Pharmacology, Biochemistry and Behavior 94 (2009) 131–147

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Gene expression changes in the nucleus accumbens of alcohol-preferring rats following chronic ethanol consumption

Richard L. Bell [a,f,g,*], Mark W. Kimpel [a,f], Jeanette N. McClintick [b,e], Wendy N. Strother [a,f], Lucinda G. Carr [c], Tiebing Liang [c], Zachary A. Rodd [a,f,g], R. Dayne Mayfield [h], Howard J. Edenberg [b,d,e], William J. McBride [a,f]

[a] Department of Psychiatry, Indiana University Purdue University at Indianapolis, Indianapolis, IN 46202, USA
[b] Department of Biochemistry and Molecular Biology, Indiana University Purdue University at Indianapolis, Indianapolis, IN 46202, USA
[c] Department of Medicine, Indiana University Purdue University at Indianapolis, Indianapolis, IN 46202, USA
[d] Department of Medical and Molecular Genetics, Indiana University Purdue University at Indianapolis, Indianapolis, IN 46202, USA
[e] Center for Medical Genomics, Indiana University Purdue University at Indianapolis, Indianapolis, IN 46202, USA
[f] Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202-4887, USA
[g] Department of Psychology, Purdue School of Science, Indiana University Purdue University at Indianapolis, Indianapolis, IN 46202, USA
[h] Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, Austin, TX, 78712, USA

ARTICLE INFO

Article history:
Received 25 November 2008
Received in revised form 21 July 2009
Accepted 28 July 2009
Available online 8 August 2009

Keywords:
Alcohol-preferring rats
Nucleus accumbens
Gene expression
Microarrays
Alcohol drinking
Self-administration
Ethanol responsive genes
Ethanol withdrawal

ABSTRACT

The objective of this study was to determine the effects of binge-like alcohol drinking on gene expression changes in the nucleus accumbens (ACB) of alcohol-preferring (P) rats. Adult male P rats were given ethanol under multiple scheduled access (MSA; three 1-h dark cycle sessions/day) conditions for 8 weeks. For comparison purposes, a second ethanol drinking group was given continuous/daily alcohol access (CA; 24 h/day). A third group was ethanol-naïve (W group). Average ethanol intakes for the CA and MSA groups were approximately 9.5 and 6.5 g/kg/day, respectively. Fifteen hours after the last drinking episode, rats were euthanized, the brains extracted, and the ACB dissected. RNA was extracted and purified for microarray analysis. The only significant differences were between the CA and W groups ($p<0.01$; Storey false discovery rate = 0.15); there were 374 differences in named genes between these 2 groups. There were 20 significant Gene Ontology (GO) categories, which included negative regulation of protein kinase activity, anti-apoptosis, and regulation of G-protein coupled receptor signaling. Ingenuity® analysis indicated a network of transcription factors, involving oncogenes (*Fos*, *Jun*, *Junb* had higher expression in the ACB of the CA group), suggesting increased neuronal activity. There were 43 genes located within rat QTLs for alcohol consumption and preference; 4 of these genes (*Tgfa*, *Hspa5*, *Mtus1* and *Creb3l2*) are involved in anti-apoptosis and increased transcription, suggesting that they may be contributing to cellular protection and maintaining high alcohol intakes. Overall, these findings suggest that chronic CA drinking results in genomic changes that can be observed during the early acute phase of ethanol withdrawal. Conversely, chronic MSA drinking, with its associated protracted withdrawal periods, results in genomic changes that may be masked by tight regulation of these genes following repeated experiences of ethanol withdrawal.

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 in lines or strains of mice and rats with divergent responses to ethanol has provided important clues toward identifying genes and gene networks involved in vulnerability to high ethanol drinking behavior. Given this, examining changes in gene expression following chronic ethanol drinking will, presumably, provide information to identify genes and gene networks involved in maintaining this behavior, as well as the consequences of chronic ethanol exposure.

Many innate genetic expression differences between high and low ethanol-consuming rodent lines have been identified. For example, Edenberg et al. (2005) examined differences in gene expression in the hippocampus of inbred alcohol-preferring (iP) and inbred alcohol-non-preferring (iNP) rats, and reported differences for genes involved in cell growth and adhesion, cellular stress reduction and anti-oxidation, protein trafficking, cellular signaling pathways, and synaptic function. In a subsequent study, Kimpel et al. (2007) reported on innate differences in gene expression between iP and iNP rats in 5 CNS regions, including the nucleus accumbens (ACB).

* Corresponding author. Indiana University School of Medicine, Institute of Psychiatric Research, 791 Union Dr., Indianapolis, IN 46202-4887, USA. Tel.: +1 317 278 4629; fax: +1 317 274 1365.
E-mail address: ribell@iupui.edu (R.L. Bell).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.07.019

These authors indicated that genes associated with anti-apoptosis, axon guidance, nerve transmission as well as synaptic structure and function displayed expression differences between the rat strains. Worst et al. (2005) reported on the transcriptome analysis of the frontal cortex from ethanol-naïve AA (Alko, alcohol) and ANA (Alko, non-alcohol) rats, with mRNA level differences found that could reflect altered neurotransmitter release. Using a whole brain analysis of inbred long-sleep and inbred short-sleep mice, Xu et al. (2001) detailed expression differences for metabolic-associated genes with higher levels seen in the long-sleep mice. In a comprehensive transcriptome meta-analysis of gene expression differences across a number of different mouse strains, Mulligan et al. (2006) identified several *cis*-regulated candidate genes for an ethanol preference QTL on chromosome 9.

Alterations in gene expression produced by exposure to ethanol have been reported in a few studies. Acute ethanol injections (6 g/kg; i.p.) produced gene expression changes associated with cell signal regulation, gene regulation, and homeostasis/stress responses in the whole brain from C57BL/6 J and DBA/2 J, high- and low-ethanol drinking mice, respectively (Treadwell and Singh, 2004). Kerns et al. (2005) reported that acute i.p. ethanol injections altered the expression of genes involved in glucocorticoid signaling, neurogenesis, myelination, neuropeptide signaling, and retinoic acid signaling in the ACB, prefrontal cortex and VTA of C57BL/6 J and DBA/2 J mice. Differences in the expression levels of genes coding for oxido-reductases and ADP-ribosylation factors have also been found in the dorsal hippocampus of Lewis rats given 12% ethanol or water for 15 months (Saito et al., 2002). In a recent study, Bowers et al. (2006) reported that chronic ethanol consumption, in a liquid diet, altered the expression of over 100 genes in the cerebellum of PKCgamma wild-type and mutant mice. In contrast, Saito et al. (2004) in a previous study found no statistically significant effects of chronic free-choice ethanol drinking on gene expression in the striatum of C57BL/6By mice. The above studies were conducted using ethanol injections or 24-h free- or forced-choice drinking. A recent study from our laboratory (Rodd et al., 2008) reported that operant ethanol self-administration produced approximately 500 significant changes in gene expression in the ACB when measured 24 h after the last 1-h operant session, whereas saccharin self-administration produced less than 60 significant changes, suggesting that chronic ethanol consumption was producing persisting effects on gene expression in the ACB of P rats. However, it is important to determine if the effects of ethanol drinking alone (absence of operant responding) produce similar changes in gene expression in limbic regions that are involved in regulating ethanol drinking.

In an initial study from our laboratory, Bell et al. (2006a) examined protein expression changes in the ACB and amygdala of iP rats given 24-h continuous access (CA) or multiple scheduled access (MSA; four 1-h sessions during the dark cycle) to ethanol for 6 weeks. The results of this study indicated that ethanol drinking conditions differentially changed protein expression in the ACB and amygdala. However, a relatively insensitive 2-dimensional gel electrophoresis procedure was used in this study and only the most abundant proteins found in tissue from the whole ACB or amygdala could be detected. The microarray procedure offers a potentially more sensitive method to measure changes resulting from ethanol drinking under different conditions of availability, which produce different patterns of ethanol intake and associated blood alcohol levels (c.f., Bell et al., 2006a,b). Therefore, the objective of this study was to examine changes in gene expression associated with chronic ethanol drinking under binge-like ethanol drinking conditions. For comparison purposes, the effects of 24-h free-choice drinking on gene expression were also determined. Gene expression changes were determined the next day after the binge-like group's last scheduled access period of the previous day. Ethanol was removed from both groups at the same time to control for the length of ethanol deprivation before brain tissue was harvested. The hypothesis to be tested was that chronic binge-like ethanol drinking would produce significant persisting effects on gene expression in the ACB of P rats that would not be observed with 24-h continuous ethanol access drinking.

2. Method

2.1. Animals and ethanol drinking procedures

Subjects were adult (>90 days old), ethanol-naïve, male P rats from the S52 generation. The rats were single-housed in hanging stainless steel wire-mesh (bottom and front) cages on a reverse 12 h/12 h dark–light-cycle (light offset at 1000 h). Animals had ad libitum access to food. Rats were randomly divided into three groups ($n=9$/group): the 1st group had access to water as their sole fluid, the 2nd group had continuous/daily, concurrent, free-choice access to 15% and 30% (v/v) ethanol and water, and the 3rd group had bout-like, concurrent access to 15% and 30% ethanol, with water available ad libitum. The bout-like group experienced a multiple scheduled access (MSA) protocol, such that they received three 1-h access periods each separated by 2 h starting at the beginning of the dark cycle (i.e., 1000–1100, 1300–1400, and 1600–1700 h). The MSA animals were given ethanol access in 5-day blocks (Monday–Friday), with each block separated by 2 days without ethanol. Measurements of water and ethanol intake, and body weights were taken Monday through Friday at 0900 h; ethanol intakes, for MSA animals, were also taken at the end of each 1-h access period. After the MSA group's 1st day of re-exposure to ethanol access, of the 9th week, both groups of ethanol drinking rats had ethanol removed at 1700 h. To ensure that ethanol blood levels were absent and the deprivation period was equivalent for both groups, all rats were killed the next day (15 h after removing ethanol). Rats were killed by decapitation and their brains processed for microarray analyses, as described below.

2.2. Brain dissections

Rats were killed by decapitation within the same 2-h 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. During the 7th and 8th weeks, the rats in the MSA group dissected on the 2nd day had ethanol access moved to Tuesday through Saturday to preserve the 5-day a week schedule. 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 equipments used to obtain tissue were treated with RNAse Zap (Ambion, Inc. Austin, TX) to prevent RNA degradation. The ACB was 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). Dissected tissue was 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, as discussed previously (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 deemed excellent.

2.3. Microarray procedures

Separate preparations of total RNA were made from the ACB of individual animals. 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 were 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-h 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.

2.4. Statistical and neuroinformatic analyses of microarray data

Each GeneChip® was scanned using an Affymetrix Model 3000 scanner and underwent image analysis using Affymetrix GCOS software. Microarray data are available at the National Center for Biotechnology Information's Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/, under series accession no. GSE13524 [GSM341183…GSM341211] (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), with these further expanded by the authors using the R language. Expression data from the 27 arrays of the ACB region were normalized 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 $\log_2(1000)$. Because the primary objective was identifying genes that could be subjected to further bioinformatic analysis, all probesets currently annotated by Affymetrix as "expressed sequence tags" or whose gene names contained the words "riken", "predicted", or "similar to" were filtered out. Next, probe sets with a very low likelihood of actual expression in our samples were removed, with this accomplished by 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 as well. Linear modeling to calculate gene-wise p-values for the contrasts of the CA versus W group and the MSA versus W 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 0.15. An FDR of 0.15 was used as a cutoff because this allowed a significant number of genes to be included in the Gene Ontology (GO) and Ingenuity® Pathways Analysis to help identify networks of genes that changed. This FDR value is a reasonably stringent cutoff value that provides a good balance between allowing more thorough gene network analyses (limiting beta-error) without including too many false positives (limiting alpha-error) in the analyses. Thus, to facilitate discussion of the present results in the context of our laboratory's previously published work with microarray data (c.f., Edenberg et al., 2005; Kimpel et al., 2007; Rodd et al., 2008), we have used the same standard statistical procedures used previously, and by the field (Gentleman et al., 2004; Irizarry et al., 2003; Smyth, 2004), to determine the effects of the two different ethanol drinking conditions on changes in gene expression.

Testing for over-representation of Gene Ontology (GO) biologic process categories (Harris et al., 2004; Ashburner et al., 2000) 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. GO categories were called significant at $p<0.05$. In addition, network analyses were conducted with *Ingenuity® Pathway Analysis (Ingenuity® Systems, www.Ingenuity.com)*. Briefly, a data set containing gene identifiers and corresponding fold-changes was uploaded into the application. Each gene identifier was mapped to its corresponding gene object in the Ingenuity® Pathways Knowledge Base. An FDR cutoff of 0.15 was set to identify genes with expression levels that were significantly altered. These genes, called focus genes, were overlaid onto a global molecular network developed from information contained in the Ingenuity® Pathways Knowledge Base. Networks of these focus genes were then algorithmically generated based on their connectivity.

2.5. Quantitative Real-Time PCR

Real-Time PCR was carried out using SybrGreen chemistry and the ABI Prism 7300 Sequence Detection System (Applied Biosystems Inc. Foster City, CA). The amplification primers were designed using Vector NTI (Invitrogen, Carlsbad, CA). Total RNA, isolated for the microarray analyses, was treated with DNase I for these analyses. Following reverse transcription of the RNA (SuperScript™ III First-Strand Synthesis System for RT-PCR, Invitrogen, Carlsbad, CA), 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 7300 Sequence Detection System was performed using the standard curve method (Applied Biosystems, User Bulletin #2; http://www.appliedbiosystems.com). Quantitative RT-PCR (qRT-PCR) measurements were conducted on genes to verify differences observed with microarray hybridization. These genes were selected on the basis of significant differential expression, relatively large fold-changes, and the availability of primers.

3. Results

3.1. Ethanol drinking

Daily ethanol intakes (mean ± S.E.M.) of the CA group averaged 9.6 ± 0.9 g/kg/day across the 8 weeks, whereas daily ethanol intakes of the MSA group averaged 6.4 ± 0.3 g/kg/day, with hourly intakes ranging between 1.7 and 2.7 g/kg (Fig. 1, upper panel). Overall, the distributions of drinking scores for the two groups did not overlap (Fig. 1, lower panel: the boxplot depicts the means, interquartile ranges, and the most extreme scores). In the present study, the average daily intakes of the CA group and average hourly intakes of the MSA are higher than previously reported (Bell et al., 2006a), despite the fact that the MSA group had access to only three 1-h sessions compared with four 1-h access sessions in the Bell et al. (2006a) study. These differences may have been due to the use of selectively bred P rats in the present study versus inbred P rats used in the previous study and/or the use of male P rats in the present study versus the use of female inbred P rats in the previous study.

3.2. Effects of ethanol on gene expression in the ACB

Comparison of the CA versus W group indicated 406 probe sets of named genes were significantly ($p<0.01$; FDR ≤ 0.15) different between

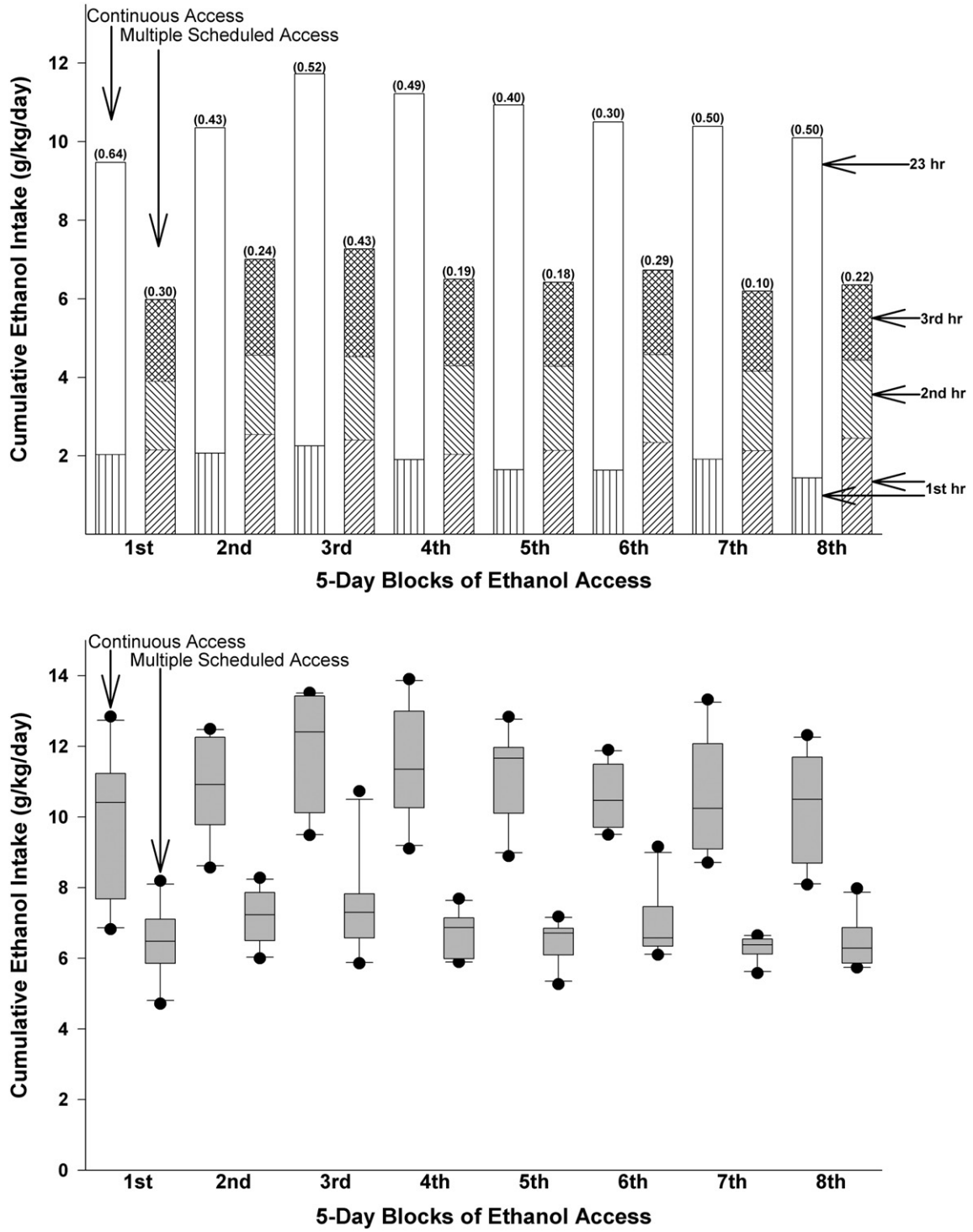

Fig. 1. The upper panel depicts the 5-day average ethanol intake values (±SEM of the total daily intake indicated in the parentheses) for the first and subsequent 23 hours of the continuous access (CA) P rats and the 5-day average ethanol intake values for the three 1-hour access periods of the multiple scheduled access (MSA) P rats. The box plot in the lower panel indicates that the distributions (CA versus MSA) of drinking scores, for the most part, did not overlap, which includes the most extreme scores. Note: the 5-day averages represent the days that ethanol (15% and 30% concurrent with water) was available to both CA and MSA P rats.

the two groups, with 233 probe sets higher and 173 probe sets lower in the CA group (Table 1). These 406 significantly different probe sets represented 374 uniquely named genes. Most of the gene expression differences (Table 1) were in the range of 1.1- to 1.3-fold.

There were 20 significant Gene Ontology (GO) Biological Processes categories with over-representation of gene differences between the CA and W groups (Table 2, which lists GO category name and GO identification number, as well as member gene names and symbols). Some of these GO categories included (a) 'anti-apoptosis' with a total of 10 gene expression differences, 5 higher (*Frag1*, *Hrpap20*, *Sod2*, *Tgfa*, *Zfp91*) and 5 lower (*Adam17*, *Foxo1a*, *Hspa5*, *Hdh*, *Tsc22d3*) in the CA group; (b) 'negative regulation of programmed cell death' with a total of 13 gene expression differences, 7 higher (*Btg2*, *Frag1*, *Hrpap20*, *Scg2*, *Sod2*, *Tgfa*, *zfp91*) and 6 lower (*Adam17*, *Angptl4*, *fox01a*, *Hspa5*, *Hdh*, *Tsc22d3*) in the CA group; (c) 'microtubule-based movement' with a total of 8 gene expression differences, 3 higher (*Actr10*, *Kif2*, *Tubb2b*)

and 5 lower (*Hdh*, *Kif11*, *Kif1b*, *Kif6*, *Klc1*) in the CA group; (d) 'regulation of kinase activity' with a total of 11 gene expression differences, with 7 higher (*Cdc42*, *Dusp6*, *Pkib*, *Spdy1*, *Spry2*, *Tgfa*) and 4 lower (*Cav*, *Dgka*, *Plce1*, Rgs3) in the CA group; (e) 'negative regulation of protein kinase activity' with a total of 5 gene expression differences, 3 higher (*Dusp6*, *Pkib*, *Spry2*) and 2 lower (*Cav*, *Rgs3*) in the CA group; (f) 'regulation of MAPK activity' with a total of 7 gene expression differences, 4 higher (*Chrna7*, *Dusp6*, *Spry2*, *Tgfa*) and 3 lower (*cav*, *Plce1*, *Rgs3*) in the CA group; (g) 'regulation of G-protein coupled receptor protein signaling pathway' with a total of 6 genes displaying decreased expression levels (*Ece1*, *Plce1*, *Rasgrp4*, *Ramp2*, *Rgs3*, *Rgs9*) in the CA group; and (h) 'anatomical structure formation' with a total of 11 gene expression differences, 7 higher (*Btg1*, *Ctgf*, *Nr4a3*, *Pofut1*, *Scg2*, *Scye1*, *Tgfa*) and 4 lower (*Angptl4*, *Tek*, *Hdh*, *Tie1*) in the CA group.

Among the named genes that were significantly different between the CA and W groups (Table 1), there were 43 genes located within rat QTLs for alcohol consumption and preference (Bice et al., 1998; Carr et al., 1998, 2003; Foroud et al., 2003). There were 11 genes located within Alc18 on chromosome 4, 6 genes located within Alc15 on chromosome 2, and 5 genes located within Alc8 on chromosome 3. Four or fewer genes were located within each of the following: Alc6 on chromosome 12, Alc11 on chromosome 16, Alc17 on chromosome 6, Alc5 on chromosome 10, Alc10 on chromosome 12, and Alc20 on chromosome 8 (Table 1).

Comparison of the MSA and W groups revealed 87 probe sets (representing 84 individual named genes) were significantly different at $p<0.01$. However, because this number is less than 1% of the identifiable genes on the Affymetrix GeneChip® (Rat Genome 230 2.0 array GeneChips) and the overall estimated FDR rate was greater than 0.90, these differences could have occurred by chance alone (data not shown). Of the 84 named genes, there were 35 differences in common with differences observed between the CA vs. W groups, some of which included *Arc*, *Cav2*, *Fos*, *Jun*, *Junb*, *Nos3*, *Pik3c3*, and *Plce1* (indicated in bold type in Table 1).

Comparison of the CA versus MSA group revealed 51 significant differences ($p<0.01$). However, the FDR-values for these findings were approximately 0.4, suggesting a high number of false positives. Therefore, this analysis indicated there were few genes with significant expression differences between the two alcohol drinking groups, even though there were a significant number of differences between the CA versus W group but not between the MSA versus W group.

3.3. RT-PCR confirmation

Eight genes from the list of significant genes in Table 1 were selected for RT-PCR. The criteria for selection were fold change (at least 20% difference), that the gene was neurobiologically interesting (e.g., Fos and Jun changes may be related to neuronal activity), and the availability of primers. The microarray results indicated that 6 of the 8 genes were up regulated in the alcohol drinking group and 2 of the genes were down regulated. RT-PCR confirmed the direction and magnitude of the changes observed with the microarray analysis between the CA and W control groups (Table 3).

3.4. Supplemental tables

See supplemental tables A and B for more complete information on data for gene expression differences in the ACB between the CA, MSA and W groups.

4. Discussion

The major finding of this study was that chronic, continuous/daily ethanol drinking under 24-h free-choice conditions (CA) altered the expression of over 370 uniquely named genes in the ACB of P rats, whereas intermittent ethanol drinking, using a multiple scheduled access protocol (MSA), with three 1-h sessions each day for 5 days per week did not produce a significant number of gene expression differences. These results do not support our hypothesis that binge-like alcohol drinking would produce significant changes in gene expression in the ACB of P rats. The disparity in findings could be due to the higher daily intakes of the CA group (~9.5 g/kg/day) versus the MSA group (~6.5 g/kg/day). However, the MSA group consumed their ethanol in distinct bouts of 1.7–2.7 g/kg/h with most of the intake expected to occur within the first 15 min of each access session (Bell et al., 2006b), mimicking binge-like drinking, with BACs approximating 80 mg% or greater (Bell et al., 2006b, 2008). The relative lack of effect of ethanol in the MSA group versus the W group suggests that gene expression in the ACB may be tightly regulated, such that, with intermittent ethanol exposure under a regimented protocol (i.e., with an inherently strong time-of-day conditioning component), the genetic machinery may adjust to ethanol-induced alterations (e.g., neuroadaptations) and be able to maintain new steady-state protein levels with basal levels of gene expression. This conclusion is supported by the finding that there were very few differences (approximately 50) between the 2 alcohol drinking groups. This suggests that the MSA procedure may be producing similar changes as the CA procedure but the effects of binge drinking may be much smaller compared with the effects of 24-h continuous access drinking. It is noteworthy that ~40% of the genes with significant differences in expression between the MSA and W groups were also similarly different between the CA and W groups. Therefore, increasing power, by increasing the number of animals in the MSA and W groups, in future studies may result in detecting a significant number of differences in gene expression between these two groups.

The lack of a significant number of differences in gene expression between the MSA and W groups of the present study, such that the number of gene expression differences (with *p*-values less than 0.01) was less than that expected by chance (i.e., less than 1% of the total number of genes), appears to disagree with a recent genomic study examining inbred P rats in a 1-h operant ethanol self-administration procedure (Rodd et al., 2008). These authors reported that inbred P rats responding for ethanol displayed a significant number (>200) of gene expression differences, in the ACB, compared to a water control group, when animals were killed the day after the last operant session. This lack of agreement suggests that multiple factors, other than temporal (i.e., time-of-day) conditioning and ethanol alone, are influencing the number of gene expression differences between the effects of MSA sessions of oral self-administration of ethanol per day and daily sessions of operant ethanol self-administration, compared with their respective controls. Some of these factors may include the role of Pavlovian and instrumental conditioning or lever pressing in the operant study (Rodd et al., 2008), as well as total ethanol consumed or expected peak BACs achieved and reduced conditioning to environmental cues found in the home-cage setting of the present study (i.e., animals were habituated to the wire-mesh home cages before ethanol was made available).

Although 24-h free-choice ethanol access is not regimented in the same manner as the MSA procedure, the same routine of body and fluid measurements are used each day and, based upon the results between the MSA and W groups, the expectation is that the genetic machinery of the CA group would also adjust to chronic ethanol drinking conditions, with a corresponding modest number of gene expression changes. The chronic ethanol drinking conditions experienced by the CA group should produce tolerance (Gatto et al., 1987; Lumeng and Li, 1986; Stewart et al., 1991) and possibly dependence (Kampov-Polevoy et al., 2000; Waller et al., 1982). Even though similar studies have yet to be conducted with P rats consuming ethanol under MSA conditions, it is anticipated that tolerance, and possibly dependence, would also develop in the MSA group, because BACs of 80 mg% or greater are expected during each ethanol access session, when using the present MSA protocol (Bell et al., 2006b, 2008; Murphy et al., 2002). Gene expression was measured 15 h after

Table 1
Significant effects of alcohol drinking under 24-h continuous access on changes in gene expression in the nucleus accumbens of P rats.

Symbol	Gene description	CA/W	FDR q	p-value	QTL	Chr	LOD
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	1.08	0.15	0.010			
Pfkfb2	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	1.11	0.11	0.002			
Acta1	Actin, alpha 1, skeletal muscle	1.12	0.12	0.004			
Actl6a	Actin-like 6A	1.10	0.12	0.003			
Abt1	Activator of basal transcription 1	1.07	0.12	0.005			
–	Activity and neurotransmitter-induced early gene 1 (ania-1) mRNA, 3'UTR	1.23	0.12	0.004			
Arc	**Activity regulated cytoskeletal-associated protein**	**1.53**	**0.01**	**0.000**			
Adamts1	A disintegrin-like and metallopeptidse (reprolysin type) with thrombospondin type 1 motif, 1	1.37	0.09	0.001			
Arf6	ADP-ribosylation factor 6	1.17	0.14	0.008			
Arl1	ADP-ribosylation factor-like 1	1.08	0.11	0.002			
Afg3l2	AFG3(ATPase family gene 3)-like 2 (yeast)	1.08	0.12	0.004			
Appbp2	Amyloid beta precursor protein (cytoplasmic tail) binding protein 2	1.17	0.13	0.006			
Apex1	Apurinic/apyrimidinic endonuclease 1	1.07	0.15	0.011			
Actr10	ARP10 actin-related protein 10 homolog (S. cerevisiae)	1.09	0.15	0.011			
Btg1	**B-cell translocation gene 1, anti-proliferative**	**1.10**	**0.14**	**0.008**			
Btg2	**B-cell translocation gene 2, anti-proliferative**	**1.35**	**0.02**	**0.000**			
Btg2	B-cell translocation gene 2, anti-proliferative	1.66	0.00	0.000			
St6gal2	Beta galactoside alpha 2,6 sialyltransferase 2	1.26	0.09	0.001			
Bex1	Brain expressed X-linked 1	1.09	0.13	0.006			
Bckdhb	Branched chain keto acid dehydrogenase E1, beta polypeptide	1.11	0.11	0.003			
C1galt1c1	C1GALT1-specific chaperone 1	1.08	0.15	0.011			
Cdh20	Cadherin 20	1.11	0.12	0.004			
Crcp	Calcitonin gene-related peptide-receptor component protein	1.07	0.14	0.008	Alc10	12	2.2
Cant1	Calcium activated nucleotidase 1	1.07	0.12	0.005			
Cacybp	Calcyclin binding protein	1.18	0.11	0.003			
Chst1	Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	1.13	0.15	0.011			
Cdv1	Carnitine deficiency-associated gene expressed in ventricle 1	1.18	0.15	0.009	Alc6	12	4.7
Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	1.13	0.14	0.008			
Cdc42	Cell division cycle 42 homolog (S. cerevisiae)	1.09	0.15	0.010			
Cep57	Centrosomal protein 57	1.11	0.15	0.010			
Chrna7	Cholinergic receptor, nicotinic, alpha polypeptide 7	1.11	0.15	0.010			
Cbx3	Chromobox homolog 3 (HP1 gamma homolog, Drosophila)	1.09	0.11	0.003	Alc18	4	9.2
Coq3	Coenzyme Q3 homolog, methyltransferase (yeast)	1.10	0.10	0.002			
Cirbp	Cold inducible RNA binding protein	1.16	0.14	0.008			
Commd10	COMM domain containing 10	1.16	0.13	0.006			
Commd3	COMM domain containing 3	1.06	0.12	0.004			
Ctgf	Connective tissue growth factor	1.24	0.09	0.001			
Crh	Corticotropin releasing hormone	1.24	0.12	0.004			
Ddx21a	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21a	1.16	0.09	0.001			
Dnd1	Dead end homolog 1 (zebrafish)	1.13	0.10	0.001			
Dhrs7b	Dehydrogenase/reductase (SDR family) member 7B	1.06	0.14	0.007			
Dlc1	Deleted in liver cancer 1	1.14	0.11	0.003	Alc11	16	3.2
Dnttip1	Deoxynucleotidyltransferase, terminal, interacting protein 1	1.09	0.13	0.006			
Dld	Dihydrolipoamide dehydrogenase	1.13	0.15	0.010			
Dcx	Doublecortin	1.20	0.14	0.007			
Dr1	Down-regulator of transcription 1	1.13	0.09	0.001			
Dph5	DPH5 homolog (S. cerevisiae)	1.11	0.14	0.007	Alc15	2	4.1
Dsm-1	**D-serine modulator-1**	**1.06**	**0.13**	**0.006**			
Dusp1	**Dual specificity phosphatase 1**	**1.42**	**0.00**	**0.000**	**Alc5**	**10**	**2.4**
Dusp1	**Dual specificity phosphatase 1**	**1.42**	**0.02**	**0.000**			
Dusp6	**Dual specificity phosphatase 6**	**1.18**	**0.07**	**0.000**			
Dusp6	**Dual specificity phosphatase 6**	**1.13**	**0.10**	**0.001**			
Dusp6	**Dual specificity phosphatase 6**	**1.13**	**0.14**	**0.008**			
Ep300	E1A binding protein p300	1.17	0.11	0.002			
Egln3	EGL nine homolog 3 (C. elegans)	1.10	0.15	0.011			
Efna3	Ephrin A3	1.13	0.10	0.002			
Eif2a	Eukaryotic translation initiation factor 2A	1.18	0.14	0.009			
Eif2s1	Eukaryotic translation initiation factor 2, subunit 1 alpha	1.12	0.10	0.002			
Nme1	Expressed in non-metastatic cells 1	1.05	0.15	0.011			
Fos	**FBJ murine osteosarcoma viral oncogene homolog**	**1.72**	**0.00**	**0.000**			
Frag1	FGF receptor activating protein 1	1.13	0.14	0.007			
Fkbp1b	FK506 binding protein 1b	1.14	0.09	0.001			
Galk2	Galactokinase 2	1.11	0.12	0.003			
Gtf2a2	General transcription factor IIa 2	1.08	0.15	0.009			
Gpm6a	Glycoprotein m6a	1.11	0.15	0.009	Alc11	16	3.2
Gpr85	G protein-coupled receptor 85	1.11	0.12	0.005			
Gdf11	Growth differentiation factor 11	1.09	0.12	0.004			
Grb2	Growth factor receptor bound protein 2	1.07	0.11	0.003			
Gng10	Guanine nucleotide binding protein (G protein), gamma 10	1.08	0.12	0.003			
Gucy1b3	Guanylate cyclase 1, soluble, beta 3	1.09	0.15	0.010			
Hes1	Hairy and enhancer of split 1 (Drosophila)	1.17	0.15	0.009			
Hsf2	Heat shock factor 2	1.13	0.10	0.001			
Havcr2	Hepatitis A virus cellular receptor 2	1.18	0.15	0.010			
Hnrpab	Heterogeneous nuclear ribonucleoprotein A/B	1.10	0.11	0.003			

Table 1 (*continued*)

Symbol	Gene description	CA/W	FDR q	p-value	QTL	Chr	LOD
Hnrpk	Heterogeneous nuclear ribonucleoprotein K	1.08	0.15	0.009			
Hdac2	Histone deacetylase 2	1.13	0.13	0.005			
Hdac5	Histone deacetylase 5	1.14	0.11	0.003			
Homer1	**Homer homolog 1 (Drosophila)**	**1.71**	**0.01**	**0.000**			
Hrpap20	Hormone-regulated proliferation associated protein 20	1.11	0.13	0.005			
Hagh	Hydroxyacyl glutathione hydrolase	1.09	0.14	0.007	Alc5	10	2.4
Hcn1	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1	1.33	0.11	0.003			
Ier2	Immediate early response 2	1.33	0.01	0.000			
Itga6	Integrin, alpha 6	1.11	0.11	0.003			
Itgb3bp	Integrin beta 3 binding protein (beta3-endonexin)	1.14	0.12	0.004			
Ift74	Intraflagellar transport 74 homolog (Chlamydomonas)	1.14	0.13	0.006			
Nyw1	Ischemia related factor NYW-1	1.15	0.07	0.000			
Idi1	**Isopentenyl-diphosphate delta isomerase**	**1.14**	**0.02**	**0.000**			
Icmt	Isoprenylcysteine carboxyl methyltransferase	1.07	0.13	0.006			
Junb	**Jun-B oncogene**	**1.27**	**0.00**	**0.000**			
Jun	**Jun oncogene**	**1.15**	**0.14**	**0.009**			
Jun	**Jun oncogene**	**1.23**	**0.01**	**0.000**			
Kab	KARP-1 binding protein 1	1.10	0.11	0.002			
Khdrbs2	KH domain containing, RNA binding, signal transduction associated 2	1.35	0.11	0.002			
Kif2	Kinesin heavy chain family, member 2	1.13	0.12	0.003			
Lactb2	Lactamase, beta 2	1.14	0.14	0.007			
Lactb2	Lactamase, beta 2	1.10	0.15	0.011			
Lap3	Leucine aminopeptidase 3	1.20	0.15	0.010			
Lrrc23	Leucine-rich repeat containing 23	1.15	0.13	0.006			
LOC246187	Liver regeneration-related protein	1.12	0.11	0.002			
Mgat2	Mannoside acetylglucosaminyltransferase 2	1.16	0.11	0.002			
Mkks	McKusick-Kaufman syndrome protein	1.07	0.12	0.004			
Med4	Mediator of RNA polymerase II transcription, subunit 4 homolog (yeast)	1.11	0.12	0.004			
Mtf2	Metal response element binding transcription factor 2	1.39	0.13	0.005			
Mtx2	Metaxin 2	1.16	0.10	0.001			
Mbd1	Methyl-CpG binding domain protein 1	1.12	0.14	0.008			
Mtfmt	Mitochondrial methionyl-tRNA formyltransferase	1.10	0.14	0.008			
Mcl1	Myeloid cell leukemia sequence 1	1.14	0.14	0.007			
Mx2	Myxovirus (influenza virus) resistance 2	1.21	0.04	0.000			
Ndufa9	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9	1.17	0.11	0.003			
Ndufv3l	NADH dehydrogenase (ubiquinone) flavoprotein 3-like	1.13	0.12	0.004			
LOC493574	Notch1-induced protein	1.24	0.11	0.003			
Nip7	Nuclear import 7 homolog (S. cerevisiae)	1.15	0.09	0.001			
Nrbf2	Nuclear receptor binding factor 2	1.15	0.09	0.001			
Nr4a1	**Nuclear receptor subfamily 4, group A, member 1**	**1.62**	**0.00**	**0.000**			
Nr4a3	**Nuclear receptor subfamily 4, group A, member 3**	**1.46**	**0.04**	**0.000**			
Nup155	**Nucleoporin 155**	**1.13**	**0.14**	**0.008**			
Nupl1	Nucleoporin like 1	1.15	0.11	0.003			
Nap1l2	Nucleosome assembly protein 1-like 2	1.25	0.14	0.007			
Nap1l3	Nucleosome assembly protein 1-like 3	1.17	0.10	0.001			
Numbl	Numb-like	1.16	0.15	0.010			
Orc6l	Origin recognition complex, subunit 6-like (S. cerevisiae)	1.20	0.04	0.000			
Orc6l	Origin recognition complex, subunit 6-like (S. cerevisiae)	1.22	0.12	0.004			
Pspc1	Paraspeckle protein 1	1.13	0.15	0.010			
Psip1	PC4 and SFRS1 interacting protein 1	1.39	0.08	0.000			
Ppig	Peptidylprolyl isomerase G	1.22	0.10	0.001			
Phf12	PHD finger protein 12	1.10	0.12	0.004			
Phf12	PHD finger protein 12	1.12	0.15	0.010			
Pigk	Phosphatidylinositol glycan, class K	1.10	0.14	0.008			
Pfkl	Phosphofructokinase, liver, B-type	1.11	0.15	0.010			
Pgam5	Phosphoglycerate mutase family member 5	1.10	0.11	0.003			
Pik3c3	**Phosphoinositide-3-kinase, class 3**	**1.07**	**0.13**	**0.005**			
Plaa	Phospholipase A2, activating protein	1.12	0.11	0.002			
Ppcs	Phosphopantothenoylcysteine synthetase	1.08	0.15	0.009			
Prps2	Phosphoribosyl pyrophosphate synthetase 2	1.22	0.09	0.001			
Prpsap2	Phosphoribosyl pyrophosphate synthetase-associated protein 2	1.11	0.10	0.001			
Psph	Phosphoserine phosphatase	1.13	0.13	0.005	Alc10	12	2.2
Plagl1	Pleiomorphic adenoma gene-like 1	1.14	0.14	0.008			
Pcyox1	Prenylcysteine oxidase 1	1.20	0.11	0.002	Alc18	4	9.2
Pnrc1	Proline rich 2	1.16	0.11	0.003			
Psmc6	Proteasome (prosome, macropain) 26 S subunit, ATPase, 6	1.28	0.09	0.001			
Psmc6	Proteasome (prosome, macropain) 26 S subunit, ATPase, 6	1.21	0.03	0.000			
Psmc6	Proteasome (prosome, macropain) 26 S subunit, ATPase, 6	1.20	0.09	0.001			
Psmd12	Proteasome (prosome, macropain) 26 S subunit, non-ATPase, 12	1.12	0.10	0.001			
Psmd6	Proteasome (prosome, macropain) 26 S subunit, non-ATPase, 6	1.07	0.12	0.003			
Psma4	Proteasome (prosome, macropain) subunit, alpha type 4	1.09	0.09	0.001			
Pkib	Protein kinase inhibitor beta, cAMP dependent, catalytic	1.23	0.11	0.003			
Pkib	Protein kinase inhibitor beta, cAMP dependent, catalytic	1.36	0.15	0.010			
Prkra	Protein kinase, interferon inducible double stranded RNA dependent activator	1.11	0.12	0.004			
Pofut1	Protein O-fucosyltransferase 1	1.13	0.15	0.010			
Ppp2ca	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	1.20	0.08	0.000			

(continued on next page)

Table 1 (*continued*)

Symbol	Gene description	CA/W	FDR q	p-value	QTL	Chr	LOD
Ppp2ca	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	1.10	0.04	0.000			
Pcdha1 /// Pcdha10 /// Pcdha11 /// Pcdha12 /// Pcdha13 /// Pcdha2 /// Pcdha3 /// Pcdha4 /// Pcdha5 /// Pcdha6 /// Pcdha7 /// Pcdha8 /// Pcdha9 /// Pcdhac1 /// Pcdhac2	protocadherin alpha 4 /// protocadherin alpha 13 /// protocadherin alpha 10 /// protocadherin alpha 12 /// protocadherin alpha 3 /// protocadherin alpha 8 /// protocadherin alpha 1 /// protocadherin alpha 2 /// protocadherin alpha 5 /// protocadherin alpha 6 /// protocadherin alpha 7 /// protocadherin alpha 9 /// protocadherin alpha subfamily C, 1 /// protocadherin alpha subfamily C, 2 /// protocadherin alpha 11	1.15	0.15	0.010			
P2ry2	Purinergic receptor P2Y, G-protein coupled 2	1.13	0.10	0.002			
LOC289809	Putatative 28 kDa protein	1.24	0.10	0.001			
Rab1	RAB1, member RAS oncogene family	1.10	0.14	0.007			
Rab21	RAB21, member RAS oncogene family	1.14	0.14	0.007			
Rab35	RAB35, member RAS oncogene family	1.14	0.13	0.006			
Rab3c	**RAB3C, member RAS oncogene family**	**1.11**	**0.14**	**0.007**			
Rad17	RAD17 homolog (S. pombe)	1.13	0.11	0.003			
Rdx	Radixin	1.12	0.12	0.003			
Rae1	RAE1 RNA export 1 homolog (S. pombe)	1.07	0.14	0.008			
Ripx	Rap2 interacting protein x	1.12	0.10	0.001			
Rdbp	RD RNA binding protein	1.09	0.10	0.001			
Rcn2	Reticulocalbin 2	1.14	0.10	0.002			
Rbbp6	Retinoblastoma binding protein 6	1.17	0.12	0.003			
Rnf149	Ring finger protein 149	1.15	0.14	0.007			
Rnf2	Ring finger protein 2	1.14	0.13	0.005			
–	RM2 mRNA, partial sequence	1.39	0.04	0.000			
Rbm13	RNA binding motif protein 13	1.13	0.14	0.007			
Rbm14	RNA binding motif protein 14	1.11	0.15	0.011			
Rbm17	RNA binding motif protein 17	1.08	0.14	0.007			
Rbm3	RNA binding motif protein 3	1.14	0.11	0.002			
Rg9mtd1	RNA (guanine-9-) methyltransferase domain containing 1	1.10	0.14	0.008			
Rpo1-1	RNA polymerase 1-1	1.08	0.15	0.011			
Rwdd3	RWD domain containing 3	1.18	0.14	0.008	Alc15	2	4.1
Rwdd3	RWD domain containing 3	1.17	0.14	0.009			
Sec23ip	SEC23 interacting protein	1.06	0.15	0.009			
Scg2	Secretogranin 2	1.12	0.13	0.006			
Siah1a	Seven in absentia 1A	1.13	0.14	0.007			
Siah2	Seven in absentia 2	1.13	0.12	0.004			
Srp54	Signal recognition particle 54	1.16	0.12	0.004			
Six3	Sine oculis homeobox homolog 3 (Drosophila)	1.19	0.13	0.006	Alc17	6	3.1
Ssbp1	Single-stranded DNA binding protein 1	1.16	0.09	0.001	Alc18	4	9.2
Ssbp1	Single-stranded DNA binding protein 1	1.15	0.09	0.001			
Scye1	**Small inducible cytokine subfamily E, member 1**	**1.08**	**0.15**	**0.011**	**Alc15**	**2**	**4.1**
Slc4a10	Solute carrier family 4, sodium bicarbonate transporter-like, member 10	1.15	0.14	0.007			
Slc6a15	Solute carrier family 6 (neurotransmitter transporter), member 15	1.10	0.14	0.009			
Snx16	Sorting nexin 16	1.13	0.13	0.006			
Snx16	Sorting nexin 16	1.13	0.11	0.003			
Spg3a	Spastic paraplegia 3A homolog (human)	1.12	0.11	0.003			
Spdy1	Speedy homolog 1 (Drosophila)	1.17	0.14	0.007	Alc17	6	3.1
Sf4	Splicing factor 4	1.09	0.14	0.009	Alc11	16	3.2
Spon1	Spondin 1	1.12	0.10	0.001			
Spry2	Sprouty homolog 2 (Drosophila)	1.10	0.15	0.011			
Sod2	Superoxide dismutase 2, mitochondrial	1.14	0.10	0.001			
Syncrip	Synaptotagmin binding, cytoplasmic RNA interacting protein	1.19	0.15	0.010			
Taf9l	TAF9-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31 kDa	1.21	0.11	0.002			
Tfpt	TCF3 (E2A) fusion partner	1.11	0.11	0.003			
Tcp11l2	t-complex 11 (mouse) like 2	1.11	0.14	0.007			
Tgif	TG interacting factor	1.21	0.02	0.000			
Txnl1	Thioredoxin-like 1	1.13	0.14	0.008			
Txnl2	Thioredoxin-like 2	1.08	0.15	0.010			
Tmsbl1	Thymosin beta-like protein 1	1.22	0.11	0.003			
Tceal8	Transcription elongation factor A (SII)-like 8	1.09	0.13	0.006			
Tceb3	Transcription elongation factor B (SIII), polypeptide 3	1.10	0.10	0.002			
Tgfa	Transforming growth factor alpha	1.13	0.14	0.008	Alc18	4	9.2
Tgfa	Transforming growth factor alpha	1.14	0.15	0.010			
Tomm20	Translocase of outer mitochondrial membrane 20 homolog (yeast)	1.07	0.12	0.005			
Tmem17	Transmembrane protein 17	1.10	0.12	0.003			
Tmeff1	Transmembrane protein with EGF-like and two follistatin-like domains 1	1.13	0.11	0.002			
Tpm3	**Tropomyosin 3, gamma**	**1.11**	**0.07**	**0.000**			
Tpm3	**Tropomyosin 3, gamma**	**1.15**	**0.15**	**0.010**			
Tubb2b	Tubulin, beta 2b	1.10	0.10	0.001			
Uchl5	Ubiquitin carboxyl-terminal hydrolase L5	1.11	0.04	0.000			
Ube2d2	Ubiquitin-conjugating enzyme E2D 2	1.17	0.14	0.007			
Ube2d3	Ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	1.07	0.14	0.007	Alc15	2	4.1
LOC500954	Ubiquitin-Like 5 Protein	1.12	0.09	0.001			
Ufc1	Ufm1-conjugating enzyme 1	1.07	0.13	0.005			

Table 1 (*continued*)

Symbol	Gene description	CA/W	FDR q	p-value	QTL	Chr	LOD
Vps33a	Vacuolar protein sorting 33A (yeast)	1.21	0.13	0.006	Alc6	12	4.7
Vkorc1l1	Vitamin K epoxide reductase complex, subunit 1-like 1	1.14	0.10	0.001			
Wdfy1	WD repeat and FYVE domain containing 1	1.11	0.11	0.003			
Wdr39	WD repeat domain 39	1.08	0.14	0.009			
Wdr77	WD repeat domain 77	1.18	0.11	0.002	Alc15	2	4.1
Wdr77	WD repeat domain 77	1.12	0.10	0.001			
Wrnip1	Werner helicase interacting protein 1	1.12	0.11	0.003			
Zcchc10	Zinc finger, CCHC domain containing 10	1.11	0.10	0.001			
Zcrb1	Zinc finger CCHC-type and RNA binding motif 1	1.20	0.10	0.001			
Zipro1	Zinc finger proliferation 1	1.14	0.12	0.004			
Zfp297	Zinc finger protein 297	1.09	0.15	0.010			
Zfp367	Zinc finger protein 367	1.18	0.12	0.004			
Zfp403	Zinc finger protein 403	1.15	0.14	0.009			
Zfp451	Zinc finger protein 451	1.10	0.15	0.010			
Zfp91	Zinc finger protein 91	1.13	0.09	0.001			
Htr4	5-hydroxytryptamine (serotonin) receptor 4	−1.14	0.11	0.002			
Nt5e	5' nucleotidase, ecto	−1.15	0.10	0.002			
Actn1	Actinin, alpha 1	−1.12	0.09	0.001			
Actn1	Actinin, alpha 1	−1.16	0.14	0.007			
Apeh	Acylpeptide hydrolase	−1.07	0.15	0.011			
Adam17	A disintegrin and metalloproteinase domain 17 (tumor necrosis factor, alpha, converting enzyme)	−1.08	0.15	0.010			
Akap8l	A kinase (PRKA) anchor protein 8-like	−1.12	0.15	0.010			
Aldh6a1	Aldehyde dehydrogenase family 6, subfamily A1	−1.13	0.10	0.001			
Amt	Aminomethyltransferase (glycine cleavage system protein T)	−1.10	0.12	0.004			
Angptl4	Angiopoietin-like 4	−1.24	0.01	0.000			
Armc5	Armadillo repeat containing 5	−1.11	0.10	0.002			
Aspa	Aspartoacylase	−1.17	0.13	0.005			
Atp6v0e1	ATPase, H + transporting, V0 subunit E isoform 1	−1.13	0.14	0.009	Alc5	10	2.4
Abcg2	ATP-binding cassette, subfamily G (WHITE), member 2	−1.14	0.13	0.006	Alc18	4	9.2
St6gal1	Beta galactoside alpha 2,6 sialyltransferase 1	−1.15	0.14	0.007			
Cast	Calpastatin	−1.10	0.15	0.010			
Cml3	Camello-like 3	−1.16	0.02	0.000	Alc18	4	9.2
Creb3l2	cAMP responsive element binding protein 3-like 2	−1.17	0.02	0.000	Alc18	4	9.2
Car11	Carbonic anhydrase 11	−1.10	0.11	0.002			
Cpt1c	Carnitine palmitoyltransferase 1c	−1.09	0.14	0.009			
Csnk1g1	Casein kinase 1, gamma 1	−1.13	0.12	0.005			
Catna1	Catenin (cadherin-associated protein), alpha 1	−1.08	0.14	0.009			
Cav	Caveolin	−1.18	0.13	0.005			
Cav2	**Caveolin 2**	**−1.19**	**0.11**	**0.002**			
Cd97	CD97 antigen	−1.22	0.08	0.000			
Cdc37l1	Cell division cycle 37 homolog (S. cerevisiae)-like 1	−1.07	0.14	0.007			
Ccr5	Chemokine (C-C motif) receptor 5	−1.11	0.12	0.004			
Cldn1	Claudin 1	−1.15	0.15	0.011			
Cldn5	Claudin 5	−1.16	0.11	0.002			
Creld2	Cysteine-rich with EGF-like domains 2	−1.10	0.12	0.004			
Cyp4f2	Cytochrome P450, family 4, subfamily F, polypeptide 2	−1.11	0.11	0.002			
Dock9	Dedicator of cytokinesis 9	−1.21	0.10	0.001			
Degs2	Degenerative spermatocyte homolog 2 (Drosophila), lipid desaturase	−1.16	0.07	0.000			
Dhrs3	Dehydrogenase/reductase (SDR family) member 3	−1.18	0.11	0.003			
Dsp	Desmoplakin	−1.15	0.10	0.002			
Dgka	Diacylglycerol kinase, alpha	−1.12	0.15	0.009			
Dlgap2	Discs, large (Drosophila) homolog-associated protein 2	−1.26	0.11	0.002			
Dlgh1	**Discs, large homolog 1 (Drosophila)**	**−1.09**	**0.09**	**0.001**			
Dscr1l1	**Down syndrome critical region gene 1-like 1**	**−1.18**	**0.05**	**0.000**			
Dscr1l1	**Down syndrome critical region gene 1-like 1**	**−1.19**	**0.11**	**0.003**			
Eltd1	EGF, latrophilin and seven transmembrane domain containing 1	−1.29	0.01	0.000			
Ehd2	EH-domain containing 2	−1.27	0.09	0.001			
Ecam	Endothelial cell adhesion molecule	−1.18	0.07	0.000	Alc20	8	2
Tek	Endothelial-specific receptor tyrosine kinase	−1.13	0.15	0.009			
Ece1	Endothelin converting enzyme 1	−1.10	0.13	0.005			
Epim	Epimorphin	−1.14	0.14	0.008	Alc10	12	2.2
Epn2	Epsin 2	−1.09	0.14	0.009			
Eef2k	Eukaryotic elongation factor-2 kinase	−1.11	0.14	0.007			
Ecm1	Extracellular matrix protein 1	−1.19	0.13	0.005			
Fbxl20	F-box and leucine-rich repeat protein 20	−1.09	0.15	0.010			
Fkbp5	**FK506 binding protein 5**	**−1.14**	**0.10**	**0.001**			
Fkbp5	**FK506 binding protein 5**	**−1.18**	**0.07**	**0.000**			
Fmo2	Flavin containing monooxygenase 2	−1.25	0.12	0.003			
Foxo1a	Forkhead box O1A	−1.15	0.13	0.006			
Fut2	Fucosyltransferase 2 (secretor status included)	−1.15	0.13	0.005			
Galt	Galactose-1-phosphate uridyl transferase	−1.08	0.11	0.003			
Gja1	Gap junction membrane channel protein alpha 1	−1.11	0.15	0.010			
Gpt1	Glutamic pyruvic transaminase 1, soluble	−1.10	0.11	0.002			
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble)	−1.22	0.10	0.001			
Gpd1	Glycerol-3-phosphate dehydrogenase 1 (soluble)	−1.18	0.10	0.001			

(*continued on next page*)

Table 1 (*continued*)

Symbol	Gene description	CA/W	FDR q	p-value	QTL	Chr	LOD
Gramd3	**GRAM domain containing 3**	**−1.09**	**0.15**	**0.011**			
Gramd3	**GRAM domain containing 3**	**−1.13**	**0.11**	**0.003**			
Garnl1	GTPase activating RANGAP domain-like 1	−1.25	0.12	0.004			
Gimap6	GTPase, IMAP family member 6	−1.11	0.11	0.003	Alc18	4	9.2
Gng7	Guanine nucleotide binding protein, gamma 7	−1.12	0.15	0.010			
Hspa5	Heat shock 70 kDa protein 5 (glucose-regulated protein)	−1.10	0.11	0.002	Alc8	3	2.7
Hspa1b	Heat shock 70kD protein 1B (mapped)	−1.28	0.15	0.010			
Hbp1	High mobility group box transcription factor 1	−1.07	0.15	0.010			
Homer1	**Homer homolog 1 (Drosophila)**	**−1.72**	**0.00**	**0.000**			
Hip1	Huntingtin interacting protein 1	−1.14	0.10	0.002	Alc10	12	2.2
Hdh	Huntington disease gene homolog	−1.15	0.11	0.002			
Hyal2	Hyaluronoglucosaminidase 2	−1.17	0.09	0.001			
Igj	Immunoglobulin joining chain	−1.11	0.15	0.009			
Irs3	Insulin receptor substrate 3	−1.14	0.09	0.001			
Ifngr1	Interferon gamma receptor 1	−1.07	0.15	0.010			
Irf3	**Interferon regulatory factor 3**	**−1.11**	**0.12**	**0.004**			
Il16	Interleukin 16	−1.09	0.13	0.005			
Nyw1	Ischemia related factor NYW-1	−1.14	0.11	0.002			
Jam2	Junction adhesion molecule 2	−1.13	0.14	0.008			
Kif11	**Kinesin family member 11**	**−1.12**	**0.12**	**0.005**			
Kif1b	Kinesin family member 1B	−1.10	0.15	0.010			
Kif6	Kinesin family member 6	−1.09	0.14	0.009			
Klc1	Kinesin light chain 1	−1.08	0.14	0.008			
Lphn2	Latrophilin 2	−1.08	0.14	0.007			
Leng8	Leukocyte receptor cluster (LRC) member 8	−1.12	0.14	0.006			
Lims2	**LIM and senescent cell antigen like domains 2**	**−1.23**	**0.01**	**0.000**			
LMO7	LIM domain only protein 7	−1.19	0.12	0.004			
Lcn7	Lipocalin 7	−1.24	0.09	0.001			
Lsr	Lipolysis stimulated lipoprotein receptor	−1.21	0.14	0.006			
Lef1	**Lymphoid enhancer binding factor 1**	**−1.12**	**0.09**	**0.001**	**Alc15**	**2**	**4.1**
Man2a1	Mannosidase 2, alpha 1	−1.16	0.09	0.001			
Mcpt1	Mast cell protease 1	−1.14	0.10	0.001			
Mxi1	Max interacting protein 1	−1.07	0.14	0.008			
Mbc2	Membrane bound C2 domain containing protein	−1.15	0.14	0.008			
Mpp4	Membrane protein, palmitoylated 4 (MAGUK p55 subfamily member 4)	−1.08	0.12	0.005			
Mgea5	Meningioma expressed antigen 5 (hyaluronidase)	−1.14	0.11	0.003			
Mat2a	Methionine adenosyltransferase II, alpha	−1.11	0.14	0.007			
Mettl7b	Methyltransferase like 7B	−1.12	0.12	0.004			
Mtus1	Mitochondrial tumor suppressor 1	−1.09	0.13	0.005	Alc11	16	3.2
Mobp	Myelin-associated oligodendrocytic basic protein	−1.22	0.10	0.001			
Ntrk2	Neurotrophic tyrosine kinase, receptor, type 2	−1.67	0.00	0.000			
Nexn	Nexilin	−1.15	0.14	0.008			
Ng3	Ng3 protein	−1.11	0.11	0.003			
Nmnat1	Nicotinamide nucleotide adenylyltransferase 1	−1.11	0.15	0.011			
Nos3	**Nitric oxide synthase 3, endothelial cell**	**−1.21**	**0.11**	**0.003**			
Nxf1	Nuclear RNA export factor 1	−1.08	0.14	0.007			
Numb	Numb gene homolog (Drosophila)	−1.12	0.12	0.004			
Ocln	Occludin	−1.14	0.10	0.002			
Pthr1	Parathyroid hormone receptor 1	−1.20	0.07	0.000			
Per2	Period homolog 2 (Drosophila)	−1.17	0.04	0.000			
Ppap2b	Phosphatidic acid phosphatase type 2B	−1.12	0.11	0.002			
Plce1	**Phospholipase C, epsilon 1**	**−1.12**	**0.14**	**0.009**			
Plcg1	Phospholipase C, gamma 1	−1.12	0.12	0.004			
RGD1303232	Phytn_dehydro and Pyr_redox domain containing protein RGD1303232	−1.10	0.13	0.006			
Pkp2	Plakophilin 2	−1.14	0.10	0.001			
Podxl	Podocalyxin-like	−1.11	0.12	0.004	Alc18	4	9.2
Pola2	**Polymerase (DNA directed), alpha 2**	**−1.16**	**0.13**	**0.005**			
Polm	Polymerase (DNA directed), mu	−1.11	0.13	0.006			
Kcnt1	Potassium channel, subfamily T, member 1	−1.12	0.14	0.008	Alc8	3	2.7
Kcnq3	Potassium voltage-gated channel, subfamily Q, member 3	−1.22	0.10	0.001			
Plod1	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	−1.13	0.15	0.009			
Col4a1	Procollagen, type IV, alpha 1	−1.14	0.12	0.004			
Psrc2	Proline/serine-rich coiled-coil 2	−1.07	0.14	0.008			
Pcsk7	Proprotein convertase subtilisin/kexin type 7	−1.11	0.11	0.003			
Ptpn5	Protein tyrosine phosphatase, non-receptor type 5	−1.08	0.15	0.009			
Ptprg	Protein tyrosine phosphatase, receptor type, G	−1.15	0.10	0.001			
Ptprv	Protein tyrosine phosphatase, receptor type, V	−1.10	0.14	0.007			
Pxk	PX domain containing serine/threonine kinase	−1.08	0.13	0.006			
Rad9b	RAD9 homolog B (S. cerevisiae)	−1.19	0.11	0.002	Alc6	12	4.7
Ralgds	Ral guanine nucleotide dissociation stimulator	−1.16	0.09	0.001	Alc8	3	2.7
Rap1ga1	RAP1, GTPase activating protein 1	−1.11	0.14	0.007			
Rasd2	RASD family, member 2	−1.17	0.10	0.001			
Rasd2	RASD family, member 2	−1.20	0.08	0.000			
Rasgrp4	**RAS guanyl releasing protein 4**	**−1.16**	**0.07**	**0.000**			
Ramp2	Receptor (calcitonin) activity modifying protein 2	−1.11	0.13	0.006			
Rgs8	Regulator of G-protein signaling 8	−1.12	0.14	0.008			

Table 1 (*continued*)

Symbol	Gene description	CA/W	FDR q	p-value	QTL	Chr	LOD
Rgs9	Regulator of G-protein signaling 9	−1.15	0.11	0.002			
Rgs3	Regulator of G-protein signaling 3	−1.12	0.14	0.007			
Rcn2	Reticulocalbin 2	−1.10	0.12	0.004			
Arhgap17	Rho GTPase activating protein 17	−1.09	0.13	0.006			
Serpina1	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	−1.12	0.14	0.009			
Shank1	SH3 and multiple ankyrin repeat domains 1	−1.14	0.15	0.010			
Cr16	SH3 domain binding protein CR16	−1.13	0.13	0.005	Alc18	4	9.2
Sipa1l1	**Signal-induced proliferation associated 1 like 1**	**−1.21**	**0.04**	**0.000**			
Srpr	Signal recognition particle receptor ('docking protein')	−1.10	0.15	0.010	Alc20	8	2
Stat4	Signal transducer and activator of transcription 4	−1.18	0.12	0.004			
Smr2	SMR2	−1.12	0.15	0.010			
Slc14a2	Solute carrier family 14 (urea transporter), member 2	−1.21	0.11	0.003			
Slc29a1	**Solute carrier family 29 (nucleoside transporters), member 1**	**−1.10**	**0.10**	**0.002**			
Slc2a1	**Solute carrier family 2 (facilitated glucose transporter), member 1**	**−1.16**	**0.04**	**0.000**			
Slc9a3r2	**Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 2**	**−1.14**	**0.07**	**0.000**	**Alc5**	**10**	**2.4**
Slco2b1	Solute carrier organic anion transporter family, member 2b1	−1.16	0.11	0.002			
Sox18	SRY-box containing gene 18	−1.16	0.12	0.005			
Sts	Steroid sulfatase	−1.11	0.12	0.003			
Stom	Stomatin	−1.13	0.14	0.007	Alc8	3	2.7
Sulf2	Sulfatase 2	−1.13	0.14	0.007			
Sdc1	Syndecan 1	−1.13	0.10	0.002	Alc17	6	3.1
Tec	Tec protein tyrosine kinase	−1.09	0.15	0.011			
Ttc7	Tetratricopeptide repeat domain 7	−1.12	0.14	0.006	Alc17	6	3.1
Tef	Thyrotroph embryonic factor	−1.14	0.14	0.008			
pur-beta	Transcription factor Pur-beta	−1.13	0.15	0.009			
Tgfbr3	Transforming growth factor, beta receptor III	−1.14	0.13	0.005			
Tmed7	Transmembrane emp24 protein transport domain containing 7	−1.11	0.15	0.010			
Tsc22d3	TSC22 domain family 3	−1.10	0.09	0.001			
Tyrp1	Tyrosinase-related protein 1	−1.13	0.12	0.004			
Tie1	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	−1.21	0.10	0.002			
Ubadc1	Ubiquitin associated domain containing 1	−1.11	0.15	0.010	Alc8	3	2
Utrn	Utrophin	−1.10	0.09	0.001			
Vamp1	Vesicle-associated membrane protein 1	−1.12	0.13	0.005			
Vwa1	Von Willebrand factor A domain containing 1	−1.13	0.12	0.004			
Wnk4	**WNK lysine deficient protein kinase 4**	**−1.21**	**0.02**	**0.000**			
Wbp1	WW domain binding protein 1	−1.08	0.12	0.004	Alc18	4	9.2
Wbp1	WW domain binding protein 1	−1.08	0.14	0.008			

CA/W = fold change of continuous access group to water group; Chr = chromosome number; QTL = rat alcohol preference QTL; LOD = LOD score for QTL; Bold type indicates genes in common with MSA vs. W differences.

the MSA group's last drinking episode when ethanol was also removed from the CA group. It is noteworthy that 10 of these hours occurred during the daily light-cycle, when P rats normally drink limited amounts of ethanol (Bell et al., 2006b,c; Murphy et al., 1986). Nevertheless, it is likely that the CA group experienced symptoms of its first protracted withdrawal at this time point (Kampov-Polevoy et al., 2000; Waller et al., 1982). Therefore, the gene expression differences observed between the CA and W groups may be due in part to ethanol withdrawal. It would be difficult to resolve the issue of withdrawal effects from continuous chronic alcohol drinking without undertaking a more detailed time-course study with this alcohol drinking protocol. However, since the MSA group did not show a significant number of differences in gene expression compared to the W control group (suggesting little effect of repeated BACs, that exceeded 50 mg% per access period, five days per week), the differences between the CA and W groups may reflect the effects of removal of the ethanol. A recent study (Bell et al., 2009) indicated significant behavioral changes (alterations in motor activity and rearing behavior) occur between 9 and 13 h after removal of ethanol in P rats that had 24-h continuous/daily free-choice access to ethanol for approximately 6 months. These studies suggest some behavioral alterations are occurring after removal of ethanol from P rats given continuous access, and there may be a relationship between the changes in gene expression within the ACB observed in the present study and these changes in general motor activity.

A proteomics study of the ACB using similar drinking procedures with inbred P rats indicated that the levels of 12 proteins were altered by MSA drinking compared to the W group and 8 proteins were altered by CA drinking compared to the W group (Bell et al., 2006a). None of the proteins that were different in the ACB between the CA and W groups of the proteomics study (Bell et al., 2006a) were found to be different between the CA and W groups in the gene expression data of the present study (Table 1). These results suggest that a direct relationship between changes in mRNA and protein levels may not be necessary (for an example of dissociations between DNA, RNA and protein levels in the brain after ethanol exposure see Babu et al., 1994) within this brain region. This could be due to a number of factors, not the least of which is procedural differences between the studies, but, in addition, proteins may be synthesized in other regions and transported to the ACB. Another possibility is that there is temporal discontinuity between changes in the expression levels of mRNA and protein, such that protein levels may increase (or decrease) leading to their accumulation (or reduction) because of post-translational modifications and/or changes in chaperoning or trafficking.

The Gene Ontology (GO) analysis indicated several significant biological processes categories. The categories of 'anti-apoptosis' and 'negative regulation of programmed cell death' suggest that cellular changes may have occurred to counter any neurotoxic effects of chronic ethanol exposure. A number of the genes identified in the CA group of the present study as having significantly changed expression levels (Table 1) and were members of over-represented GO categories (Table 2) have also been reported in the literature as genes altered by or associated with high ethanol consumption. For example, (a) *Btg2* gene expression, elevated in the CA group, is greater in inbred P versus inbred NP rats as well (Edenberg et al., 2005); (b) *Scg2* gene expression, elevated in the CA group, is also increased in the frontal

Table 2
Gene Ontology (GO) categories, with names and identification numbers, displaying significant over-representation of genes, and the respective gene names and their symbols.

GO category/GOBPID	Genes	Symbol
Blood vessel development/ GO:0001568	Angiopoietin-like 4	Angptl4
	B-cell translocation gene 1, anti-proliferative	Btg1
	Connective tissue growth factor	Ctgf
	Endothelial-specific receptor tyrosine kinase	Tek
	Forkhead box O1A	Foxo1a
	Gap junction membrane channel protein alpha 1	Gja1
	Neurotrophic tyrosine kinase, receptor, type 2	Ntrk2
	Phosphatidic acid phosphatase type 2B	Ppap2b
	Protein O-fucosyltransferase 1	Pofut1
	Secretogranin 2	Scg2
	Small inducible cytokine subfamily E, member 1	Scye1
	Transforming growth factor alpha	Tgfa
	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	Tie1
Regionalization/ GO:0003002	Activity regulated cytoskeleton-associated protein	Arc
	B-cell translocation gene 2, anti-proliferative	Btg2
	E1A binding protein p300	Ep300
	Huntington disease gene homolog	Hdh
	Lymphoid enhancer binding factor 1	Lef1
	Protein O-fucosyltransferase 1	Pofut1
	Ring finger protein 2	Rnf2
Negative regulation of protein kinase activity/ GO:0006469	Caveolin	Cav
	Dual specificity phosphatase 6	Dusp6
	Protein kinase inhibitor beta, cAMP dependent, catalytic	Pkib
	Regulator of G-protein signaling 3	Rgs3
	Sprouty homolog 2	Spry2
Isoprenoid metabolic process/ GO:0006720	3-hydroxy-3-methylglutaryl-coenzyme A reductase	Hmgcr
	Dehydrogenase/reductase (SDR family) member 3	Dhrs3
	Isopentenyl-diphosphate delta isomerase	Idi1
	Notch1-induced protein	
	Phytn-dehydro and Pyr-redox domain containing protein	
One-carbon compound metabolic process/ GO:0006730	B-cell translocation gene 1, anti-proliferative	Btg1
	Carbonic anhydrase 11	Car11
	FBJ murine osteosarcoma viral oncogene homolog	Fos
	Isoprenylcysteine carboxyl methyltransferase	Icmt
	Methionine adenosyltransferase II, alpha	Mat2a
	Methyl-CpG binding domain protein 1	Mbd1
	Protein tyrosine phosphatase, receptor type, G	Ptprg
Anti-apoptosis/ GO:0006916	A disintegrin and metalloproteinase domain 17	Adam17
	FGF receptor activating protein 1	Frag1
	Forkhead box O1A	Foxo1a
	Heat shock 70 kDa protein 5 (glucose-regulated protein)	Hspa5
	Hormone-regulated proliferation associated protein 20	Hrpap20
	Huntington disease gene homolog	Hdh
	Superoxide dismutase 2, mitochondrial	Sod2
	Transforming growth factor alpha	Tgfa
	TSC22 domain family 3	Tsc22d3
	Zinc finger protein 91	Zfp91
Microtubule-based movement/ GO:0007018	Actin-related protein 10 homolog	Actr10
	Huntington disease gene homolog	Hdh
	Kinesin family member 1B	Kif1b
	Kinesin family member 6	Kif6
	Kinesin family member 11	Kif11
	Kinesin heavy chain family, member 2	Kif2
	Kinesin light chain 1	Klc1
	Tubulin, beta 2b	Tubb2b
Gamete generation/ GO:0007276	3-hydroxy-3-methylglutaryl-coenzyme A reductase	Hmgcr
	A disintegrin-like and metallopeptidse with thrombospondin type 1 motif, 1	Adamts1
	B-cell translocation gene 1, anti-proliferative	Btg1
	Carnitine deficiency-associated gene expressed in ventricle 1	Cdv1
	Dead end homolog 1	Dnd1
	Heat shock factor 2	Hsf2
	Hydroxyacyl glutathione hydrolase	Hagh
	Kinesin family member 6	Kif6
	McKusick-Kaufman syndrome protein	Mkks
	Nitric oxide synthase 3, endothelial cell	Nos3
	Seven in absentia 1A	Siah1a
	Zinc finger protein 403	Zfp403
Gastrulation/ GO:0007369	Camello-like 3	Cml3
	Huntington disease gene homolog	Hdh
	Nuclear receptor subfamily 4, group A, member 3	Nr4a3
	Phosphatidic acid phosphatase type 2B	Ppap2b
	Ring finger protein 2	Rnf2
Heart development/ GO:0007507	E1A binding protein p300	Ep300
	Endothelial-specific receptor tyrosine kinase	Tek
	Gap junction membrane channel protein alpha 1	Gja1
	Histone deacetylase 5	Hdac5
	McKusick-Kaufman syndrome protein	Mkks
	Plakophilin 2	Pkp2
	Protein O-fucosyltransferase 1	Pofut1
	Transforming growth factor, beta receptor 3	Tgfbr3
Regulation of G-protein coupled receptor protein signaling/ GO:0008277	Endothelin converting enzyme 1	Ece1
	Phospholipase C, epsilon 1	Plce1
	RAS guanyl releasing protein 4	Rasgrp4
	Receptor (calcitonin) activity modifying protein 2	Ramp2
	Regulator of G-protein signaling 3	Rgs3
	Regulator of G-protein signaling 9	Rgs9
Protein catabolic process/ GO:0030163	Calpastatin	Cast
	Gap junction membrane channel protein alpha 1	Gja1
	Prenylcysteine oxidase 1	Pcyox1
	Proteasome (prosome, macropain) 26 S subunit, ATPase, 6	Psmc6
	Proteasome (prosome, macropain) 26 S subunit, non-ATPase, 12	Psmd12
	Proteasome (prosome, macropain) subunit, alpha type 4	Psma4
	Seven in absentia 1A	Siah1a
	Seven in absentia 2	Siah2
	Ubiquitin associated domain containing 1	Ubadc1
	Ubiquitin carboxyl-terminal hydrolase L5	Uchl5
	Ubiquitin-conjugating enzyme E2D 3	Ube2d3
Protein modification by small protein conjugation/ GO:0032446	Calcyclin binding protein	Cacybp
	LIM domain only protein 7	Lmo7
	Retinoblastoma binding protein 6	Rbbp6
	Ring finger protein 2	Rnf2
	Seven in absentia 1A	Siah1a
	Seven in absentia 2	Siah2
	Ubiquitin associated domain containing 1	Ubadc1
Negative regulation of programmed cell death/ GO:0043069	A disintegrin and metalloproteinase domain 17	Adam17
	Angiopoietin-like 4	Angptl4

Table 2 (*continued*)

GO category/GOBPID	Genes	Symbol
Negative regulation of programmed cell death/ GO:0043069	B-cell translocation gene 2, anti-proliferative	Btg2
	FGF receptor activating protein 1	Frag1
	Forkhead box O1A	Foxo1a
	Heat shock 70 kDa protein 5 (glucose-regulated protein)	Hspa5
	Hormone-regulated proliferation associated protein 20	Hrpap20
	Huntington disease gene homolog	Hdh
	Secretogranin 2	Scg2
	Superoxide dismutase 2, mitochondrial	Sod2
	Transforming growth factor alpha	Tgfa
	TSC22 domain family 3	Tsc22d3
	Zinc finger protein 91	Zfp91
Regulation of MAPK activity/ GO:0043405	Caveolin	Cav
	Cholinergic receptor, nicotinic, alpha polypeptide 7	Chrna7
	Dual specificity phosphatase 6	Dusp6
	Phospholipase C, epsilon 1	Plce1
	Regulator of G-protein signaling 3	Rgs3
	Sprouty homolog 2	Spry2
	Transforming growth factor alpha	Tgfa
Biopolymer methylation/ GO:0043414	B-cell translocation gene 1, anti-proliferative	Btg1
	B-cell translocation gene 2, anti-proliferative	Btg2
	FBJ murine osteosarcoma viral oncogene homolog	Fos
	Isoprenylcysteine carboxyl methyltransferase	Icmt
	Methyl-CpG binding domain protein 1	Mbd1
Regulation of kinase activity/ GO:0043549	Caveolin	Cav
	Cell division cycle 42 homolog	Cdc42
	Cholinergic receptor, nicotinic, alpha polypeptide 7	Chrna7
	Diacylglycerol kinase, alpha	Dgka
	Dual specificity phosphatase 6	Dusp6
	Phospholipase C, epsilon 1	Plce1
	Protein kinase inhibitor beta, cAMP dependent, catalytic	Pkib
	Regulator of G-protein signaling 3	Rgs3
	Speedy homolog 1	Spdy1
	Sprouty homolog 2	Spry2
	Transforming growth factor alpha	Tgfa
Cell maturation/ GO:0048469	Actin, alpha 1, skeletal muscle	Acta1
	Calpastatin	Cast
	Gap junction membrane channel protein alpha 1	Gja1
	Growth differentiation factor 11	Gdf11
	Hairy and enhancer of split 1	Hes1
	Parathyroid hormone receptor 1	Pthr1
Anatomical structure formation/ GO:0051603	Angiopoietin-like 4	Angptl4
	B-cell translocation gene 1, anti-proliferative	Btg1
	Connective tissue growth factor	Ctgf
	Endothelial-specific receptor tyrosine kinase	Tek
	Huntington disease gene homolog	Hdh
	Nuclear receptor subfamily 4, group A, member 3	Nr4a3
	Protein O-fucosyltransferase 1	Pofut1
	Secretogranin 2	Scg2
	Small inducible cytokine subfamily E, member 1	Scye1
	Transforming growth factor alpha	Tgfa
	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	Tie1

cortex, but decreased in the motor cortex of alcoholics (Mayfield et al., 2002); (c) *Tgfa*, with gene expression increased in the CA group, over-expressing mice display greater ethanol preference than their wild-type counterparts (Hilakivi-Clarke and Goldberg, 1995); and (d) a gene moderately similar to *Zfp91* is altered in the prefrontal cortex of alcoholics (Flatscher-Bader et al., 2005), with *Zfp91* gene expression

Table 3
Quantitative RT-PCR confirmation of microarray results for respective genes.

Gene symbol	CA expression	W expression	qRT-PCR fold Δ	Microarray fold Δ
Fos	2.02	1.46	1.38[a]	1.72
Homer1	3.60	2.13	1.69[a]	1.71
Nr4a1	2.62	1.27	2.07[a]	1.62
Dusp1	1.16	0.78	1.49[a]	1.42
Junb	3.31	1.80	1.84[a]	1.27
Mobp	1.23	1.50	−1.22[a]	−1.22
Jun	1.01	0.93	1.09[a]	1.23
Ntrk2	3.18	6.09	−1.92[a]	−1.67

CA = Continuous access group; W = Water control group.
[a] indicates qRT-PCR probability value < 0.001.

increased in the CA group of the present study as well. The results of the GO analysis (Table 2) also suggest that significant changes are occurring in intracellular signaling systems, involving protein kinase activity, G-protein coupled receptor protein signaling, and MAPK activity. These changes in intracellular signaling systems may indicate that major neuronal alterations occurred in the ACB of the CA group.

Several of the kinase activity-related genes (Table 2) identified as having altered expression levels in the CA group (Table 1) have been implicated in alcohol abuse. For instance, (a) *Cav2* gene expression, which was reduced in the present study as was gene expression of the family member *Cav*, is increased in the ACB of iP rats after operant self-administration of ethanol (Rodd et al., 2008); (b) *Dusp6* gene expression, which was increased in the CA group of the present study, is greater in iNP than iP rats (Kimpel et al., 2007), with gene expression differences also found between high and low alcohol-consuming mice (Kerns et al., 2005); and (c) *Pkib* gene expression is increased in the frontal cortex of alcoholics vs. nonalcoholics (Liu et al., 2006), which was elevated in the CA group of the present study as well. Interestingly, inhibition of PKA in the ACB shell increases ethanol intake (Misra and Pandey, 2006), and family member *Pkia* (cAMP dependent, regulatory) gene expression is decreased in the frontal and motor cortices of alcoholics (Mayfield et al., 2002).

Chrna7 gene expression was increased in the CA group (Table 1) and was identified in the over-represented GO category "regulation of MAPK activity" (Table 2). It is noteworthy that several reports support a role for *Chrna7* in substance abuse, for instance, (a) *Chrna7* knockout mice display greater sensitivity to lower dose ethanol-induced motor activation and higher dose ethanol-induced hypothermia and loss of righting reflex compared with their wild-type counterparts (Bowers et al., 2005); (b) *Chrna7* has been proposed to reduce ethanol-induced neurotoxicity (de Fiebre and de Fiebre, 2003); and (c) a significant association between the *Chrna7* gene, altered cognitive (response inhibition and sustained attention) function (Rigbi et al., 2008) or psychological characteristics (Greenbaum et al., 2006), and smoking behavior have been reported in humans.

Although the *Arc* gene was identified under the GO category 'regionalization' (Table 2), this gene was one of the most cited genes both detected as significantly changed in the present study (its gene expression was increased 1.5-fold in the CA group, Table 1) and implicated in substance abuse for morphine (Ammon et al., 2003), amphetamine (Gonzalez-Nicolini and McGinty, 2002), cocaine (Freeman et al., 2002; Samaha et al., 2004) and nicotine (Schochet et al., 2005; Samaha et al., 2005). *Arc* is an immediate early gene found in soma and dendrites and is involved in, or associated with, synaptic modification and learning/memory (e.g., Guzowski et al., 2006). In a recent study (Pandey et al., 2008), the *BDNF-Arc* signaling pathway has been implicated in both alcohol dependence and the comorbid expression of anxiety with alcohol abuse.

Among the 43 genes that were located within rat QTLs for alcohol consumption and preference, some were evident in certain GO categories and gene networks. *Tgfa* (located within Alc18 on chromosome 4) appears to be associated with ethanol preference in

mice (Hilakivi-Clarke and Goldberg, 1995), anti-apoptosis (Table 2) and up-regulation of Fos-related transcription factors (Fig. 2). *Hspa5* (located within Alc8 on chromosome 3) is also involved in anti-apoptosis (Table 2). *Mtus1* (located within Alc11 on chromosome 16) and *Creb3l2* (located within Alc18 on chromosome 4) are involved in transcription (Fig. 3). The anti-apoptosis involvement and increased transcription functions of these genes suggest that increased cellular protection may be occurring in the ACB of the CA group, which could be factors contributing to high alcohol intakes.

Ingenuity® pathway analyses uncovered networks overlapping and extending those detected with the GO analysis. In agreement with the GO biological processes category of 'anti-apoptosis' genes, the *Ingenuity*® pathway analysis revealed a network of 11 genes involved in apoptosis, 8 of which were reduced in the CA group (*Cast*, *Ccr5*, *Ece1*, *Nos3*, *Ntrk2*, *Plce1*, *Slc2a1*, *Tgfbr3*). Several of these genes have been implicated in alcoholism and drug abuse, including reports that (a) *Cast* gene expression is reduced in the frontal cortex of alcoholics vs. nonalcoholics (Liu et al., 2006); (b) female, but not male, *Ccr5* knockout mice display greater ethanol intake, but not preference, as well as ethanol-induced conditioned taste aversion than their wild type counterparts (Blednov et al., 2005); and (c) *Slc2a1* (facilitated glucose transporter) gene expression is increased in the ACB of iP rats operantly self-administering ethanol, although, at the same time, family member *Slc2a3* (facilitated glucose transporter) gene expression is decreased (Rodd et al., 2008). Regarding *Ntrk2*, its gene expression is decreased in the frontal and motor cortices of alcoholics (Mayfield et al., 2002). Moreover, single nucleotide polymorphism (SNP)-based analyses implicate the *Ntrk2* gene in alcohol dependence (Xu et al., 2007). The *Ntrk2* gene has been implicated in nicotine abuse as well (Beuten et al., 2007; Sun et al., 2007). Several genes responding to glucocorticoid receptor signaling were also altered in the CA versus W groups with 3 genes having reduced expression levels (*Fkbp5*, *Hspa12b*, *Tsc22d3*), whereas only one was increased (*Dusp1*).

In addition to the oncogenes, *Fos*, *Jun* and *Junb*, there were several other genes in the oncogene network that were up regulated in the ACB of the CA group compared with the control animals (See Fig. 2). These genes included *Ctgf*, *Tgfa*, *Plagl1*, *Spry2* and *Rdbp*. The up-regulation of *Fos* and other transcription factors in the CNS are often associated with increased neuronal activity (Greenberg et al., 1986; Herrera and Robertson, 1996; Morgan and Curran, 1989). *Fos* (along with *Jun* and *JunB*) is regulated by, or mediates the effects of, ethanol, for example, *Fos* is induced in the ACB shell by acute ethanol and 80% of the *Fos*-positive cells labeled for GAD as well (Leriche et al., 2008). *Fos* expression has also been associated with morphine (Taracha et al., 2008), cocaine (Zhang et al., 2006) and nicotine (Schochet et al., 2005) abuse. *Ctgf* and *Tgfa* are growth factors and their increased expression may indicate neuronal alterations are occurring as well. These results are in agreement with the GO analysis (Table 2), suggesting that anatomical structural alterations may be occurring in the CA group. It is noteworthy that CNS *Ctgf* gene expression levels differ between high alcohol-consuming AA vs low alcohol-consuming ANA rats (Sommer et al., 2006) and this gene has been linked with cocaine abuse (Mash et al., 2007), and, as indicated above, *Tgfa* over-expressing mice display greater ethanol preference than their wild-type counterparts (Hilakivi-Clarke and Goldberg, 1995).

The higher expression of *Plagl1* [a zinc finger protein (Yang et al., 2005)] and *Rdbp* [a nuclear RNA binding protein (Surowy et al., 1990)] are also consistent with increased transcription associated with neuronal activity, with *Plag1* gene expression increased in the accumbens of iP rats operantly self-administering ethanol (Rodd et al., 2008). Moreover, the higher expression (Table 1) of several members of the oncogene family (*Rab1*, *Rab3c*, *Rab21*, *Rab35*) and RNA binding motif proteins (*Rbm3*, *Rbm13*, *Rbm17*) are also consistent with an

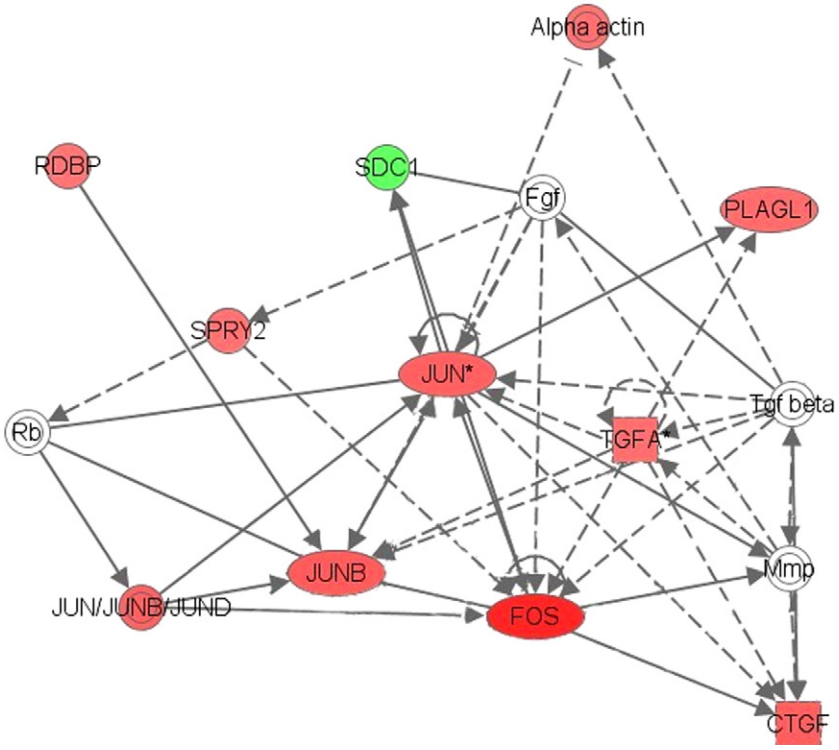

Fig. 2. Abridged Ingenuity® network analysis revealed up-regulation of Fos-related transcription factors. Red indicates up-regulation and green down-regulation of the associated genes. Genes not colored indicate no change in expression but they are highly linked to genes that did change. Solid lines indicate a direct interaction between genes, whereas dashed lines indicate an indirect interaction between the genes. [For interpretation of the references to color in this figure, the reader is referred to the web version of the article] Abbreviations used: Ctgf—connective tissue growth factor; Fos—FBJ osteosarcoma oncogene; Fgf—fibroblast growth factor; Jun—Jun oncogene; Junb—Jun B oncogene; Mmp—matrix metallopeptidase; Plagl1—pleiomorphic adenoma gene-like 1; Rdbp—RD RNA binding protein; Rb—retinoblastoma; Spry2—sprouty homolog 2; Sdc1—syndecan 1; Tgfa—transforming growth factor alpha.

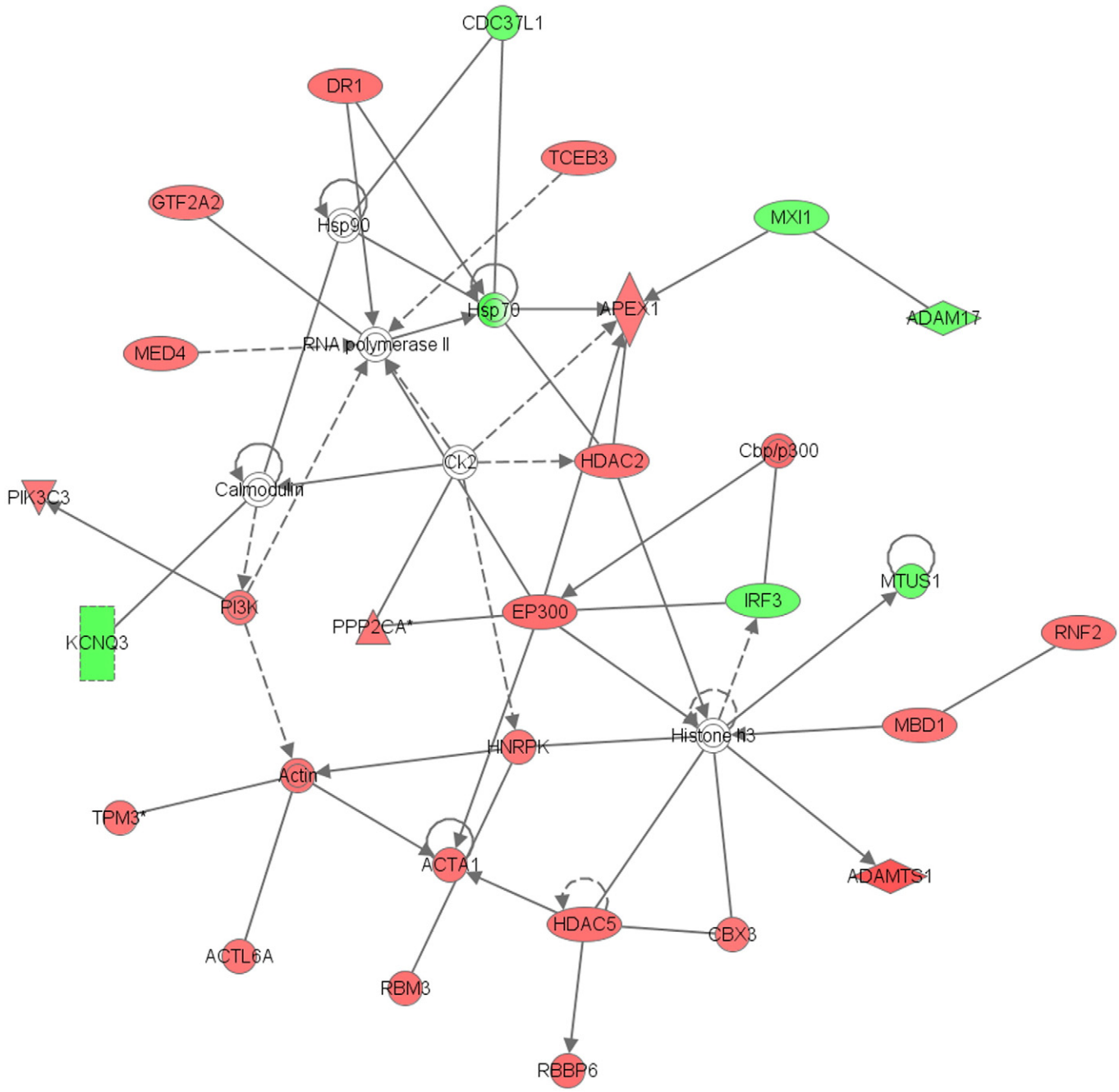

Fig. 3. Ingenuity® network analysis revealed primarily up-regulation of interacting pathways involved in transcription, calcium signaling, oxidative stress response and glucocorticoid receptor signaling. Red indicates up-regulation and green down-regulation of the associated genes. Genes not colored indicate no change in expression but they are highly linked to genes that did change. Solid lines indicate a direct interaction between genes, whereas dashed lines indicate an indirect interaction between the genes. [For interpretation of the references to color in this figure, the reader is referred to the web version of the article] Abbreviations used: Acta1—actin alpha 1 skeletal muscle; Actl6a—actin-like 6A; Adam17—a disintegrin and metallopeptidase domain 17; Adamts1—a disintegrin-like and metallopeptidase; Apex1—a purinic/pyrimidinic endonuclease 1; Cdc37l1—cell division cycle 37 homolog-like 1; Cbx3—chromobox homolog 3; Cbp/p300—CREB binding protein; Dr1—down-regulator of transcription 1; Ep300—E1A binding protein p300; Gtf2a2—general transcription factor IIA 2; Hsp70—heat shock protein 70; Hnrpk—heterogeneous nuclear ribonucleoprotein K; Hdac2—histone deacetylase 2; Hdac5—histone deacetylase 5; Irf3—interferon regulatory factor 3; Kcnq3—potassium voltage-gated channel subfamily Q member 3; Mxl1—Max interacting protein 1; Med4—mediator of RNA polymerase II transcription subunit 4 homolog; Mbd1—methyl-CpG binding domain protein 1; Mtus1—mitochondrial tumor suppressor 1; Pi3k—phosphatidylinositol 3-kinase; Pik3c3—phosphoinositide-3-kinase class 3; Ppp2ca—protein phosphatase 2 catalytic subunit alpha isoform; Rbbp6—retinoblastoma binding protein 6; Rnf2—ring finger protein 2; Rbm3—RNA binding motif protein 3; Tceb3—transcription elongation factor B polypeptide 3; Tpm3—tropomyosin 3 gamma.

interpretation of increased transcription activity. Fig. 3 shows a network of genes involved in calcium signaling, oxidative stress response, and transcription. In the calcium signaling network, there were 4 genes (*Acta1*, *Ep300*, *Hdac5*, *Tpm3*), and, in the oxidative stress network, there were 4 genes (*Acta1*, *Bex1*, *Ep300*, *Pik3c3*) that were up regulated in the CA group. In the transcription network, there were 12 genes that were different between the CA and W groups with 10 genes higher (*Cbx3*, *Dr1*, *Ep300*, *Hdac2*, *Hdac5*, *Mbd1*, *Med4*, *Rbbp6*, *Rnf2*, *Tceb3*) and only 2 genes lower (*Irf3*, *Mxi1*) in the CA group; these results are consistent with the findings for the oncogenes (Fig. 2) and also support an interpretation of increased transcription.

A number of the genes in Fig. 3 have been implicated in alcohol abuse, such as (a) *Acta1* gene expression differences are found between iP and iNP rats (Kimpel et al., 2007); (b) *Irf3* gene expression

differences are found between iP and iNP rats (Kimpel et al., 2007); (c) *Tceb3* gene expression is increased in the frontal cortex of cirrhotic alcoholics vs. controls (Liu et al., 2007); and (d) *Tpm3* gene expression is decreased in the ACB of iP rats operantly self-administering ethanol (Rodd et al., 2008), with *Tpm3* protein expression levels decreased in the amygdala of chronic ethanol drinking iP rats, but increased in the ACB of iP rats given multiple scheduled access sessions of ethanol per day as well (Bell et al., 2006a).

Although not identified by the GO or Ingenuity® analyses, *Crh* gene expression was increased in the chronic ethanol drinking P rats of the present study and has been implicated in substance abuse (c.f., Heilig and Koob, 2007; Koob and Le Moal, 2008). For example, (a) *Crh* knockout mice display greater ethanol preference and limited access ethanol intake than their wild-type counterparts (Olive et al., 2003); (b) *Crh* over-expressing mice display lower ethanol preference and reduced 24-h ethanol intake than their wild-type counterparts (Palmer et al., 2004); (c) chronic ethanol drinking increases prepro*Crh* mRNA in the CNS of Sprague–Dawley rats (Lack et al., 2005); and (d) *Crh* levels predict intensity of craving and probability of relapse to drug use after acute detoxification (Kiefer and Wiedemann, 2004; see also Goeders, 2002a,b).

In summary, the results of the present study suggest that, under intermittent ethanol drinking conditions, gene expression levels may reach a near normal steady-state level, which may be sufficient to maintain altered protein levels in the ACB. Because gene expression was determined 15 h after removal of ethanol in the CA group, these changes may also reflect withdrawal-responsive genes rather than purely ethanol responsive genes. Nevertheless, because a number of the genes identified as significant in the present study have also been described in the literature on drug and/or alcohol abuse, these genes may serve as candidates for continued research into the neurobiology of drug and/or alcohol abuse.

Acknowledgements

The present study was supported in part by AA07611, AA13521 [INIA Project], AA13522 [INIA Project], AA016652 [INIA Project], AA16660 [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.2009.07.019.

References

Ammon S, Mayer P, Riechert U, Tischmeyer H, Hollt V. Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal. Brain Res Mol Brain Res 2003;112:113–25.
Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25:25–9.
Babu PP, Kumari LR, Vemuri MC. Differential changes in cell morphology, macromolecular composition and membrane protein profiles of neurons and astrocytes in chronic ethanol treated rats. Mol Cell Biochem 1994;130:29–40.
Barrett T, Suzek TO, Troup DB, Wilhite SE, Ngau WC, Ledoux P, et al. NCBI GEO: mining millions of expression profiles—database and tools. Nucleic Acids Res 2005;33: D562–6 Database issue.
Bell RL, Kimpel MW, Rodd ZA, Strother WN, Bai F, Peper CL, et al. Protein expression changes in the nucleus accumbens and amygdala of inbred alcohol-preferring rats given either continuous or scheduled access to ethanol. Alcohol 2006a;40:3-17.
Bell RL, Rodd ZA, Lumeng L, Murphy JM, McBride WJ. The alcohol-preferring P rat and animal models of excessive alcohol drinking. Addict Biol 2006b;11:270–88.
Bell RL, Rodd ZA, Sable HJK, Schultz JA, Hsu CC, Lumeng L, et al. Daily patterns of ethanol drinking in peri-adolescent and adult alcohol-preferring (P) rats. Pharmacol Biochem Behav 2006c;83:35–46.
Bell RL, Rodd ZA, Schultz JA, Peper CL, Lumeng L, Murphy JM, et al. Effects of short deprivation and re-exposure intervals on the ethanol drinking behavior of selectively bred high alcohol-consuming rats. Alcohol 2008;42:407–16.
Bell RL, Hauser SR, Smith RJ, McConnell KK, Rodd ZA, McBride WJ. Evaluating withdrawal-like behavior 8 to 15 hours after cessation of chronic 24-hr free-choice ethanol drinking in alcohol-preferring (P) rats. Alcohol Clin Exp Res 2009;33(S1): 139A.
Beuten J, Ma JZ, Payne TJ, Dupont RT, Lou XY, Crews KM, et al. Association of specific haplotypes of neurotrophic tyrosine kinase receptor 2 gene (NTRK2) with vulnerability to nicotine dependence in African-Americans and European-Americans. Biol Psychiatry 2007;61:48–55.
Bice P, Foroud T, Bo R, Castelluccio P, Lumeng L, Li TK, et al. Genomic screen for QTLs underlying alcohol consumption in the P and NP rat lines. Mamm Genome 1998;9: 949–55.
Blednov YA, Bergeson SE, Walker D, Ferreira VM, Kuziel WA, Harris RA. Perturbation of chemokine networks by gene deletion alters the reinforcing actions of ethanol. Behav Brain Res 2005;165:110–25.
Bowers BJ, McClure-Begley TD, Keller JJ, Paylor R, Collins AC, Wehner JM. Deletion of the alpha7 nicotinic receptor subunit gene results in increased sensitivity to several behavioral effects produced by alcohol. Alcohol Clin Exp Res 2005;29:295–302.
Bowers BJ, Radcliffe RA, Smith AM, Miyamoto-Ditmon J, Wehner JM. Microarray analysis identifies cerebellar genes sensitive to chronic ethanol treatment in PKCgamma mice. Alcohol 2006;40:19–33.
Carr LG, Foroud T, Bice P, Gobbett T, Ivashina J, Edenberg H, et al. A quantitative trait locus for alcohol consumption in selectively bred rat lines. Alcohol Clin Exp Res 1998;22:884–7.
Carr LG, Habegger K, Spence J, Ritchotte A, Liu L, Lumeng L, et al. Analyses of quantitative trait loci contributing to alcohol preference in HAD1/LAD1 and HAD2/LAD2 rats. Alcohol Clin Exp Res 2003;27:1710–7.
de Fiebre NEC, de Fiebre CM. Alpha-7 nicotinic acetylcholine receptor-mediated protection against ethanol-induced neurotoxicity. Alcohol 2003;31:149–53.
Edenberg HJ, Strother WN, McClintick JN, Tian H, Stephans M, Jerome RE, et al. Gene expression in the hippocampus of inbred alcohol-preferring and -nonpreferring rats. Genes Brain Behav 2005;4:20–30.
Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30:207–10.
Flatscher-Bader T, van der Brug M, Hwang JW, Gochee PA, Matsumoto I, Niwa S, et al. Alcohol-responsive genes in the frontal cortex and nucleus accumbens of human alcoholics. J Neurochem 2005;93:359–70.
Foroud T, Ritchotte A, Spence J, Liu L, Lumeng L, Li TK, et al. Confirmation of alcohol preference quantitative trait loci in the replicate high alcohol drinking and low alcohol drinking rat lines. Psychiatr Genet 2003;13:155–61.
Freeman WM, Brebner K, Lynch WJ, Patel KM, Robertson DJ, Roberts DC, et al. Changes in rat frontal cortex gene expression following chronic cocaine. Brain Res Mol Brain Res 2002;104:11–20.
Gatto GJ, Murphy JM, Waller MB, McBride WJ, Lumeng L, Li T-K. Chronic ethanol tolerance through free-choice drinking in the P line of alcohol-preferring rats. Pharmacol Biochem Behav 1987;28:111–5.
Gentleman RC. Using GO for statistical analysis. Proc COMPSTAT 2004:171–80.
Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5:R80.
Goeders NE. Stress and cocaine addiction. J Pharmacol Exp Ther 2002a;301:785–9.
Goeders NE. The HPA axis and cocaine reinforcement. Psychoneuroendocrin 2002b;27: 13–33.
Gonzalez-Nicolini V, McGinty JF. Gene expression profile from the striatum of amphetamine-treated rats: a cDNA array and in situ hybridization histochemical study. Brain Res Gene Expr Patterns 2002;1:193–8.
Greenbaum L, Kanyas K, Karni O, Merbl Y, Olender T, Horowitz A, et al. Why do young women smoke? I. Direct and interactive effects of environment, psychological characteristics and nicotine cholinergic receptor genes. Mol Psychiatry 2006;11:312–22.
Greenberg ME, Ziff EG, Greene LA. Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science 1986;234:80–3.
Guzowski JF, Miyashita T, Chawla MK, Sanderson J, Maes LI, Houston FP, et al. Recent behavioral history modifies coupling between cell activity and Arc gene transcription in hippocampal CA1 neurons. Proc Natl Acad Sci USA 2006;103:1077–82.
Harris MA, Clark J, Ireland A, Lomax J, Ashburner M, Foulger R, et al. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 2004;32:D258–61 Database issue.
Heilig M, Koob GF. A key role for corticotrophin-releasing factor in alcohol dependence. Trends Neurosci 2007;30:399–406.
Herrera DG, Robertson HA. Activation of c-fos in the brain. Prog Neurobiol 1996;50:83-107.
Hilakivi-Clarke L, Goldberg R. Gonadal hormones and aggression-maintaining effect of alcohol in male transgenic transforming growth factor-alpha mice. Alcohol Clin Exp Res 1995;19:708–13.
Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249–64.
Kampov-Polevoy AB, Matthews DB, Gause L, Morrow AL, Overstreet DH. P rats develop physical dependence on alcohol via voluntary drinking: changes in seizure thresholds, anxiety, and patterns of alcohol drinking. Alcohol Clin Exp Res 2000;24:278–84.
Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, et al. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2 J versus C57BL/6 J mice. J Neurosci 2005;25:2255–66.
Kiefer F, Wiedemann K. Neuroendocrine pathways of addictive behavior. Addict Biol 2004;9:205–12.
Kimpel MW, Strother WN, McClintick JN, Carr LG, Edenberg HJ, McBride WJ. Functional gene expression differences between inbred alcohol-preferring (iP) and -non-preferring (iNP) rats in five brain regions. Alcohol 2007;41:95-132.
Koob GF, Le Moal M. Review. Neurobiological mechanisms for opponent motivational processes in addiction. Philos Trans R Soc Lond B Biol Sci 2008;363:3113–23.

Lack AK, Floyd DW, McCool BA. Chronic ethanol ingestion modulates proanxiety factors expressed in rat central amygdala. Alcohol 2005;36:83–90.
Leriche M, Mendez M, Zimmer L, Berod A. Acute ethanol induces Fos in GABAergic and non-GABAergic forebrain neurons: a double-labeling study in the medial prefrontal cortex and extended amygdala. Neuroscience 2008;153:259–67.
Liu J, Lewohl JM, Harris RA, Iyer VR, Dodd PR, Randall PK, et al. Patterns of gene expression in the frontal cortex discriminate alcoholic from nonalcoholic individuals. Neuropsychopharmacology 2006;30:1574–82.
Liu J, Lewohl JM, Harris RA, Dodd PR, Mayfield RD. Altered gene expression profiles in the frontal cortex of cirrhotic alcoholics. Alcohol Clin Exp Res 2007;31:1460–6.
Lumeng L, Li T-K. The development of metabolic tolerance in the alcohol-preferring P rats: comparison of forced and free-choice drinking of ethanol. Pharmacol Biochem Behav 1986;25:1013–20.
Mash DC, Ffrench-Mullen J, Adi N, Qin Y, Buck A, Pablo J. Gene expression in human hippocampus from cocaine abusers identifies genes which regulate extracellular matrix remodeling. PLoS ONE 2007;2:e1187.
Mayfield RD, Lewohl JM, Dodd PR, Herlihy A, Liu J, Harris RA. Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. J Neurochem 2002;81:802–13.
McClintick JN, Jerome RE, Nicholson CR, Crabb DW, Edenberg HJ. Reproducibility of oligonucleotide arrays using small samples. BMC Genomics 2003;4:1-15.
Misra K, Pandey SC. The decreased cyclic-AMP dependent-protein kinase A function in the nucleus accumbens: a role in alcohol drinking but not in anxiety-like behaviors in rats. Neuropsychopharmacology 2006;31:1406–19.
Morgan JI, Curran T. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. Topics Neurosci 1989;12:459–62.
Mulligan MK, Ponomerav I, Hitzemann RJ, Belknap JK, Tabakoff B, Harris RA, et al. Toward understanding the genetics of alcohol drinking through transcriptome meta-analysis. Proc Natl Acad Sci USA 2006;103:6368–73.
Murphy JM, Gatto GJ, Waller MB, McBride WJ, Lumeng L, Li T-K. Effects of scheduled access on ethanol intake by the alcohol-preferring (P) line of rats. Alcohol 1986;3:331–6.
Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, et al. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. Behav Genet 2002;32:363–88.
Olive MF, Mehmert KK, Koenig HN, Camarini R, Kim JA, Nannini MA, et al. A role for corticotropin releasing factor (CRF) in ethanol consumption, sensitivity, and reward as revealed by CRF-deficient mice. Psychopharmacology 2003;165:181–7.
Palmer AA, Sharpe AL, Burkhart-Kasch S, McKinnon CS, Coste SC, Stenzel-Poore MP, et al. Corticotropin-releasing factor overexpression decreases ethanol drinking and increases sensitivity to the sedative effects of ethanol. Psychopharmacology 2004;176: 386–97.
Pandey SC, Zhang H, Ugale R, Prakash A, Xu T, Misra K. Effector immediate-early gene arc in the amygdala plays a critical role in alcoholism. J Neurosci 2008;28:2589–600.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1998.
Rigbi A, Kanyas K, Yakir A, Greenbaum L, Pollak Y, Ben-Asher E, et al. Why do young women smoke? V. Role of direct and interactive effects of nicotinic cholinergic receptor gene variation on neurocognitive function. Genes Brain Behav 2008;7:164–72.
Rodd ZA, Kimpel MW, Edenberg HJ, Bell RL, Strother WN, McClintick JN, et al. Differential gene expression in the nucleus accumbens with ethanol self-administration in inbred alcohol-preferring rats. Pharmacol Biochem Behav 2008;89:491–8.
Saito M, Smiley J, Toth R, Vadasz C. Microarray analysis of gene expression in rat hippocampus after chronic ethanol treatment. Neurochem Res 2002;27:1221–9.
Saito M, Szakall I, Toth R, Kovacs KM, Oros M, Prasad VV, et al. Mouse striatal transcriptome analysis: effects of oral self-administration of alcohol. Alcohol 2004;32: 223–41.
Samaha AN, Mallet N, Ferguson SM, Gonon F, Robinson TE. The rate of cocaine administration alters gene regulation and behavioral plasticity: implications for addiction. J Neurosci 2004;24:6362–70.
Samaha AN, Yau WY, Yang P, Robinson TE. Rapid delivery of nicotine promotes behavioral sensitization and alters its neurobiological impact. Biol Psychiatry 2005;57:351–60.
Schochet TL, Kelley AE, Landry CF. Differential expression of arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. Neurosci 2005;135:285–97.
Smyth GK. Linear models and empirical Bayes methods for assessing differntial expression in microarray experiments. Stat Appl Genet Mol Biol 2004;3(1).
Sommer W, Hyytia P, Kiianmaa K. The alcohol-preferring AA and alcohol-avoiding ANA rats: neurobiology of the regulation of alcohol drinking. Addict Biol 2006;11:289–309.
Stewart RB, McBride WJ, Lumeng L, Li T-K, Murphy JM. Chronic alcohol consumption in alcohol-preferring P rats attenuates subsequent conditioned taste aversion produced by ethanol injections. Psychopharmacology 1991;105:530–4.
Sun D, Huang W, Hwang YY, Zhang Y, Zhang Q, Li MD. Regulation by nicotine of Gpr51 and Ntrk2 expression in various rat brain regions. Neuropsychopharmacology 2007;32: 110–6.
Surowy CS, Hoganson G, Gosink J, Strunk K, Spritz RA. The human RD protein is closely related to nuclear RNA-binding proteins and has been highly conserved. Gene 1990;90:299–302.
Taracha E, Chrapusta SJ, Lehner M, Skorzewska A, Maciejak P, Szyndler J, et al. Morphine and methadone pre-exposure differently modify brain regional Fos protein expression and locomotor activity responses to morphine challenge in the rat. Drug Alcohol Depend 2008;97:21–32.
Treadwell JA, Singh SM. Microarray analysis of mouse brain gene expression following acute ethanol treatment. Neurochem Res 2004;29:357–69.
Waller MB, McBride WJ, Lumeng L, Li T-K. Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. Pharmacol Biochem Behav 1982;16:501–7.
Worst TJ, Tan JC, Robertson DJ, Freeman WM, Hyytia P, Kiianmaa K, et al. Transcriptome analysis of frontal cortex in alcohol-preferring and -non-preferring rats. J Neurosci Res 2005;80:529–38.
Xu Y, Ehringer M, Yang F, Sikela JM. Comparison of global brain gene expression profiles between inbred long-sleep and inbred short-sleep mice by high-density gene array hybridization. Alcohol Clin Exp Res 2001;25:810–8.
Xu K, Anderson TR, Neyer KM, Lamparella N, Jenkins G, Zhou Z, et al. Nucleotide sequence variation within the human tyrosine kinase B neurotrophin receptor gene: association with antisocial alcohol dependence. Pharmacogenomics 2007;7:368–79.
Yang MC, Weissler JC, Terada LS, Deng F, Yang YS. Pleiomorphic adenoma gene-like-2, a zinc finger protein, transactivates the surfactant protein-C promoter. Am J Respir Cell Mol Biol 2005;32:35–43.
Zhang J, Zhang L, Jiao H, Zhang Q, Zhang D, Lou D, et al. c-Fos facilitates the acquisition and extinction of cocaine-induced persistent changes. J Neurosci 2006;26:13287–96.