expression changes in the ACB and amygdala of inbred P rats that had been chronically drinking alcohol. These investigators reported that ethanol drinking altered 14 proteins in the ACB and 27 proteins in the amygdala (Bell et al., 2006). There was no overlap between the proteins altered in the ACB by chronic alcohol drinking (Bell et al., 2006) and those altered by repeated ethanol injections in the present study (Table 2). This is likely due to differences in the alcohol-exposure protocols and analyzing the entire ACB versus the ACB-shell.

Comparison of the present findings with P rats injected with ethanol (Table 2) and proteomics studies with autopsied alcoholic brains (Matsuda-Matsumoto et al., 2007; Alexander-Kaufman et al., 2006, 2007; Lewohl et al., 2004) indicated few proteins or classes of proteins in common between the P rats and human studies. In the present study (Table 2) and the results of Matsuda-Matsumoto et al. (2007) for the hippocampus, only guanine nucleotide-binding (Gn) proteins were altered by ethanol treatment in both the present study and the post mortem study.

Comparison of the present proteomics findings (Table 2) with gene expression changes in the ACB of inbred P rats self-administering 15% ethanol (Rodd et al., 2008) indicated no common ethanol-induced differences. The lack of overlap between the operant ethanol self-administration study and the present study may likely be due, in part, to the differences in the alcohol exposure protocols.

A previous study (Kimpel et al., 2007) indicated that there were significant differences in the expression of several genes in the ACB between inbred P and NP rats. However, in the present study, there were no innate differences in protein levels in the ACB-sh between the selectively bred P and NP rats. The apparent discrepancy between the gene expression data and the current protein findings may be due to a combination of factors, including: (a) differences in mRNA may not necessarily translate into similar differences in local protein levels; (b) changes in mRNA reflect changes occurring primarily in the local glial and neurons, whereas protein levels in a given region reflect both local synthesis and protein transport; and (c) the entire ACB was analyzed in the gene expression study, whereas only a sub-region of the ACB was studied in the present study.

Over 875 proteins were identified (with a confidence level of greater than 99% with 2 independent peptides); there were another 1700 proteins detected and identified with a lower confidence level in the individual micropunch samples. With an improved database and better bioinformatics, this proteomics approach could yield significantly more information.

In summary, the present results indicate that the repeated systemic administration of a moderate dose of ethanol produces differential effects on protein expression in the ACB-shell between P, NP and W rats, suggesting significant strain×ethanol interactions. Ethanol produced effects on proteins involved in synaptic function within the ACB-shell of NP rats that were not observed for P and W rats; these alterations might be factors contributing to the low alcohol drinking characteristics of the NP line.

Acknowledgements

Supported in part by AA07611, AA016652 [INIA project], AA013522 [INIA project] and INGEN® (which is partially funded by Lilly Endowment Inc.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbb.2008.12.019.

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