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Differential gene expression in the nucleus accumbens with ethanol self-administration in inbred alcohol-preferring rats

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Abstract

The current study examined the effects of operant ethanol (EtOH) self-administration on gene expression kin the nucleus accumbens (ACB) and amygdala (AMYG) of inbred alcohol-preferring (iP) rats. Rats self-trained on a standard two-lever operant paradigm to administer either water–water, EtOH (15% v/v)–water, or saccharin (SAC; 0.0125% g/v)–water. Animals were killed 24 h after the last operant session, and the ACB and AMYG dissected; RNA was extracted and purified for microarray analysis. For the ACB, there were 513 significant differences at the $p<0.01$ level in named genes: 55 between SAC and water; 215 between EtOH and water, and 243 between EtOH and SAC. In the case of the AMYG ($p<0.01$), there were 48 between SAC and water, 23 between EtOH and water, and 63 between EtOH and SAC group. Gene Ontology (GO) analysis indicated that differences in the ACB between the EtOH and SAC groups could be grouped into 15 significant ($p<0.05$) categories, which included major categories such as synaptic transmission, cell and ion homeostasis, and neurogenesis, whereas differences between the EtOH and water groups had only 4 categories, which also included homeostasis and synaptic transmission. Several genes were in common between the EtOH and both the SAC and water groups in the synaptic transmission (e.g., *Cav2*, *Nrxn3*, *Gabrb2*, *Gad1*, *Homer1*) and homeostasis (*S100b*, *Prkca*, *Ftl1*) categories. Overall, the results suggest that changes in gene expression in the ACB of iP rats are associated with the reinforcing effects of EtOH.

Keywords: Microarrays; Gene expression; Ethanol self-administration; Alcohol-preferring rats; Nucleus accumbens; Amygdala

1. Introduction

Microarray analysis has emerged as a tool to study the multiple complex effects of pharmacological treatments on changes in gene expression. Examining innate differences and changes in gene expression in response to ethanol (EtOH) in lines or strains of mice and rats with divergent responses to ethanol could provide important clues toward identifying genes and gene networks involved in vulnerability to high alcohol drinking. Further, examining changes in gene expression resulting from chronic EtOH drinking could provide clues to identifying genes and gene networks involved in maintaining high alcohol drinking behavior. Thus far, changes in gene expression under operant EtOH self-administration conditions have not been conducted with rats that have been bred for high alcohol drinking behavior.

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Animal models have been used to study the influence of genetic factors on the effects of alcohol and on alcohol drinking behavior (reviewed by Bell et al., 2005; McBride and Li, 1998; Murphy et al., 2002). Selective breeding programs have developed lines of rats with divergent alcohol drinking behaviors. The results of these studies provide convincing data that genetics can markedly influence alcohol drinking behavior. Many studies have been conducted with these rat lines and, thus far, the overall results suggest that differences in the complex interactions of a number of neurotransmitter systems and multiple intracellular events in several CNS regions may contribute to a predisposition for high alcohol drinking behavior (reviewed by Bell et al., 2005; McBride and Li, 1998; Murphy et al., 2002).

Innate genetic expression differences between high and low alcohol consuming rodent lines have been indicated in several studies. Edenberg et al. (2005) examined differences in gene expression in the hippocampus (HIP) of inbred alcohol-preferring (iP) and inbred alcohol-non-preferring (iNP) rats, and reported differences in expression of genes involved in cell growth and adhesion, cellular stress reduction and anti-oxidation, protein trafficking, cellular signaling pathways, and synaptic function. Worst et al. (2005) reported on the transcriptome analysis in the frontal cortex of alcohol-naïve AA (Alko, alcohol) and ANA (Alko, non-alcohol) rats, and found differences between the AA and ANA rats in mRNA levels that could alter transmitter release (e.g., vesicle-associated membrane protein 2, syntaxin 1, syntaxin binding protein). In the whole brain analysis of inbred long-sleep and inbred short-sleep mice, expression of genes encoding for tyrosine protein kinase and ubiquitin carboxyl terminal hydrolase were higher in the brain of long-sleep mice (Xu et al., 2001). In a comprehensive transcriptome meta-analysis of different mice strains, Mulligan et al. (2006) identified several cis-regulated candidate genes for an alcohol preference QTL on chromosome 9.

Alterations in gene expression produced by exposure to alcohol have been reported in a few studies. Acute EtOH injections (6 g/kg; i.p.) produced changes in whole brain of C57BL/6J and DBA/2J mice (high and low alcohol drinkers, respectively) in expression of genes involved in regulating cell signaling, gene regulation, and homeostasis/stress response (Treadwell and Singh, 2004). Kerns et al. (2005) reported that acute i.p. ethanol injections altered, in the nucleus accumbens (ACB), prefrontal cortex and ventral tegmental area (VTA) of C57BL/6J and DBA/2J mice, expression of genes involved in glucocorticoid signaling, neurogenesis, myelination, neuropeptide signaling, and retinoic acid signaling. Differences were found in the dorsal HIP of Lewis rats given 12% EtOH or water for 15 months in expression of genes coding for oxido-reductases and ADP-ribosylation factors (Saito et al., 2002). In contrast, Saito et al. (2004) found no statistically significant effects of chronic free-choice alcohol drinking on gene expression in the striatum of C57BL/6By mice. The above studies were conducted using EtOH injections or 24-hour free-choice drinking. Moreover, other then the study of Kerns et al. (2005) using i.p. EtOH injections, none of the other studies reported data on limbic regions that are involved in mediating alcohol drinking. Therefore, it would be important to determine the effects of alcohol drinking on changes in gene expression in limbic regions that are involved in regulating alcohol drinking.

The nucleus accumbens (ACB) and amygdala (AMYG) are considered to be involved in mediating the reinforcing effects of EtOH and EtOH drinking (c.f., Koob et al., 1998; McBride and Li, 1998). Therefore, it would be important to determine changes in gene expression in these two limbic structures following EtOH self-administration. The objectives of the present study were to determine changes in gene expression associated with operant EtOH self-administration by inbred P rats. The use of operant procedures allowed determining the effects of the reinforcing effects of EtOH on gene expression under a controlled pattern of EtOH access and intake. Previous studies did not use operant techniques, nor did these studies use a controlled pattern of EtOH intake. Moreover, previous EtOH drinking studies did not examine changes in gene expression in the ACB and AMYG. In addition, a group self-administering saccharin (SAC) was used for comparison purposes to provide data on changes associated with learning the operant procedure, and motor activity related to lever responses. The present study was designed to test the hypothesis that EtOH self-administration would produce regional changes within the ACB and AMYG of iP rats in the expression of genes associated with intracellular signaling and synaptic transmission, and that these changes would be different from changes observed with SAC and water self-administration.

2. Methods

To reduce genetic variability, inbred adult (90–100 days old) male rats from the iP (5C) strains were used in these experiments. Inbreeding by brother–sister mating was initiated after the S30 generation of mass selection; the inbred strain was in the F37 generation for these experiments. Rats were maintained on a 12-hour reversed light–dark cycle (lights off at 0900 h). Food and water were available *ad libitum* throughout the experiment, except during operant testing. 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 Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

EtOH-naïve iP rats were self-trained on a standard two-lever operant paradigm using daily 1-hour sessions, as previously described for P rats (Rodd-Henricks et al., 2002a,b). Rats ($n=6$/group) were allowed to self-administer either water–water, EtOH (15% v/v)–water, or SAC (0.0125% g/v)–water. The fixed-ratio (FR) requirement was increased on the EtOH and SAC levers, and on one of the levers in the water–water group, until a concurrent FR5–FR1 schedule of reinforcement was reached. Operant sessions were conducted over a 10-week

period. A computer controlled the operant programs and recorded all data; the number of responses on both levers and the number of reinforcements obtained were recorded throughout all sessions. Sessions were 60 min in duration, occurring daily during the dark cycle. All operant sessions were conducted between 1100 and 1700. Previous research indicated that approximately 90–95% of the predicted fluid intake is consumed during the 60-min sessions (Rodd et al., 2003).

Animals were killed by decapitation approximately 24 h after the last operant session. In this study, the 24-hour time point was chosen to allow (a) comparison of the EtOH group with the other two groups without EtOH being present; and (b) detection of changes in gene expression associated with self-administration behavior separated from a pharmacological response to EtOH.

Rats were killed within the same 2-hour time frame over 2 days with equal number of animals from each group being killed on each day to minimize differences in time of sacrifice and dissection, and maintain the experimental balance across groups. The head was immediately placed in a cold box maintained at −15 °C, where the brain was rapidly removed and placed on a glass plate for dissection. All equipment used to obtain tissue was treated with RNAse Zap (Ambion, Inc. Austin, TX) to prevent RNA degradation. The ACB and AMYG were dissected according to the coordinates of Paxinos and Watson (1998). Briefly, the ACB was dissected from a 2-mm section generated by a coronal cut at 2 mm anterior to the optic chiasm (Bregma 1.70 mm) and a coronal cut at the optic chiasm (Bregma −0.26 mm). The AMYG was dissected by a cut at the lateral borders of the lateral hypothalamus (Bregma −2.12 mm) and ventral of the rhinal fissure, with cortical tissue then trimmed at the lateral edges of the dissected slice. Dissected tissues were immediately homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) and processed according to the manufacturer's protocol, but with twice the suggested ratio of Trizol to tissue (Edenberg et al., 2005). Ethanol precipitated RNA was further purified through RNeasy® columns (Qiagen, Valencia, CA) according to the manufacturer's protocol. The yield, concentration and purity of the RNA were determined by running a spectrum from 210 to 350 nm, and analyzing the ratio of large and small ribosomal RNA bands using an Agilent Bioanalyzer. Yields and purity of the RNA were excellent.

2.1. Microarray procedures

Separate preparations of total RNA were made from individual CNS regions from each animal. Samples were not pooled. Standard Affymetrix protocols (GeneChip® Expression Analysis Technical Manual, Rev. 5 and updates) were used to synthesize biotinylated cRNA, starting with 5 μg total RNA from each region, using the Affymetrix kits for cDNA synthesis, in vitro transcription and sample cleanup. Fifteen micrograms of fragmented, biotinylated cRNA from each independent sample were mixed into 300 μl of hybridization cocktail, of which 200 μl was used for each hybridization. Hybridization was for 17 h at 42 °C. Samples were hybridized to the Affymetrix GeneChip® (Rat Genome 230 2.0 array GeneChips). Washing and scanning of the GeneChips were carried out according to standard protocols, as previously described (Edenberg et al., 2005; McClintick et al., 2003).

To minimize potential systematic errors, all stages of the experiment were balanced across experimental groups. That is, equal numbers of animals in each group were sacrificed within the same 2-hour time frame each day, and equal numbers of RNA preparations from the representative groups were processed through the labeling, hybridization, washing and scanning protocols on a given day, in a counterbalanced order, using premixes of reagents.

2.2. Statistical and neuroinformatics analysis of microarray data

Each GeneChip® was scanned using an Affymetrix Model 3000 scanner and underwent image analysis using Affymetrix GCOS software. Microarray data will be available from the National Center for Biotechnology Information's Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/, (Barrett et al., 2005; Edgar et al., 2002). Raw .cel files were then imported into the statistical programming environment R (R: A language and environment for statistical computing Ver 2.2.0; R Foundation for Statistical Computing, 2005) for further analysis with tools available from the Bioconductor Project (Gentleman et al., 2004), themselves further expanded by the authors using the R language. Expression data from the 18 arrays of each region were normalized within-region and converted to log(2) using the Robust Multi-chip Average (RMA) method (Irizarry et al., 2003) implemented in the Bioconductor package RMA. As a standardization step to facilitate later comparisons with other experiments, expression levels were scaled such that the mean expression of all arrays was $\log_2(1000)$. As we were primarily concerned with identifying genes that could be subjected to further bioinformatic analysis, all probesets currently annotated by Affymetrix as "expressed sequence tags" or whose gene names contain the words "riken", "predicted", or "similar to" were filtered out. We next filtered out probe sets with a very low likelihood of actual expression in our samples, accomplished with the Bioconductor package "genefilter." Probe sets that did not have at least 25% of samples with normalized scaled expression greater than 64 were filtered out. Linear modeling to calculate gene-wise p values for the contrasts of the EtOH group versus water group, SAC group versus water group, and EtOH group versus SAC group was performed using the package Limma (Smyth, 2004); probe sets were considered to be statistically significant at $p<0.01$, with a false discovery rate (FDR) less than 0.3.

Testing for over-representation of Gene Ontology (Harris et al., 2004; Ashburner et al., 2000) biologic process (GO) categories was performed using the Bioconductor package GOstats (Gentleman, 2004). Briefly, for each gene set tested, a list of unique Entrez-Gene identifiers was constructed. This list was then compared to the list of all known Entrez-Gene identifiers that are represented on the Affymetrix chipset Rat Genome 230 2.0. Identification of over-represented GO categories was then accomplished within GOstats using the hypergeometric distribution. To filter out uninteresting categories, only those categories

with greater than 9 and less than 300 genes represented on the chipset were included in the analysis, as were categories with less than 5 significant genes. GO categories were called significant at $p<0.05$. Co-citation and network analyses were conducted with *Ingenuity®*.

2.3. Quantitative real-time PCR

Real-Time PCR was carried out using SybrGreen chemistry and the ABI Prism 7700 Sequence Detection System (Applied Biosystems). The amplification primers were designed using Primer Express software (Applied Biosystems). Total RNA, isolated for the microarray analyses, was employed for these analyses. Following reverse transcription of the RNA (TaqMan Reverse Transcription Reagents, Applied Biosystems), an aliquot of each reverse transcription reaction was amplified in triplicate. This reaction was repeated to generate 6 values for each test group. Two control reactions were run for each RNA preparation: 1) a reverse transcription and PCR reaction with no added RNA to control for contamination of the reagents; and 2) a PCR reaction without the reverse transcription reaction in the presence of RNA to detect DNA contamination of the RNA preparation. To correct for sample-to-sample variation, an endogenous control (GAPDH) was amplified with the target and served as an internal reference to normalize the data. Relative quantification of data from the ABI Prism 7700 Sequence Detection System was performed using the standard curve method (Applied Biosystems, User Bulletin #2; htpp://www.appliedbiosystems.com). Quantitative RT-PCR (qRT-PCR) measurements were conducted on genes to verify differences observed with microarray hybridization. Genes were selected on the basis of significant differential expression, relatively large fold changes, and the availability of primers.

3. Results

Average responses on the FR5 lever indicated that there was a significant group effect ($F_{2,15}$ values > 162.54, p values < 0.001); post-hoc comparisons indicated that the SAC group responded significantly more than the EtOH and water groups, and the EtOH group responded significantly more than the water group (Fig. 1). Responding by the SAC group was approximately 1.5-fold higher than the EtOH group and 25-fold higher than the water group. Responding on the alternate lever for water was low for all 3 groups and was comparable to responses on the FR5 lever by the water group (~20 responses/session).

The average number of SAC reinforcements was 104, which would produce intakes of approximately 10 ml of 0.0125% SAC per session. The average number of EtOH reinforcements was 61, which would produce intakes of approximately 6 ml of 15% EtOH per session. Given that the average body weight was 410 g at the end of testing, the amount of EtOH consumed would be equivalent to approximately 1.7 g/kg/session. This level of EtOH self-administering was reached for at least 21 consecutive days. Previous research indicated that this level of intake would result in blood ethanol concentrations greater than 80 mg% in the P rat (c.f. Murphy et al., 2002; Rodd-Henricks et al., 2001).

3.1. Gene expression in the ACB

Comparing across the 3 groups, there were 513 differences in named gene expression in the ACB, with 55 differences between the SAC and water groups, 215 differences between the EtOH and water groups, and 243 differences between the EtOH and SAC groups. Most of the differences were in the range of 1.15 to 1.25-fold.

There were 55 differences ($p<0.01$) in gene expression in the SAC versus the water group, with 31 genes having higher and 24 genes having lower expression in the SAC group (Table 1). However, with a FDR of 0.87, these differences could have occurred by chance alone.

Table 2 lists the genes that were significantly different between the EtOH and water groups. Among the 215 named genes listed, 131 genes had higher and 84 genes lower expression levels in the EtOH compared to the water group. Several neurotransmitter receptors had lower expression levels in the EtOH group; these included the *Htr2a*, *Htr5a*, *Gabrb1*, *Gabrb2*, *Grm1*, and *Sstr1*, whereas only *P2ry13* had higher expression in the EtOH group.

There were approximately 243 significant differences in named genes ($p<0.01$) between the EtOH and SAC groups (Table 3), with 148 genes having higher and 95 genes having lower expression in the EtOH versus the SAC group. Genes for several transmitter receptors had lower expression in the EtOH group than the SAC group; these included *Gabrb2. Gabrb3*, *Gria2*, *Gria3* and *Oprk1*; only the expression of the *Tacr3* gene was higher in the EtOH than SAC group.

There were 4 significant GO categories that differed between the EtOH and water groups, and 15 GO categories that differed between the EtOH and SAC groups (Table 4). General categories such as cell and ion transport and homeostasis, and synaptic transmission appeared in both lists of GO categories.

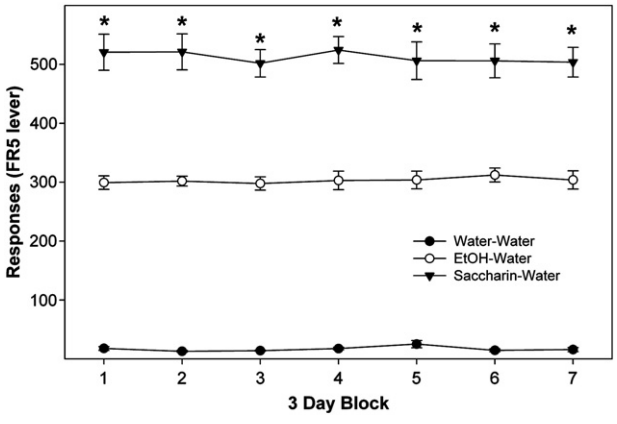

Fig. 1. Responses per session on the lever paired with ethanol, saccharin or water (FR5 lever) by the 3 groups of iP rats ($n=6$/group). Data are the means ± SEM. Responding by the saccharin group was significantly higher than responding by other 2 groups; responding by the EtOH group was significantly higher than responding by the water group. Lever presses on the alternate lever for water (FR1 lever) are not shown but are comparable to the lever presses by the water group on the FR5 lever (~20 responses/session).

Table 1
Genes that were different in the nucleus accumbens of iP rats between the saccharin and water groups at $p<0.01$ (FDR>0.8)

Gene symbol	Name	Fold change	Limma *p*-value
Nt5dc2	5′-nucleotidase domain containing 2	−1.11	0.009
Ar	Androgen receptor	−1.15	0.005
Aqp11	Aquaporin 11	−1.14	0.006
Bcl2l1	Bcl2-like 1	−1.15	0.001
Clstn2	Calsyntenin 2	−1.13	0.009
Csnk1d	Casein kinase 1, delta	−1.12	0.008
C8b	Complement component 8, beta polypeptide (mapped)	−1.11	0.004
Cpne9	Copine family member IX	−1.13	0.005
Cxxc4	CXXC finger 4	−1.17	0.006
Doc2a	Double C2, alpha	−1.14	0.003
Dusp1	Dual specificity phosphatase 1	−1.33	0.009
Gsk3b	Glycogen synthase kinase 3 beta /// glycogen synthase kinase 3 beta	−1.14	0.007
Gna11	Guanine nucleotide binding protein, alpha 11 /// guanine nucleotide binding protein, alpha 11	−1.18	0.003
Bat5	HLA-B associated transcript 5	−1.11	0.002
Homer1	Homer homolog 1 (*Drosophila*)	−2.00	0.001
Jun	Jun oncogene /// Jun oncogene	−1.13	0.009
Numb	Numb gene homolog (*Drosophila*)	−1.17	0.005
Col2a1	Procollagen, type II, alpha 1	−1.15	0.002
Pdcd8	Programmed cell death 8	−1.16	0.003
Pcsk1	Proprotein convertase subtilisin/kexin type 1	−1.13	0.002
Scrg1	Scrapie responsive gene 1	−1.14	0.008
Scamp5	Secretory carrier membrane protein 5	−1.17	0.004
Tmed3	Transmembrane emp24 domain containing 3 /// transmembrane emp24 domain containing 3	−1.13	0.009
Tnfaip6	Tumor necrosis factor alpha induced protein 6	−1.10	0.009
Arpc1b	Actin related protein 2/3 complex, subunit 1B	1.16	0.004
Adra2c	Adrenergic receptor, alpha 2c	1.15	0.005
Cacnb1	Calcium channel, voltage-dependent, beta 1 subunit	1.13	0.008
Cast	Calpastatin	1.13	0.006
Cnksr3	Cnksr family member 3	1.17	0.006
Coil	Coilin	1.20	0.010
Cfb	Complement factor B /// complement factor B	1.20	0.007
Ddx27	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	1.17	0.006
H2afx	Dolichyl-phosphate (UDP-*N*-acetylglucosamine) *N*-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	1.13	0.007
Eef2k	Eukaryotic elongation factor-2 kinase	1.18	0.009
Eif4a2	Eukaryotic translation initiation factor 4A2	1.15	0.004
Fkbp11	FK506 binding protein 11 /// FK506 binding protein 11	1.13	0.005
Gpnmb	Glycoprotein (transmembrane) nmb /// glycoprotein (transmembrane) nmb	1.14	0.004
Gpm6b	Glycoprotein m6b	1.26	0.006
Gbp2	Guanylate nucleotide binding protein 2	1.19	0.001
Ifitm3	Interferon induced transmembrane protein 3	1.32	0.002
Neurod1	Neurogenic differentiation 1	1.16	0.006
Nexn	Nexilin	1.30	0.002
Nexn	Nexilin	1.24	0.005
Nfs1	Nitrogen fixation gene 1 (*S. cerevisiae*)	1.12	0.005
Ppig	Peptidylprolyl isomerase G	1.20	0.008
Pola2	Polymerase (DNA directed), alpha 2	1.21	0.003
Kcnd1	Potassium voltage-gated channel, Shal-related family, member 1	1.15	0.002
Ptprc	Protein tyrosine phosphatase, receptor type, C /// protein tyrosine phosphatase, receptor type, C	1.21	0.003
Rimbp2	RIM binding protein 2 /// RIM binding protein 2	1.11	0.008
RT1-Aw2 ///	RT1 class Ib, locus Aw2 /// RT1 class Ia, locus A2 /// RT1 class I, A3	1.21	0.001
Snrpb	Small nuclear ribonucleoprotein polypeptides B and B1	1.21	0.001
Slc15a3	Solute carrier family 15, member 3	1.16	0.008
Tada1l	Transcriptional adaptor 1 (HFI1 homolog, yeast) like	1.14	0.002
Usf2	Upstream transcription factor 2	1.24	0.000
Wwp1 ///	AWW domain containing E3 ubiquitin protein ligase 1 /// adipose differentiation related protein	1.12	0.005

Additional major GO categories in the EtOH versus SAC contrast included endocytosis, neurogenesis and ensheathment of neurons. Several genes listed in the synaptic transmission category for both EtOH contrasts included *Grm1*, *Rims1*, *Htr2a*, *Htr5a, Gria2*, *Gria3*, *Sv2a*, *Scn2b*, *Gad1*, *Gad2*, *Camk4*, *Gabrb1*, *Gabrb2*, *Gabrb3*, *Cav2*, *Nrxn3*, *S100b* and *Oprk1* (Tables 1 and 2).

There were 73 genes that were significantly changed in the same direction in the EtOH group versus both the water and SAC groups, with 40 genes having higher and 33 genes lower expression in the EtOH group (Table 5). There were 11 genes within the synaptic transmission category that were in common in both contrasts, with 7 genes (*Cav2*, *Homer1*, *Nrxn3*, *Pik4ca*, *Plp*, *S100b* and *Sv2a*) having higher, and 4 genes (*Camk4*, *Gabrb2*, *Gad1* and *Syt6*) having lower expression in the EtOH group. There were 7 genes within a combined homeostasis/transport category that were in common in the EtOH group versus the SAC and water groups, with 5 genes (*S100b*, *Sv2a*, *Clcn3*, *Ftl1 and Alb*) having higher and only 2 genes (*Prkca* and *Atp2b4*) having lower expression in the EtOH group.

3.2. Gene expression in the AMYG

In the AMYG, comparing across the 3 groups, there were 134 differences ($p<0.01$) in the expression of named genes, with 48 differences between the SAC and water groups, 23 differences between the EtOH and water groups, and 63 differences between the EtOH and SAC groups (Table 6). However, because of the high FDR, these differences could have occurred by chance alone.

3.3. Quantitative RT-PCR confirmation

Because there were more significant differences and more significant GO categories between the EtOH versus SAC group than between the EtOH versus water group, genes selected for qRT-PCR confirmation (Table 7) were chosen from the EtOH–SAC comparison (Table 3). Among the 12 genes tested, 9 were confirmed as changing significantly in the same direction as the microarray values (Table 7). Of the remaining 3 genes, *Map1b* changed in the same direction with both measures (however, the RT-PCR values were not statistically different), *Camk4* was not

Table 2
Genes that were significantly different in the nucleus accumbens of iP rats between the ethanol and water groups at $p<0.01$ (FDR = 0.2–0.3)

Gene symbol	Name	Fold change	Limma *p*-value
Pdpk1	3-phosphoinositide dependent protein kinase-1	−1.45	0.003
Htr2a	5-hydroxytryptamine (serotonin) receptor 2A	−1.27	0.007
Htr5a	5-hydroxytryptamine (serotonin) receptor 5A	−1.18	0.009
Ahi1	Abelson helper integration site 1	−1.31	0.006
Adar	Adenosine deaminase, RNA-specific	−1.14	0.006
Atrx	Alpha thalassemia/mental retardation syndrome X-linked homolog (human)	−1.22	0.006
Appbp2	Amyloid beta precursor protein (cytoplasmic tail) binding protein 2	−1.14	0.007
Agtr1a	Angiotensin II receptor, type 1 (AT1A)	−1.16	0.006
Amh	Anti-Mullerian hormone	−1.17	0.009
Ap1gbp1	AP1 gamma subunit binding protein 1	−1.13	0.006
Alg2	Asparagine-linked glycosylation 2 homolog (yeast, alpha-1,3-mannosyltransferase)	−1.22	0.004
Alg2	Asparagine-linked glycosylation 2 homolog (yeast, alpha-1,3-mannosyltransferase)	−1.21	0.008
Atxn3	Ataxin 3	−1.17	0.000
Atp2b4	ATPase, Ca++ transporting, plasma membrane 4	−1.27	0.001
Blnk	B-cell linker	−1.12	0.005
Bcl2l1	Bcl2-like 1	−1.22	0.000
Bid	BH3 interacting domain death agonist /// BH3 interacting domain death agonist	−1.14	0.003
Cacna2d1	Calcium channel, voltage-dependent, alpha2/delta subunit 1	−1.29	0.002
Cacnb4	Calcium channel, voltage-dependent, beta 4 subunit	−1.20	0.006
Camk4	Calcium/calmodulin-dependent protein kinase IV	−1.38	0.000
Clstn2	Calsyntenin 2	−1.16	0.002
Csnk1e	Casein kinase 1, epsilon	−1.17	0.007
Cstf1	Cleavage stimulation factor, 3′ pre-RNA, subunit 1	−1.14	0.008
Clock	Clock homolog (mouse)	−1.17	0.006
Cxxc4	CXXC finger 4	−1.26	0.000
Ccnh	Cyclin H	−1.21	0.002
Cftr	Cystic fibrosis transmembrane conductance regulator homolog	−1.13	0.005
Cyp11b1	Cytochrome P450, sub-family 11B, polypeptide 1 /// cytochrome P450, sub-family 11B, polypeptide 1	−1.21	0.005
Dusp12	Dual specificity phosphatase 12	−1.15	0.008
Gabrb1	Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 1	−1.15	0.003
Gabrb2	Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 2	−1.32	0.004
Grm1	Glutamate receptor, metabotropic 1	−1.19	0.000
Gad1	Glutamic acid decarboxylase 1	−1.25	0.003
Gsk3b	Glycogen synthase kinase 3 beta /// glycogen synthase kinase 3 beta	−1.13	0.009
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide	−1.27	0.000
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide /// guanine nucleotide binding protein, alpha q polypeptide	−1.33	0.000
Impact	Imprinted and ancient	−1.18	0.005
Kifc3	Kinesin family member C3	−1.19	0.001
Mkks	McKusick–Kaufman syndrome protein	−1.15	0.004
Map1b	Microtubule-associated protein 1b	−1.34	0.000
Mapk8ip3	Mitogen-activated protein kinase 8 interacting protein 3	−1.23	0.006
Mllt10	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 10	−1.21	0.001
Myh8	Myosin, heavy polypeptide 8, skeletal muscle, perinatal	−1.10	0.010
Nmt1	*N*-myristoyltransferase 1	−1.14	0.005
Nedd4a	Neural precursor cell expressed, developmentally down-regulated gene 4A	−1.17	0.009
2610020o0	Nuclear NF-kappaB activating protein	−1.23	0.002
Npap60	Nuclear pore associated protein	−1.15	0.001
Npap60	Nuclear pore associated protein	−1.14	0.003
P34	p34 protein	−1.14	0.004
Pnma1	Paraneoplastic antigen MA1	−1.16	0.008
Pip5k2b	Phosphatidylinositol-4-phosphate 5-kinase, type II, beta	−1.21	0.008
Prps2	Phosphoribosyl pyrophosphate synthetase 2	−1.13	0.007
Kcnk9	Potassium channel, sub-family K, member 9	−1.19	0.003
Kcns2	Potassium voltage-gated channel, delayed-rectifier, sub-family S, member 2	−1.12	0.010
Kcnh2	Potassium voltage-gated channel, sub-family H (eag-related), member 2	−1.14	0.009
Kcnq3	Potassium voltage-gated channel, sub-family Q, member 3	−1.17	0.004
Col2a1	Procollagen, type II, alpha 1	−1.13	0.004
Pcsk1	Proprotein convertase subtilisin/kexin type 1	−1.12	0.002
Prkca	Protein kinase C, alpha /// protein kinase C, alpha	−1.12	0.009
Prkab2	Protein kinase, AMP-activated, beta 2 non-catalytic subunit	−1.17	0.007
Prkacb	Protein kinase, cAMP dependent, catalytic, beta	−1.29	0.000
Ppp2r1a	Protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	−1.15	0.008
Ramp3	Receptor (calcitonin) activity modifying protein 3	−1.14	0.009
Reln	Reelin	−1.24	0.007
Rnf12	Ring finger protein 12	−1.18	0.003
Styxl1	Serine/threonine/tyrosine interacting-like 1	−1.22	0.001
Sgtb	Small glutamine-rich tetratricopeptide repeat (TPR)-containing, beta	−1.27	0.010
Slc2a3	Solute carrier family 2 (facilitated glucose transporter), member 3 /// solute carrier family 2 (facilitated glucose transporter), member 3	−1.20	0.007
Slc22a4	Solute carrier family 22 (organic cation transporter), member 4	−1.13	0.003
Sstr1	Somatostatin receptor 1	−1.24	0.001
St8sia3	ST8 alpha-*N*-acetyl-neuraminide alpha-2,8-sialyltransferase 3	−1.21	0.005
Stch	Stress 70 protein chaperone, microsome-associated, 60kD human homolog	−1.16	0.003
Syt6	Synaptotagmin VI	−1.22	0.006
Txndc13	Thioredoxin domain containing 13	−1.23	0.008
Tgfb1i4	Transforming growth factor beta 1 induced transcript 4	−1.15	0.002
Tmod2	Tropomodulin 2	−1.16	0.006
Tpm3	Tropomyosin 3, gamma	−1.12	0.004
Wars	Tryptophanyl-tRNA synthetase	−1.13	0.006
Flk	Tyrosine protein kinase FLK	−1.10	0.007
Usp11	Ubiquitin specific protease 11	−1.24	0.006
Ube4a	Ubiquitination factor E4A, UFD2 homolog (*S. cerevisiae*)	−1.12	0.010

Table 2 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Vti1a	Vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	−1.20	0.010
Wdr47	WD repeat domain 47	−1.23	0.008
Wbp4	WW domain binding protein 4	−1.12	0.004
Zfp483	Zinc finger protein 483	−1.25	0.002
Zdhhc22	Zinc finger, DHHC-type containing 22	−1.15	0.002
Akap8l	A kinase (PRKA) anchor protein 8-like	1.17	0.006
Abhd1	Abhydrolase domain containing 1	1.21	0.006
Aco2	Aconitase 2, mitochondrial	1.20	0.007
Actn1	Actinin, alpha 1	1.16	0.010
Alb	Albumin /// albumin	1.21	0.005
As3mt	Arsenic (+3 oxidation state) methyltransferase	1.13	0.006
Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A /// ATP-binding cassette, sub-family B (MDR/TAP), member 1A	1.21	0.004
Abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4 /// ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.19	0.005
Atp2b1	ATPase, Ca++ transporting, plasma membrane 1	1.22	0.001
B2m	Beta-2 microglobulin	1.14	0.004
B2m	Beta-2 microglobulin	1.15	0.008
Cdh11	Cadherin 11	1.23	0.002
Cib1	Calcium and integrin binding 1 (calmyrin)	1.12	0.006
Cacna2d3	Calcium channel, voltage-dependent, alpha 2/delta 3 subunit	1.33	0.004
Camk2b	Calcium/calmodulin-dependent protein kinase II, beta	1.10	0.009
Car6	Carbonic anhydrase 6	1.19	0.001
Cflar	CASP8 and FADD-like apoptosis regulator	1.27	0.001
Cav2	Caveolin 2	1.17	0.003
Cebpa	CCAAT/enhancer binding protein (C/EBP), alpha	1.26	0.005
Cd81	CD 81 antigen	1.10	0.006
Cd99	CD99 antigen	1.12	0.002
Cdca1	Cell division cycle associated 1	1.12	0.005
Cxcl14	Chemokine (C-X-C motif) ligand 14 /// chemokine (C-X-C motif) ligand 14	1.13	0.009
Chi3l1	Chitinase 3-like 1	1.14	0.007
Clcn3	Chloride channel 3	1.18	0.002
Ccdc5	Coiled-coil domain containing 5	1.15	0.008
Ctdsp1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	1.14	0.005
Cst3	Cystatin C	1.14	0.010
P22k15	Cystatin related protein 2	1.14	0.001
Dhx57	DEAH (Asp-Glu-Ala-Asp/His) box polypeptide 57	1.11	0.007
Ddn	Dendrin	1.16	0.009
Dcir3	Dendritic cell inhibitory receptor 3	1.14	0.010
Dscr1l1	Down syndrome critical region gene 1-like 1 /// Down syndrome critical region gene 1-like 1	1.23	0.006
Dullard	Dullard homolog (*Xenopus laevis*)	1.13	0.005
Dtnb	Dystrobrevin, beta	1.14	0.009
Efemp2	EGF-containing fibulin-like extracellular matrix protein 2	1.13	0.007
Emcn	Endomucin	1.23	0.001
Ftl1	Ferritin light chain 1 /// ferritin light chain 1	1.13	0.006
Gkap1	G kinase anchoring protein 1	1.29	0.006
Galm	Galactose mutarotase	1.13	0.003
Gjb6	Gap junction membrane channel protein beta 6	1.15	0.005

Table 2 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Glul	Glutamate-ammonia ligase (glutamine synthase) /// glutamate-ammonia ligase (glutamine synthase)	1.14	0.008
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble) /// glycerol-3-phosphate dehydrogenase 1 (soluble)	1.20	0.005
Gpm6b	Glycoprotein m6b	1.27	0.004
Csf2ra	Granulocyte-macrophage colony stimulating receptor alpha	1.15	0.010
H2afy	H2A histone family, member Y	1.14	0.001
Bat1a	HLA-B-associated transcript 1A	1.13	0.009
Homer1	Homer homolog 1 (*Drosophila*)	1.75	0.005
Hyal3	Hyaluronoglucosaminidase 3	1.12	0.003
Id4	Inhibitor of DNA binding 4 /// inhibitor of DNA binding 4	1.15	0.001
Itgb1	Integrin beta 1 (fibronectin receptor beta)	1.17	0.001
Itgb1	Integrin beta 1 (fibronectin receptor beta) /// integrin beta 1 (fibronectin receptor beta)	1.18	0.001
Klhl5	Kelch-like 5 (*Drosophila*)	1.24	0.009
Kif1a	Kinesin family member 1A	1.16	0.003
Klf15	Kruppel-like factor 15	1.14	0.006
Letm2	Leucine zipper-EF-hand containing transmembrane protein 2	1.17	0.002
Lig3	Ligase III, DNA, ATP-dependent	1.14	0.010
Man2c1	Mannosidase, alpha, class 2C, member 1	1.14	0.005
39148	Membrane-associated ring finger (C3HC4) 7	1.16	0.006
Mag	Myelin-associated glycoprotein	1.15	0.008
Mcl1	Myeloid cell leukemia sequence 1	1.12	0.009
Mrlcb	Myosin light chain, regulatory B	1.16	0.010
Nrd1	Nardilysin, *N*-arginine dibasic convertase 1	1.17	0.009
—	Nclone10 mRNA	1.21	0.002
Ndnl2	Necdin-like 2	1.20	0.004
Nrxn3	Neurexin 3	1.25	0.003
Nfia	Nuclear factor I/A	1.19	0.007
Nfib	Nuclear factor I/B	1.12	0.009
Nfib	Nuclear factor I/B	1.15	0.005
Nfkbia	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha /// nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	1.19	0.006
Numa1	Nuclear mitotic apparatus protein 1	1.14	0.002
Nr1h3	Nuclear receptor sub-family 1, group H, member 3 /// nuclear receptor sub-family 1, group H, member 3	1.15	0.007
Odz2	Odd Oz/ten-m homolog 2 (*Drosophila*)	1.27	0.010
Pctk1	PCTAIRE-motif protein kinase 1	1.16	0.001
Ppig	Peptidylprolyl isomerase G	1.22	0.004
Prdx6	Peroxiredoxin 6	1.19	0.001
Ppap2b	Phosphatidic acid phosphatase type 2B	1.15	0.002
Pik4ca	Phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	1.21	0.009
Pla2g6	Phospholipase A2, group VI	1.14	0.004
Plscr3	Phospholipid scramblase 3	1.10	0.009
Plag1	Pleiomorphic adenoma gene 1	1.18	0.004
Pola2	Polymerase (DNA directed), alpha 2	1.20	0.003
Polb	Polymerase (DNA directed), beta	1.21	0.010
Psg4	Pregnancy specific beta-1-glycoprotein 4	1.16	0.008
Col11a2	Procollagen, type XI, alpha 2 (mapped)	1.19	0.003
Pkig	Protein kinase inhibitor, gamma	1.09	0.007
Prkwnk1	Protein kinase, lysine deficient 1	1.13	0.004
Ptp4a2	Protein tyrosine phosphatase 4a2	1.11	0.007
Ptpn2	Protein tyrosine phosphatase, non-receptor type 2	1.12	0.009

(continued on next page)

Table 2 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Ptprf	Protein tyrosine phosphatase, receptor type, F	1.17	0.003
Plp	Proteolipid protein	1.15	0.006
Ptk2	PTK2 protein tyrosine kinase 2 /// PTK2 protein tyrosine kinase 2	1.12	0.007
P2ry13	Purinergic receptor P2Y, G-protein coupled, 13	1.24	0.005
Ua20	Putative UA20 protein	1.14	0.007
Rims1	Regulating synaptic membrane exocytosis 1	1.20	0.000
Rgc32	Response gene to complement 32	1.15	0.005
Rgc32	Response gene to complement 32	1.19	0.003
Rpe65	Retinal pigment epithelium 65	1.17	0.001
Arhgef1	Rho guanine nucleotide exchange factor (GEF) 1	1.19	0.007
Rnasen	Ribonuclease III, nuclear	1.14	0.005
Rpl13a	Ribosomal protein L13A /// ribosomal protein L13A	1.14	0.005
Rps29	Ribosomal protein S29	1.14	0.006
Rps3a	Ribosomal protein S3a	1.11	0.010
Rps6ka2	Ribosomal protein S6 kinase polypeptide 2	1.22	0.009
Rnf44	Ring finger protein 44	1.12	0.007
S100b	S100 protein, beta polypeptide	1.14	0.008
Scamp1	Secretory carrier membrane protein 1	1.18	0.002
Sepw1	Selenoprotein W, muscle 1	1.13	0.008
Sdccag1	Serologically defined colon cancer antigen 1	1.14	0.005
Shank1	SH3 and multiple ankyrin repeat domains 1	1.19	0.003
Shank2	SH3/ankyrin domain gene 2 /// SH3/ankyrin domain gene 2	1.17	0.004
Slc2a1	Solute carrier family 2 (facilitated glucose transporter), member 1 /// solute carrier family 2 (facilitated glucose transporter), member 1	1.15	0.004
Slc22a17	Solute carrier family 22 (organic cation transporter), member 17	1.11	0.005
Slc23a2	Solute carrier family 23 (nucleobase transporters), member 2	1.19	0.003
Slc25a25	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25 /// solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	1.15	0.003
Slc33a1	Solute carrier family 33 (acetyl-CoA transporter), member 1	1.23	0.008
Slc34a1	solute carrier family 34 (sodium phosphate), member 1	1.18	0.005
Slc4a4	Solute carrier family 4, member 4	1.22	0.001
Scd2	Stearoyl-Coenzyme A desaturase 2	1.15	0.009
Sc5d	Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (*S. cerevisiae*) /// sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (*S. cerevisiae*)	1.21	0.000
Sympk	Symplekin	1.12	0.004
Sv2a	Synaptic vesicle glycoprotein 2a	1.22	0.001
Sdc4	Syndecan 4	1.17	0.009
Tbkbp1	TBK1 binding protein 1	1.22	0.001
Thap7	THAP domain containing 7	1.16	0.001
pur-beta	Transcription factor Pur-beta /// Transcription factor Pur-beta	1.17	0.001
Tmem10	Transmembrane protein 10 /// transmembrane protein 10	1.14	0.010
Ets1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	1.22	0.000
Vcam1	Vascular cell adhesion molecule 1 /// vascular cell adhesion molecule 1	1.14	0.008
Zfp212	Zinc finger protein 212	1.11	0.004

Table 2 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Zfp36l2	Zinc finger protein 36, C3H type-like 2	1.15	0.002
Zfp423	Zinc finger protein 423	1.20	0.002
Zfand3	Zinc finger, AN1-type domain 3	1.10	0.007
Zswim6	Zinc finger, SWIM domain containing 6	1.16	0.004

changed in the RT-PCR measure, and *Nrxn3* changed significantly in both measures, but in opposite directions (Table 7). Similar to previous studies from our lab (Edenberg et al., 2005; Kimpel et al., 2007), there was a high degree of concordance between the microarray and RT-PCR results. However, the lack of agreement between the two measures for *Camk4* and *Nrxn3* suggests the results for these two genes are inconclusive.

3.4. Supplemental tables

See Supplemental Tables A and B for more complete information on data for differences in the ACB between the EtOH and water groups, and between the EtOH and SAC groups.

4. Discussion

The major findings of this study are that, compared to the water control group, EtOH self-administration, but not SAC self-administration, produced changes in named gene expression in the ACB of iP rats (Tables 1 and 2), whereas significant changes in named gene expression were not observed in the AMYG (Table 6). The effects of EtOH self-administration on gene expression in the ACB is not due to the presence of EtOH in the tissue at the time of killing, because animals were killed 24 h after the last operant session. Also, the differences between the EtOH and water groups do not appear to be due to motor activity, learning or conditioning factors associated with the operant task, because the SAC group learned the task as well as the EtOH group and responded more on the active lever than the water lever (Fig. 1), but there were no significant differences in gene expression in the ACB between the SAC and water groups (Table 1). Changes associated with the operant task may have occurred in the ACB of EtOH and SAC groups, but these changes were not detectable after 24 h, as suggested by the SAC versus water contrast (Table 1). The changes that persisted for 24 h in the ACB of the EtOH group may be due to the chronic effects of EtOH exposure and changes associated with the CNS reinforcing effects of EtOH. More robust differences between the EtOH and the other groups may have been observed with the present experimental conditions, if the ACB shell had been analyzed separately from the core, and if shorter time points had been analyzed.

The apparent lack of finding significant changes in gene expression in the AMYG between any of the groups may be due to the combination of factors, i.e., (a) changes are occurring but

Table 3
Genes that were significantly different in the nucleus accumbens of iP rats between the ethanol and saccharin groups at $p<0.01$ (FDR=0.2–0.3)

Gene symbol	Name	Fold change	Limma p-value
Pdpk1	3-phosphoinositide dependent protein kinase-1	−1.47	0.002
Ap3m2	Adaptor-related protein complex 3, mu 2 subunit	−1.11	0.004
Adar	Adenosine deaminase, RNA-specific	−1.16	0.003
Atrx	Alpha thalassemia/mental retardation syndrome X-linked homolog (human)	−1.29	0.001
Atrx	Alpha thalassemia/mental retardation syndrome X-linked homolog (human)	−1.26	0.001
Aplp2	Amyloid beta (A4) precursor-like protein 2	−1.30	0.003
App	Amyloid beta (A4) precursor protein	−1.13	0.004
App	Amyloid beta (A4) precursor protein /// amyloid beta (A4) precursor protein	−1.27	0.003
Appbp2	Amyloid beta precursor protein (cytoplasmic tail) binding protein 2	−1.14	0.007
Arih1	Ariadne ubiquitin-conjugating enzyme E2 binding protein homolog 1 (*Drosophila*)	−1.14	0.001
Actr3	ARP3 actin-related protein 3 homolog (yeast)	−1.20	0.008
Atxn3	Ataxin 3	−1.15	0.001
Atp2b4	ATPase, Ca++ transporting, plasma membrane 4	−1.25	0.002
Atp6v1b2	ATPase, H transporting, lysosomal V1 subunit B2	−1.16	0.009
Birc4	Baculoviral IAP repeat-containing 4	−1.30	0.007
Bag4	BCL2-associated athanogene 4	−1.16	0.003
Bfar	Bifunctional apoptosis regulator	−1.20	0.009
Blcap	Bladder cancer associated protein homolog (human)	−1.25	0.001
Bmp3	Bone morphogenetic protein 3	−1.11	0.009
Cacnb4	Calcium channel, voltage-dependent, beta 4 subunit	−1.31	0.004
Cacnb4	Calcium channel, voltage-dependent, beta 4 subunit	−1.20	0.006
Camk4	Calcium/calmodulin-dependent protein kinase IV	−1.23	0.002
Camk4	Calcium/calmodulin-dependent protein kinase IV	−1.32	0.001
Csen	Calsenilin, presenilin binding protein, EF hand transcription factor	−1.23	0.008
Csnk1e	Casein kinase 1, epsilon	−1.15	0.007
Cp	Ceruloplasmin /// ceruloplasmin	−1.30	0.004
Cct3	Chaperonin subunit 3 (gamma)	−1.14	0.004
Cldn1	Claudin 1 /// claudin 1	−1.15	0.005
Ccnh	Cyclin H	−1.21	0.002
Dcbld2	Discoidin, CUB and LCCL domain containing 2	−1.12	0.009
Dlgh2	Discs, large homolog 2 (*Drosophila*)	−1.17	0.010
Ddit4l	DNA-damage-inducible transcript 4-like /// DNA-damage-inducible transcript 4-like	−1.18	0.006
Dnm3	Dynamin 3	−1.17	0.010
Elavl2	ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 2 (Hu antigen B)	−1.19	0.008
Enah	Enabled homolog (*Drosophila*) /// enabled homolog (*Drosophila*)	−1.13	0.009
Extl3	Exostoses (multiple)-like 3	−1.14	0.007
Fgl2	Fibrinogen-like 2	−1.19	0.010
Gabrb2	Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 2	−1.32	0.004
Gabrb3	Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 3	−1.33	0.002
Gria2	Glutamate receptor, ionotropic, AMPA2	−1.16	0.001
Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3) /// glutamate receptor, ionotropic, AMPA3 (alpha 3)	−1.19	0.009
Gad1	Glutamic acid decarboxylase 1	−1.23	0.006
Gad2	Glutamic acid decarboxylase 2	−1.32	0.007
Gpiap1	GPI-anchored membrane protein 1	−1.31	0.002
Grb2	Growth factor receptor bound protein 2	−1.14	0.006
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide	−1.20	0.001
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide /// guanine nucleotide binding protein, alpha q polypeptide	−1.30	0.001
Hnrpm	Heterogeneous nuclear ribonucleoprotein M	−1.13	0.008
Hk1	Hexokinase 1	−1.22	0.006
Igf2r	Insulin-like growth factor 2 receptor /// insulin-like growth factor 2 receptor	−1.14	0.004
Ifitm3	Interferon induced transmembrane protein 3	−1.30	0.002
Kifc3	Kinesin family member C3	−1.15	0.007
Lmo4	LIM domain only 4	−1.28	0.007
Mak10	MAK10 homolog, amino-acid *N*-acetyltransferase subunit, (*S. cerevisiae*)	−1.11	0.009
Map1b	Microtubule-associated protein 1b	−1.37	0.000
Mllt10	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 10	−1.23	0.000
Ncam2	Neural cell adhesion molecule 2	−1.23	0.005
Nedd4a	Neural precursor cell expressed, developmentally down-regulated gene 4A	−1.18	0.006
Nxph3	Neurexophilin 3	−1.14	0.009
Neurod1	Neurogenic differentiation 1	−1.15	0.008
Nln	Neurolysin (metallopeptidase M3 family)	−1.15	0.003
2610020o0	Nuclear NF-kappaB activating protein	−1.21	0.006
Npap60	Nuclear pore associated protein	−1.18	0.000
Npap60	Nuclear pore associated protein	−1.14	0.002
Nupl1	Nucleoporin like 1	−1.16	0.008
Oprk1	Opioid receptor, kappa 1	−1.20	0.007
Otud4	OTU domain containing 4	−1.20	0.003
Osbpl2	Oxysterol binding protein-like 2	−1.21	0.005
P34	P34 protein	−1.14	0.003
Pik3r3	Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3	−1.16	0.009
Pafah1b1	Platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	−1.19	0.007
Kcnj9	Potassium inwardly-rectifying channel, sub-family J, member 9	−1.18	0.008
Pja2	Praja 2, RING-H2 motif containing	−1.16	0.001
Prkca	Protein kinase C, alpha /// protein kinase C, alpha	−1.17	0.001
Prkacb	Protein kinase, cAMP dependent, catalytic, beta	−1.29	0.000
Ppp3r1	Protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)	−1.29	0.004
Clcn4-2	Putative chloride channel 4−2	−1.15	0.005
Rasgrp1	RAS guanyl releasing protein 1	−1.31	0.004
Ramp3	Receptor (calcitonin) activity modifying protein 3	−1.14	0.008
Rp1h	Retinitis pigmentosa 1 homolog (human)	−1.16	0.005
Scamp1	Secretory carrier membrane protein 1	−1.14	0.009
Sel1h	Sel1 (suppressor of lin-12) 1 homolog (*C. elegans*)	−1.19	0.001
Styxl1	Serine/threonine/tyrosine interacting-like 1	−1.17	0.007

(continued on next page)

Table 3 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Sgtb	Small glutamine-rich tetratricopeptide repeat (TPR)-containing, beta	−1.28	0.009
Snrpb	Small nuclear ribonucleoprotein polypeptides B and B1	−1.22	0.001
Scn2b	Sodium channel, voltage-gated, type II, beta	−1.45	0.002
Slc2a3	Solute carrier family 2 (facilitated glucose transporter), member 3 /// solute carrier family 2 (facilitated glucose transporter), member 3	−1.22	0.005
Slc23a2	Solute carrier family 23 (nucleobase transporters), member 2	−1.16	0.005
Stc2	Stanniocalcin 2	−1.09	0.008
Stch	Stress 70 protein chaperone, microsome-associated, 60kD human homolog	−1.20	0.000
Strn	Striatin, calmodulin binding protein	−1.17	0.004
Syt6	Synaptotagmin VI	−1.21	0.009
Txndc13	Thioredoxin domain containing 13	−1.23	0.008
Tef	Thyrotroph embryonic factor	−1.20	0.009
Tmed5	Transmembrane emp24 protein transport domain containing 5	−1.19	0.008
Uhmk1	U2AF homology motif (UHM) kinase 1	−1.25	0.005
Ube4a	Ubiquitination factor E4A, UFD2 homolog (*S. cerevisiae)*	−1.13	0.006
Vti1a	Vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	−1.21	0.007
Wwp1 ///	AWW domain containing E3 ubiquitin protein ligase 1 /// adipose differentiation related protein	−1.12	0.004
Zfp161	Zinc finger protein 161	−1.11	0.009
Zfp260	Zinc finger protein 260	−1.13	0.002
Zfp483	Zinc finger protein 483	−1.30	0.000
Nt5c3l	5′-nucleotidase, cytosolic III-like	1.14	0.008
Arbp	Acidic ribosomal phosphoprotein P0	1.16	0.004
Alb	Albumin	1.22	0.001
Alb	Albumin /// albumin	1.35	0.000
Aspa	Aspartoacylase	1.12	0.010
Arid1b	AT rich interactive domain 1B (Swi1 like)	1.23	0.006
Abcb10	ATP-binding cassette, sub-family B (MDR/TAP), member 10	1.12	0.010
Abcc4	ATP-binding cassette, sub-family C (CFTRMRP), member 4 /// ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.24	0.001
Blnk	B-cell linker	1.16	0.002
B2m	Beta-2 microglobulin	1.12	0.009
Bckdha	Branched chain ketoacid dehydrogenase E1, alpha polypeptide	1.14	0.010
Bckdk	Branched chain ketoacid dehydrogenase kinase	1.16	0.003
Cdh11	Cadherin 11	1.23	0.003
Cflar	CASP8 and FADD-like apoptosis regulator	1.29	0.001
Ctnnb1	Catenin (cadherin associated protein), beta 1	1.25	0.009
Cav2	Caveolin 2	1.21	0.001
Cav2	Caveolin 2	1.16	0.005
Cd99	CD99 antigen	1.10	0.006
Ctbs	Chitobiase, di-*N*-acetyl-	1.12	0.006
Clcn3	Chloride channel 3	1.15	0.008
Ccdc23	Coiled-coil domain containing 23	1.10	0.005
Ckb	Creatine kinase, brain /// creatine kinase, brain	1.13	0.005
Ctdsp1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	1.12	0.009
Cugbp2	CUG triplet repeat, RNA binding protein 2	1.20	0.008
P22k15	Cystatin related protein 2	1.15	0.001

Table 3 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Cox6a1	Cytochrome *c* oxidase, subunit VIa, polypeptide 1 /// cytochrome *c* oxidase, subunit VIa, polypeptide 1	1.13	0.002
Cyp4f2	Cytochrome P450, family 4, sub-family F, polypeptide 2 /// cytochrome P450, family 4, sub-family F, polypeptide 2	1.16	0.003
Ddt	D-dopachrome tautomerase	1.14	0.007
Dedd	Death effector domain containing	1.19	0.002
Dlgh1	Discs, large homolog 1 (*Drosophila*)	1.28	0.000
Dlgh2	Discs, large homolog 2 (*Drosophila*)	1.24	0.002
Dscam	Down syndrome cell adhesion molecule	1.15	0.010
Dusp6	Dual specificity phosphatase 6	1.13	0.006
E2f5	E2F transcription factor 5 /// E2F transcription factor 5	1.19	0.004
Egr2	Early growth response 2 /// early growth response 2	1.43	0.004
Emcn	Endomucin	1.23	0.001
Edg2	Endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	1.16	0.005
Fads1	Fatty acid desaturase 1	1.19	0.002
Fasn	Fatty acid synthase /// fatty acid synthase	1.13	0.008
Fos	FBJ murine osteosarcoma viral oncogene homolog /// FBJ murine osteosarcoma viral oncogene homolog	1.52	0.003
Fcgrt	Fc receptor, IgG, alpha chain transporter	1.15	0.008
Ftl1	Ferritin light chain 1 /// ferritin light chain 1	1.15	0.003
Fau	Finkel–Biskis–Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived) protein	1.13	0.006
Fzd2	Frizzled homolog 2 (*Drosophila*)	1.13	0.003
Gtf3a	General transcription factor III A	1.15	0.009
Gpx4	Glutathione peroxidase 4 /// glutathione peroxidase 4	1.15	0.004
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble)	1.44	0.009
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble) /// glycerol-3-phosphate dehydrogenase 1 (soluble)	1.23	0.002
Gp1bb /// Sept5	Glycoprotein Ib, beta polypeptide /// septin 5	1.16	0.009
Gm2a	GM2 ganglioside activator protein	1.13	0.009
Gramd3	GRAM domain containing 3	1.17	0.001
Gamt	Guanidinoacetate methyltransferase	1.14	0.004
Hes1	Hairy and enhancer of split 1 (*Drosophila*)	1.19	0.006
Hhex	Hematopoietically expressed homeobox	1.17	0.008
Hist1h4b	Histone cluster 1, H4b /// histone cluster 1, H4b	1.13	0.004
Bat5	HLA-B associated transcript 5	1.09	0.009
Homer1	Homer homolog 1 (*Drosophila*)	3.49	0.000
Hyal3	Hyaluronoglucosaminidase 3	1.13	0.005
Hadh2	Hydroxyacyl-Coenzyme A dehydrogenase type II /// hydroxyacyl-Coenzyme A dehydrogenase type II	1.13	0.002
Hsd11b1	Hydroxysteroid 11-beta dehydrogenase 1 /// hydroxysteroid 11-beta dehydrogenase 1	1.17	0.004
Hcn1	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1	1.23	0.003
Impa2	Inositol (myo)-1(or 4)-monophosphatase 2	1.17	0.005
Ifngr	Interferon gamma receptor 1	1.14	0.005
Il12a	Interleukin 12a /// interleukin 12a	1.16	0.002
Klk6	Kallikrein 6	1.20	0.004
Klf15	Kruppel-like factor 15	1.15	0.004

Table 3 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Klf4	Kruppel-like factor 4 (gut)	1.38	0.001
Ldhd	Lactate dehydrogenase D	1.13	0.005
Ldhd	Lactate dehydrogenase D	1.15	0.001
Matr3	Matrin 3	1.21	0.002
Mkks /// Cldn1	McKusick–Kaufman syndrome protein /// Claudin 1	1.13	0.007
39143	Membrane-associated ring finger (C3HC4) 2	1.15	0.001
Mt3	Metallothionein 3 /// metallothionein 3	1.14	0.003
MAST1	Microtubule-associated serine/threonine kinase 1	1.15	0.001
Mfge8	Milk fat globule-EGF factor 8 protein	1.15	0.005
Map2k3	Mitogen-activated protein kinase kinase 3	1.16	0.002
Mag	Myelin-associated glycoprotein	1.16	0.004
Mal	Myelin and lymphocyte protein, T-cell differentiation protein	1.12	0.007
Mog	Myelin oligodendrocyte glycoprotein	1.16	0.002
Mcl1	Myeloid cell leukemia sequence 1	1.12	0.010
—	Nclone10 mRNA	1.24	0.000
Necap2	NECAP endocytosis associated 2	1.14	0.008
Nedd9	Neural precursor cell expressed, developmentally down-regulated gene 9	1.17	0.002
Nrxn3	Neurexin 3	1.31	0.001
Ntrk2	Neurotrophic tyrosine kinase, receptor, type 2	1.51	0.000
Nfia	Nuclear factor I/A	1.20	0.004
Nfib	Nuclear factor I/B	1.15	0.010
Nfkbia	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha /// nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	1.23	0.002
Nr4a3	Nuclear receptor sub-family 4, group A, member 3	1.28	0.003
Nr4a3	Nuclear receptor sub-family 4, group A, member 3 /// nuclear receptor sub-family 4, group A, member 3	1.56	0.000
Numb	Numb gene homolog (*Drosophila*)	1.18	0.002
Olig1	Oligodendrocyte transcription factor 1	1.16	0.009
Por	P450 (cytochrome) oxidoreductase /// P450 (cytochrome) oxidoreductase	1.11	0.010
Pnlip	Pancreatic lipase /// pancreatic lipase	1.18	0.001
Prdx6	Peroxiredoxin 6	1.15	0.005
Ppan	Peter pan homolog (*Drosophila*)	1.10	0.006
Pik4ca	Phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	1.13	0.009
Pitpnm1	Phosphatidylinositol transfer protein, membrane-associated 1	1.13	0.008
Pea15	Phosphoprotein enriched in astrocytes 15	1.13	0.004
Pttg1ip	Pituitary tumor-transforming 1 interacting protein	1.11	0.006
Pllp	Plasma membrane proteolipid	1.13	0.007
Plekhc1	Pleckstrin homology domain containing, family C (with FERM domain) member 1	1.16	0.004
Plag1	Pleiomorphic adenoma gene 1	1.19	0.003
Pnkp	Polynucleotide kinase 3′-phosphatase	1.14	0.001
Kcnn2	Potassium intermediate/small conductance calcium-activated channel, sub-family N, member 2 /// potassium intermediate/small conductance calcium-activated channel, sub-family N, member 2	1.17	0.007
Kcnh3	Potassium voltage-gated channel, sub-family H (eag-related), member 3	1.13	0.008
Kcnd3	Potassium voltage-gated channel, Shal-related family, member 3	1.16	0.007
Pias4	Protein inhibitor of activated STAT, 4	1.13	0.003

Table 3 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Prkwnk1	Protein kinase, lysine deficient 1	1.21	0.008
Plp	Proteolipid protein	1.14	0.009
Ua20	Putative UA20 protein	1.14	0.007
Qscn6	Quiescin Q6	1.13	0.004
Rab34	RAB34, member of RAS oncogene family	1.13	0.009
Rad23a	RAD23a homolog (*S. cerevisiae*)	1.14	0.009
Rassf4	Ras association (RalGDS/AF-6) domain family 4	1.15	0.010
Rgc32	Response gene to complement 32	1.23	0.000
Rpe65	Retinal pigment epithelium 65	1.14	0.005
Rpl10a	Ribosomal protein L10A	1.18	0.004
Rpl28	Ribosomal protein L28	1.14	0.002
Rpl29	Ribosomal protein L29	1.15	0.004
Rpl32	Ribosomal protein L32	1.19	0.001
Rps15	Ribosomal protein S15	1.20	0.003
Rps5	Ribosomal protein S5	1.13	0.005
Rnf167	Ring finger protein 167	1.11	0.004
—	RM2 mRNA, partial sequence	1.47	0.001
S100a1	S100 calcium binding protein A1	1.10	0.006
S100b	S100 protein, beta polypeptide	1.14	0.008
Scrg1	Scrapie responsive gene 1	1.18	0.002
Sepw1	Selenoprotein W, muscle 1	1.13	0.006
Sgk	Serum/glucocorticoid regulated kinase	1.29	0.006
Sh3glb1	SH3-domain GRB2-like B1 (endophilin)	1.16	0.008
Sirt2	Sirtuin (silent mating type information regulation 2 homolog) 2 (*S. cerevisiae*)	1.16	0.002
Slc23a2	Solute carrier family 23 (nucleobase transporters), member 2	1.22	0.001
Spata6	Spermatogenesis associated 6	1.11	0.008
Sc5d	Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (*S. cerevisiae*) /// sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (*S. cerevisiae*)	1.19	0.001
Srebf1	Sterol regulatory element binding factor 1 /// sterol regulatory element binding factor 1	1.14	0.003
Strn3	Striatin, calmodulin binding protein 3	1.13	0.008
Sv2a	Synaptic vesicle glycoprotein 2a	1.18	0.003
Stx5a	Syntaxin 5a	1.10	0.008
Snta1	Syntrophin, acidic 1	1.12	0.007
Tacr3	Tachykinin receptor 3	1.13	0.005
Tbkbp1	TBK1 binding protein 1	1.23	0.001
Tspan2	Tetraspanin 2	1.12	0.006
Thap7	THAP domain containing 7	1.19	0.000
Tst	Thiosulfate sulfurtransferase	1.20	0.001
Tmed3	Transmembrane emp24 domain containing 3 /// transmembrane emp24 domain containing 3	1.15	0.004
Uba52	Ubiquitin A-52 residue ribosomal protein fusion product 1	1.19	0.002
Unc13c	Unc-13 homolog C (*C. elegans*)	1.19	0.005
Ets1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	1.15	0.007
Vat1	Vesicle amine transport protein 1 homolog (T californica)	1.18	0.002
Zfp335	Zinc finger protein 335	1.13	0.006

they do not persist for 24 h, and (b) measuring the whole AMYG may mask changes occurring within distinct amygdaloid nuclei. It is also possible in the AMYG, and to a lesser extent in the ACB, only small changes in mRNA may be needed to maintain larger changes in protein levels that may have

developed with chronic drinking. Therefore, many changes may have occurred in the AMYG and ACB that are not detected with microarray analyses, but may be detected with sensitive proteomics methods.

Common differences in the EtOH group compared to both the SAC and water groups could indicate differences in the CNS reinforcing effects of EtOH, the chronic general pharmacological actions of EtOH, and conditioning factors associated with the operant EtOH sessions. In the ACB, there were 73 genes that were significantly different in the EtOH group versus both the water and SAC groups (Table 5). GO analysis indicated two general overlapping categories in the contrasts of EtOH versus water and EtOH versus SAC (Table 4), i.e., synaptic transmission and homeostasis/transport. Seven of the 11 genes that were changed in the same direction in the ACB had higher expression in the EtOH group (Table 5), suggesting increased transmission at certain synapses in the ACB. In contrast, the lower expression of *Gad1* and *Gabrb2* may indicate reduced transmission at certain GABA-A receptors. If reduced transmission is occurring at certain GABA synapses and increased transmission is occurring at non-inhibitory synapses, the net results could indicate increased excitatory synaptic function within the ACB of the EtOH group. In addition, 5 of the 7 genes in common between the EtOH and both the other two groups in the homeostasis/transport category had higher expression in the EtOH group (Table 5), suggesting that the ACB may have reached a different homeostatic state as a result of chronic EtOH self-administration.

Ingenuity® analysis indicated a network of genes, involved in intracellular signaling pathways (e.g., *Prkca*, *Gnaq*, *Prkacb*),

Table 4
Significant GO categories for EtOH versus water and EtOH versus SAC comparisons

Term	*p*-value	No. of significant genes	Total genes
I. EtOH versus water significant categories			
Anion transport	0.04	5	65
Calcium ion transport	0.02	6	72
Chemical homeostasis	0.01	10	151
Synaptic transmission	0.02	15	288
II. EtOH versus SAC significant categories			
Calcium ion homeostasis	0.01	9	92
Cell ion homeostasis	0.00	17	132
Cell maturation	0.01	6	50
Chemical homeostasis	0.00	19	178
Endocytosis	0.02	5	47
Ensheathment of neurons	0.00	7	33
Forebrain development	0.00	7	35
Membrane organization and biogenesis	0.02	9	116
Myelination	0.00	5	27
Negative regulation of transcription from RNA polymerase II promoter	0.04	6	73
Neurogenesis	0.05	15	265
Neurological process	0.00	24	272
Nucleocytoplasmic transport	0.05	5	56
Potassium ion transport	0.02	7	80
Synaptic transmission	0.00	17	233

Table 5
Genes that were significantly different and changed in the same direction in the nucleus accumbens of iP rats for the ethanol group versus both the saccharin and water groups

Symbol	Gene description	Higher (+) or lower (−) with EtOH	GO category
Pdpk1	3-phosphoinositide dependent protein kinase-1	−	
Adar	Adenosine deaminase, RNA-specific	−	
Alb	Albumin	+	h/t
Atrx	Alpha thalassemia/mental retardation syndrome X-linked homolog (human)	−	
Appbp2	Amyloid beta precursor protein (cytoplasmic tail) binding protein 2	−	
Atxn3	Ataxin 3	−	
Atp2b4	ATPase, Ca++ transporting, plasma membrane 4	−	h/t
Abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	+	
Blnk	B-cell linker	−	
B2m	Beta-2 microglobulin	+	
Cdh11	Cadherin 11	+	
Cacnb4	Calcium channel, voltage-dependent, beta 4 subunit	−	
Camk4	Calcium/calmodulin-dependent protein kinase IV	−	st
Csnk1e	Casein kinase 1, epsilon	−	
Cflar	CASP8 and FADD-like apoptosis regulator	+	
Cav2	Caveolin 2	+	st
Cd99	CD99 antigen	+	
Clcn3	Chloride channel 3	+	h/t
Ctdsp1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	+	
Ccnh	Cyclin H	−	
P22k15	Cystatin related protein 2	+	
Emcn	Endomucin	+	
Ftl1	Ferritin light chain 1	+	h/t
Gabrb2	Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 2	−	st
Gad1	Glutamic acid decarboxylase 1	−	st
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble)	+	
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide	−	
Homer1	Homer homolog 1 (*Drosophila*)	+	st
Hyal3	Hyaluronoglucosaminidase 3	+	
Kifc3	Kinesin family member C3	−	
Klf15	Kruppel-like factor 15	+	
Map1b	Microtubule-associated protein 1b	−	
Mag	Myelin-associated glycoprotein	+	
Mcl1	Myeloid cell leukemia sequence 1	+	
Mllt10	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 10	−	
—	Nclone10 mRNA	+	
Nedd4a	Neural precursor cell expressed, developmentally down-regulated gene 4A	−	
Nrxn3	Neurexin 3	+	st
Nfia	Nuclear factor I/A	+	
Nfib	Nuclear factor I/B	+	

Table 5 (*continued*)

Symbol	Gene description	Higher (+) or lower (−) with EtOH	GO category
Nfkbia	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	+	
2610020o08rik	Nuclear NF-kappaB activating protein	−	
Npap60	Nuclear pore associated protein	−	
P34	p34 protein	−	
Prdx6	Peroxiredoxin 6	+	
Pik4ca	Phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	+	st
Plag1	Pleiomorphic adenoma gene 1	+	
Prkca	Protein kinase C, alpha	−	h/t
Prkacb	Protein kinase, cAMP dependent, catalytic, beta	−	
Prkwnk1	Protein kinase, lysine deficient 1	+	
Plp	Proteolipid protein	+	st
Ua20	Putative UA20 protein	+	
Ramp3	Receptor (calcitonin) activity modifying protein 3	−	
Rgc32	Response gene to complement 32	+	
Rpe65	Retinal pigment epithelium 65	+	
S100b	S100 protein, beta polypeptide	+	st, h/t
Scamp1	Secretory carrier membrane protein 1	+	
Sepw1	Selenoprotein W, muscle 1	+	
Styxl1	Serine/threonine/tyrosine interacting-like 1	−	
Sgtb	Small glutamine-rich tetratricopeptide repeat (TPR)-containing, beta	−	
Slc2a3	Solute carrier family 2 (facilitated glucose transporter), member 3	−	
Slc23a2	Solute carrier family 23 (nucleobase transporters), member 2	+	
Sc5d	Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (*S. cerevisiae*)	+	
Stch	Stress 70 protein chaperone, microsome-associated, 60kD human homolog	−	
Sv2a	Synaptic vesicle glycoprotein 2a	+	st, h/t
Syt6	Synaptotagmin VI	−	st
Tbkbp1	TBK1 binding protein 1	+	
Thap7	THAP domain containing 7	+	
Txndc13	Thioredoxin domain containing 13	−	
Ube4a	Ubiquitination factor E4A, UFD2 homolog (*S. cerevisiae*)	−	
Vti1a	Vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	−	
Ets1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	+	
Zfp483	Zinc finger protein 483	−	

Abbreviation: st = synaptic transmission; h/t = homeostasis/transport.

that mainly had reduced expression in the EtOH group compared to the other groups (Fig. 2). These results could suggest that chronic EtOH may be reducing general cellular functions, some of which are calcium-dependent. In contrast, other genes involved in pro-inflammatory responses (e.g., *Cflar*, *Mcl1*) and histone regulation (e.g., *Thap7*, *Est1*) appear mainly to have higher expression in the ACB of the EtOH group (Fig. 2). Overall, these results suggest that chronic EtOH self-

Table 6
Genes that were different in the amygdala of iP rats between the Ethanol, Saccharin and Water groups at $p<0.01$ (FDR > 0.5)

Gene symbol	Name	Fold change	Limma *p*-value
I. Saccharin versus water (FDR = 1.0)			
Adcy3	Adenylate cyclase 3	−1.13	0.008
Anxa4	Annexin A4	−1.17	0.004
Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	−1.20	0.009
Atg7	Autophagy-related 7 (yeast)	−1.12	0.006
Dusp1	Dual specificity phosphatase 1	−1.32	0.000
Dusp1	Dual specificity phosphatase 1	−1.23	0.005
Dusp5	Dual specificity phosphatase 5	−1.23	0.009
Dusp9	Dual specificity phosphatase 9	−1.18	0.005
Ephb6	Eph receptor B6	−1.20	0.009
Foxp1	Forkhead box P1	−1.20	0.005
Hs3st2	Heparan sulfate (glucosamine) 3-*O*-sulfotransferase 2	−1.21	0.001
Hpca	Hippocalcin	−1.26	0.002
Homer1	Homer homolog 1 (*Drosophila*)	−1.97	0.001
Klf10	Kruppel-like factor 10 /// Kruppel-like factor 10	−1.28	0.000
Lgr4	Leucine-rich repeat-containing G-protein coupled receptor 4	−1.16	0.002
Map1lc3b	Microtubule-associated protein 1 light chain 3 beta	−1.28	0.000
Nr4a3	Nuclear receptor sub-family 4, group A, member 3 /// nuclear receptor sub-family 4, group A, member 3	−1.39	0.003
Pvalb	Parvalbumin	−1.18	0.003
Kcnab1	Potassium voltage-gated channel, shaker-related sub-family, beta member 1	−1.19	0.007
Kcnab1	Potassium voltage-gated channel, shaker-related sub-family, beta member 1	−1.25	0.006
Prkcb1	Protein kinase C, beta 1	−1.14	0.006
Pprf18	PRP18 pre-mRNA processing factor 18 homolog (yeast)	−1.16	0.003
Ramp3	Receptor (calcitonin) activity modifying protein 3	−1.29	0.000
—	RM2 mRNA, partial sequence	−1.21	0.010
Slit2	Slit homolog 2 (*Drosophila*)	−1.28	0.003
Trpv6	Transient receptor potential cation channel, sub-family V, member 6	−1.36	0.001
Zfand2a	Zinc finger, AN1-type domain 2A /// zinc finger, AN1-type domain 2A	−1.12	0.009
A2m	Alpha-2-macroglobulin /// alpha-2-macroglobulin	1.26	0.004
Cd44	CD44 antigen	1.21	0.004
Ceacam1	CEA-related cell adhesion molecule 1	1.17	0.006
Cybrd1	Cytochrome *b* reductase 1 /// cytochrome *b* reductase 1	1.20	0.006
Dspp	Dentin sialophosphoprotein	1.13	0.008
Dpp6	Dipeptidylpeptidase 6	1.17	0.005
Doc2g	Double C2, gamma	1.14	0.007
DLP2	Dynein-like protein 2	1.15	0.007
Fcgr2b	Fc receptor, IgG, low affinity IIb	1.29	0.004
Gja4	Gap junction membrane channel protein alpha 4	1.14	0.006
Gipr	Gastric inhibitory polypeptide receptor	1.14	0.007
Igh-1a	Immunoglobulin heavy chain 1a (serum IgG2a)	1.94	0.008
Irf3	Interferon regulatory factor 3	1.12	0.010
Kazald1	Kazal-type serine peptidase inhibitor domain 1	1.14	0.008
LMO7	LIM domain only protein 7	1.16	0.007
Phactr2	Phosphatase and actin regulator 2	1.23	0.001
Pik4cb	Phosphatidylinositol 4-kinase, catalytic, beta polypeptide	1.14	0.010

(continued on next page)

Table 6 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Arhgdib	Rho, GDP dissociation inhibitor (GDI) beta	1.13	0.009
RT1-Bb	RT1 class II, locus Bb	1.25	0.010
Srpk3	Serine/arginine-rich protein specific kinase 3	1.32	0.000
Smyd2	SET and MYND domain containing 2	1.14	0.007
Stx4a	Syntaxin 4A (placental)	1.16	0.009
Vwf	von Willebrand factor /// von Willebrand factor	1.24	0.007
II. Ethanol versus water (FDR = 1.0)			
Bfar	Bifunctional apoptosis regulator	−1.24	0.007
Cast	Calpastatin	−1.16	0.004
Eif4g2	Eukaryotic translation initiation factor 4 gamma, 2	−1.22	0.003
Gabbr1	Gamma-aminobutyric acid (GABA) B receptor 1	−1.33	0.005
Homer2	Homer homolog 2 (*Drosophila*)	−1.16	0.008
Igf2r	Insulin-like growth factor 2 receptor /// insulin-like growth factor 2 receptor	−1.19	0.005
Il1rap	Interleukin 1 receptor accessory protein	−1.15	0.009
Rab27a	RAB27A, member RAS oncogene family	−1.23	0.007
Slc30a7	Solute carrier family 30 (zinc transporter), member 7	−1.18	0.009
Tef	Thyrotroph embryonic factor	−1.20	0.006
Tgfbr1	Transforming growth factor, beta receptor 1 /// transforming growth factor, beta receptor 1	−1.18	0.004
Acvr1	Activin A receptor, type 1	1.14	0.009
Aldh5a1	Aldehyde dehydrogenase family 5, sub-family A1	1.19	0.004
Amt	Aminomethyltransferase (glycine cleavage system protein T) /// aminomethyltransferase (glycine cleavage system protein T)	1.14	0.009
Acly	ATP citrate lyase /// ATP citrate lyase	1.16	0.005
Bhlhb2	Basic helix–loop–helix domain containing, class B2	1.25	0.006
Dtnbp1	Distrobrevin binding protein 1	1.16	0.007
Ifngr	Interferon gamma receptor 1	1.14	0.005
Lpxn	Leupaxin	1.13	0.007
Rpo1-4	RNA polymerase 1–4	1.16	0.005
Serpinc1	Serine (or cysteine) peptidase inhibitor, clade C (antithrombin), member 1	1.17	0.009
Stom	Stomatin	1.21	0.008
Ttc23	Tetratricopeptide repeat domain 23	1.18	0.004
III. Ethanol versus saccharin (FDR = 0.5–0.8)			
A2m	Alpha-2-macroglobulin /// alpha-2-macroglobulin	−1.23	0.008
Atp5i	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e /// ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e	−1.19	0.002
Bckdha	Branched chain ketoacid dehydrogenase E1, alpha polypeptide	−1.17	0.007
Cacna1c	Calcium channel, voltage-dependent, L type, alpha 1C subunit	−1.20	0.003
Camk2d	Calcium/calmodulin-dependent protein kinase II, delta	−1.13	0.008
Ceacam1	CEA-related cell adhesion molecule 1	−1.19	0.004
Cops3	COP9 (constitutive photomorphogenic) homolog, subunit 3 (Arabidopsis thaliana)	−1.14	0.008
Ckap5	Cytoskeleton associated protein 5	−1.15	0.009
Dgki	Diacylglycerol kinase, iota	−1.19	0.006
Dscr1l1	Down syndrome critical region gene 1-like 1	−1.25	0.005
Fmo2	Flavin containing monooxygenase 2	−1.21	0.007
Gspt1	G1 to S phase transition 1	−1.15	0.001
Gipr	Gastric inhibitory polypeptide receptor	−1.15	0.003

Table 6 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Glt8d1	Glycosyltransferase 8 domain containing 1	−1.17	0.002
Hcr	HCR (a-helix coiled-coil rod homolog)	−1.17	0.006
Igh-1a	Immunoglobulin heavy chain 1a (serum IgG2a)	−1.92	0.009
Maea	Macrophage erythroblast attacher	−1.18	0.005
Hnt	Neurotrimin	−1.21	0.009
Ntrk1	Neurotrophic tyrosine kinase, receptor, type 1	−1.17	0.004
Pctp	Phosphatidylcholine transfer protein	−1.14	0.003
Pabpn1	Poly(A) binding protein, nuclear 1	−1.16	0.003
Psmb8	Proteosome (prosome, macropain) subunit, beta type 8 /// proteosome (prosome, macropain) subunit, beta type 8	−1.33	0.002
Pycard	PYD and CARD domain containing	−1.17	0.007
Rasgrp4	RAS guanyl releasing protein 4	−1.14	0.009
Reep4	Receptor accessory protein 4	−1.17	0.004
Serinc3	Serine incorporator 3	−1.21	0.002
Vps54	Vacuolar protein sorting 54 (yeast)	−1.15	0.008
Wdr46	WD repeat domain 46	−1.20	0.006
Nt5c3l	5′-nucleotidase, cytosolic III-like	1.22	0.002
Acvr1	Activin A receptor, type 1	1.20	0.001
Adcy3	Adenylate cyclase 3	1.14	0.005
Acly	ATP citrate lyase /// ATP citrate lyase	1.14	0.008
B3gat2	Beta-1,3-glucuronyltransferase 2 (glucuronosyltransferase S)	1.14	0.006
Cacna2d3	Calcium channel, voltage-dependent, alpha 2/delta 3 subunit /// calcium channel, voltage-dependent, alpha 2/delta 3 subunit	1.13	0.009
Crem	cAMP responsive element modulator	1.15	0.001
Ckb	Creatine kinase, brain /// creatine kinase, brain	1.13	0.007
Dlgap2	Discs, large (*Drosophila*) homolog-associated protein 2	1.15	0.002
Dusp1	Dual specificity phosphatase 1	1.36	0.000
Dusp1	Dual specificity phosphatase 1	1.28	0.001
Egr2	Early growth response 2 /// early growth response 2	1.40	0.001
Fpgt	Fucose-1-phosphate guanylyltransferase	1.18	0.006
Gcnt2	Glucosaminyl (*N*-acetyl) transferase 2, I-branching enzyme	1.13	0.006
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble) /// glycerol-3-phosphate dehydrogenase 1 (soluble)	1.22	0.004
Hs3st2	Heparan sulfate (glucosamine) 3-*O*-sulfotransferase 2	1.27	0.000
Homer1	Homer homolog 1 (*Drosophila*)	2.19	0.000
Klf10	Kruppel-like factor 10 /// Kruppel-like factor 10	1.27	0.000
Masp1	Mannan-binding lectin serine peptidase 1	1.21	0.009
Nedd9	Neural precursor cell expressed, developmentally down-regulated gene 9	1.14	0.005
Nedd9	Neural precursor cell expressed, developmentally down-regulated gene 9	1.18	0.005
Nr4a3	Nuclear receptor sub-family 4, group A, member 3	1.30	0.007
Nr4a3	Nuclear receptor sub-family 4, group A, member 3 /// nuclear receptor sub-family 4, group A, member 3	1.56	0.000
Pvalb	Parvalbumin	1.26	0.000
Kcnab1	Potassium voltage-gated channel, shaker-related sub-family, beta member 1	1.26	0.005
Pnoc	Prepronociceptin	1.23	0.008
P2ry12	Purinergic receptor P2Y, G-protein coupled 12	1.12	0.010
Ramp3	Receptor (calcitonin) activity modifying protein 3	1.30	0.000

Table 6 (*continued*)

Gene symbol	Name	Fold change	Limma *p*-value
Rgs18	Regulator of G-protein signaling 18	1.35	0.003
Rnf138	Ring finger protein 138	1.14	0.001
—	RM2 mRNA, partial sequence	1.36	0.000
Slc33a1	Solute carrier family 33 (acetyl-CoA transporter), member 1	1.14	0.008
St3gal5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	1.16	0.002
Txnl4b	Thioredoxin-like 4B	1.15	0.007
Tle4	Transducin-like enhancer of split 4, E(spl) homolog (*Drosophila*)	1.14	0.009
Trpv6	Transient receptor potential cation channel, sub-family V, member 6	1.27	0.006
Tpbg	Trophoblast glycoprotein	1.30	0.006
Tsc22d3	TSC22 domain family 3 /// TSC22 domain family 3	1.15	0.002

administration may be producing effects on multiple intracellular systems that could alter cellular function and the response of these cells to environmental alterations.

In the ACB, the two main GO categories represented were synaptic transmission and homeostasis/transport for the EtOH group versus the other two groups. In the synaptic transmission category, *Homer1*, *Sv2a* and *Cav2* had higher expression levels in the EtOH group than in the SAC and water groups (Table 5). The Homer 1 genes are part of a family of synaptic scaffolding proteins that are involved in regulating the insertion of metabotropic glutamate (mGlu) receptors into the synaptic plasma membrane (Kammermeier, 2006; Tappe and Kuner, 2006). The protein for *Cav2* can also function as a scaffolding protein and interact with mGlu receptors (Burgueno et al., 2004), as well as other receptors, e.g., dopamine D1 (Yu et al., 2004) and muscarinic (Perez-Rosello et al., 2005) receptors. The synaptic vesicle glycoprotein 2a (*Sv2a*) is involved in regulating exocytosis (Xu and Bajjalieh, 2001; Crowder et al., 1999). Overall, these changes suggest that complex neuronal alterations may be occurring to increase neuronal function at certain synapses.

Expression of *Gpd1* was elevated in the ACB of the alcohol group in the present study (Table 5); similar findings were reported for *Gpd1* in the hippocampus of C57 mice exposed to EtOH in a vapor chamber (Daniels and Buck, 2002), although opposite effects were observed for *Gpd1* in the hippocampus of rats that had been on a forced liquid diet for several months (Saito et al., 2002). An increased expression of Kruppel-like factors (*Klf*), transcription factors possibly involved in controlling neuronal morphogenesis (Laub et al., 2005), was observed in the present study in the ACB (Table 5), and in the study of Daniels and Buck (2002). The increased expression of *Klf* might reflect alterations in neuronal structure.

Some of the changes observed with EtOH self-administration in the present study have also been reported for human alcoholics. Lewohl et al. (2000) examined differences in gene expression in the frontal cortex of human alcoholics and controls, and reported reduced expression of *Gabrb2* and microtubule-associated protein 4. In the present study (Table 5), lower expression levels of *Gabrb2* and *Map1b* were observed in the ACB of the alcohol group. Flatscher-Bader et al. (2005) reported reduced expression of synaptogamin 1 (involved in exocytosis) in the ACB of human alcoholics, whereas, in the present, lower expression levels of *Syt6* were observed in the ACB of the EtOH group (Table 5). The study of Lewohl et al. (2000) reported lower expression levels of genes for many myelin proteins in the frontal cortex of alcoholics. However, in the present study, lower expression levels of genes for myelin-associated proteins were not observed, suggesting that similar signs of neuronal damage were not evident in the ACB of the iP rats self-administering EtOH, as were found for human alcoholics (Lewohl et al., 2000).

Acute EtOH administration increased expression of *Klf15* and *Nfkbia* in the whole brain of C57 and DBA mice (Treadwell and Singh, 2004), a finding also observed in the ACB of the EtOH group in the present study (Table 5), suggesting that acute EtOH administration can increase expression of genes for transcription factors and that these effects persist with chronic

Table 7
Quantitative RT-PCR confirmation of differences observed in the nucleus accumbens between EtOH and SAC groups

Gene symbol	Gene name	Microarray fold change	qRT-PCR fold change	Microarray *p*-value	qRT-PCR *p*-value
Cacnb4	Calcium channel, voltage-dependent, beta 4 subunit	−1.31	−1.28	0.004	0.003
Camk4	Calcium/calmodulin-dependent protein kinase IV	−1.23	1.01	0.002	0.42
Cflar	CASP8 and FADD-like apoptosis regulator — intron	1.29	1.04	0.001	0.036
Cflar	CASP8 and FADD-like apoptosis regulator — exon	1.29	1.05	0.001	0.001
Gabrb2	GABA-A receptor, beta 2 subunit	−1.31	−1.05	0.004	0.069
Gnaq	Guanine nucleotide binding protein, alpha q polypeptide	−1.30	−1.04	0.001	0.063
Homer1	Homer homolog 1 (*Drosophila*) — exon	−1.15	−1.33	0.089	0.075
Homer1	Homer homolog 1 (*Drosophila*) — intron	3.49	2.52	0.001	0.001
Map1b	Microtubule-associated protein 1b	−1.37	−1.04	0.001	0.12
Nrxn3	Neurexin 3	1.31	−1.31	0.001	0.001
Pdpk1	3-phosphoinositide dependent protein kinase-1	−1.47	−1.15	0.002	0.007
Prkacb	Protein kinase, cAMP dependent, catalytic, beta	−1.29	−1.08	0.001	0.030

Negative values indicate that EtOH values are lower than SAC values; positive values indicate that EtOH values are higher than SAC values.

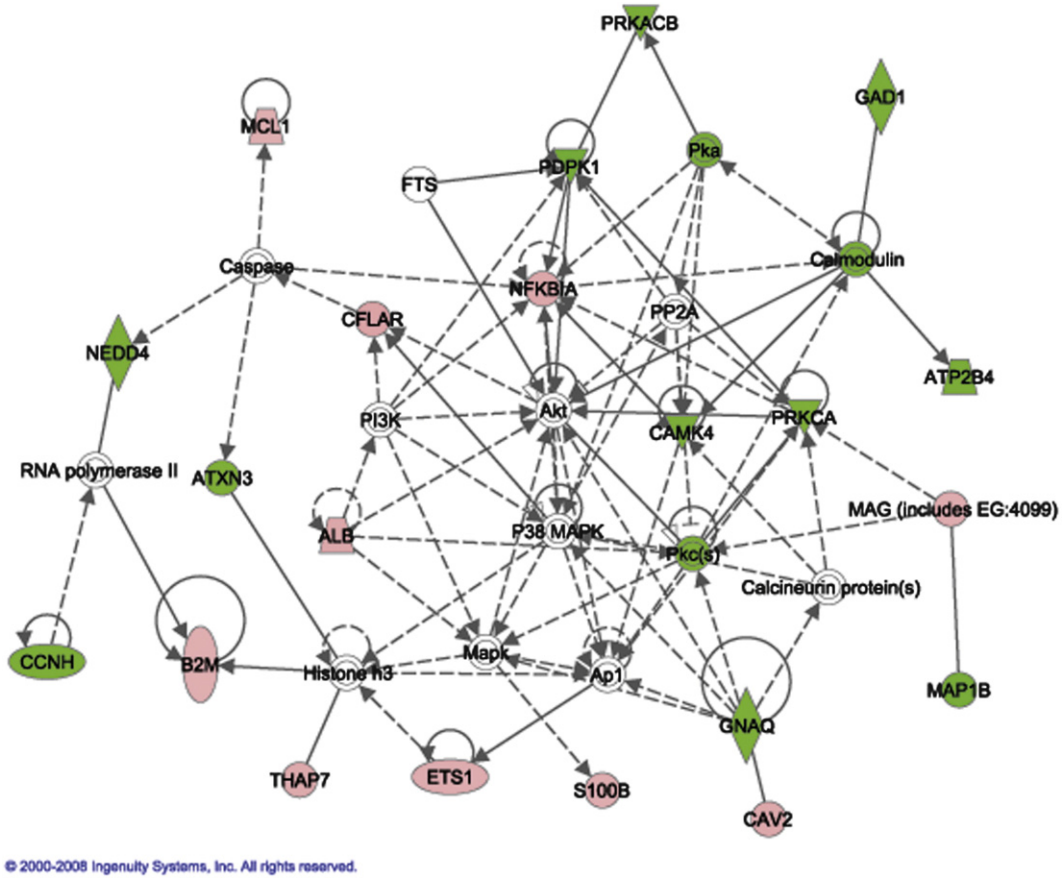

Fig. 2. *Ingenuity*® analysis showing co-citation and networks for genes that were significantly different between the ethanol group and the saccharin group. Green indicates genes that had reduced expression in the ethanol group, and red indicates genes that had higher expression in the ethanol group. Open symbols indicate that these genes were not statistically different between the ethanol group and the other two groups, but these genes were highly linked to multiple genes that were significantly changed. See Tables 2 and 3 for abbreviations of genes that changed significantly. Reduced expression of genes involved in intracellular signaling networks is depicted in green on the right hand part of the figure. Increased expression of genes involved in pro-inflammatory responses and histone regulation is shown in red on the left side. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

EtOH exposure. In contrast to the decreased expression of *Gabrb2* in the ACB of the chronic EtOH group (Table 5), acute EtOH administration increased *Gabrb1* gene expression in the ACB of mice (Kerns et al., 2005).

If there were innate differences in certain CNS regions that predispose certain individuals to high alcohol drinking behavior, then one hypothesis could be that expression of these genes is altered by EtOH. Kimpel et al. (2007) reported that there were innate differences in gene expression in 5 CNS regions, i.e., ACB, AMYG, frontal cortex, hippocampus, striatum, between the iP and iNP rats. Comparison of the expression of genes that changed in the ACB of the EtOH group versus the other 2 groups, with innate differences in gene expression between iP and iNP rats indicated a number of overlapping genes (summarized in Table 8). Sixteen named genes that differed between the iP and iNP rats also differed in the EtOH group versus both the SAC and water groups. A change in the opposite direction between innate and EtOH self-administration values might suggest that alcohol drinking is attempting to bring the expression of these genes toward a normal value. On the other hand, the expression of genes that changed in the same direction between the innate and EtOH self-administration studies might indicate that these genes are involved in vulnerability to high alcohol drinking and maintaining high alcohol drinking after it has begun. Genes that were changed in the same direction with alcohol drinking as were found between the iP versus the iNP rats (Table 8) included several genes coding for proteins involved in neurotransmission/synaptic function (e.g., *Gnaq*, *Syt6*, *Sv2a*, *Plp*). Compared to changes observed between iP and iNP rats (Kimpel et al., 2007), alcohol self-administration produced changes in the opposite direction for several of genes coding for proteins involved in synaptic transmission (e.g., *Homer1*, *Gabrb2*) or intracellular signaling (*Prkca*), suggesting that alcohol drinking may be attempting to re-establish 'normal' levels of the proteins produced by these genes.

In conclusion, the current study indicates that the ACB may be an important limbic structure regulating the reinforcing effects of EtOH in iP rats, and that changes in the expression of genes involved in synaptic transmission, homeostasis and intracellular signaling may contribute to this regulation. The study has some shortcomings, i.e., there may be a number of false positives in our analysis, and only a limited number of genes were confirmed. Future studies should be directed at

Table 8
Comparison of innate differences in gene expression between iP and iNP rats and effects of EtOH self-administration by iP rats on gene expression in the nucleus accumbens

Gene description	iP versus iNP	EtOH versus SAC and water
Proteolipid protein	Plp (+)	Plp (+)
Adenosine monophosphate deaminase/ adenosine deaminase	Ampd3 (+)	Adar (−)
3-phosphoglycerate dehydrogenase/glycerol-3-phosphate dehydrogenase	Phgdh (−)	Gdp1 (+)
Beta-2 microglobulin	B2m (−)	B2m (+)
ATPase, Ca++ transporting, plasma membrane	Atp2a2 (−)	Atp2b4 (−)
Guanine nucleotide binding protein alpha	Gnao (−)	Gnaq (−)
Homer homolog 1, 2 (*Drosophila*)	Homer2 (−)	Homer1 (+)
Microtubule-associated proteins tau, 1A/1B light chain 3, 1b	Mapt (−); Map1lc3b (+)	Map1b (−)
Casein kinase 1 delta/epsilon	Csnk1d (−)	Csnk1e (−)
Synaptogamin 6	Syt6 (−)	Syt6 (−)
Albumin	Alb (+)	Alb (+)
Ferritin heavy/light chain 1	Fth1 (+)	*Ftl1* (+)
Gamma-aminobutyric acid receptor subunit beta 1, 2	Gabrb1 (+)	Gabrb2 (−)
Response gene to complement 32	Rgc32 (+)	Rgc32 (+)
Synaptic vesicle glycoprotein 2b, 2a	Sv2b (+)	Sv2a (+)
Protein kinase C, alpha, delta, gamma	Prkcd (+) Prkcg (+)	Prkca (−)

Plus (+) symbol indicates higher expression in iP compared to iNP or higher expression in EtOH group versus SAC and Water groups; minus (−) symbol indicates the opposite.

analyzing more discrete sub-regions and nuclei within the ACB and AMYG at shorter time points after the operant sessions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbb.2008.01.023.

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