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Role of Adrenal Glucocorticoid Signaling in Prefrontal Cortex Gene Expression and Acute Behavioral Responses to Ethanol

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Abstract

Background—Glucocorticoid hormones modulate acute and chronic behavioral and molecular responses to drugs of abuse including psychostimulants and opioids. There is growing evidence that glucocorticoids might also modulate behavioral responses to ethanol. Acute ethanol activates the HPA axis, causing release of adrenal glucocorticoid hormones. Our prior genomic studies suggest glucocorticoids play a role in regulating gene expression in the prefrontal cortex (PFC) of DBA2/J (D2) mice following acute ethanol administration. However, few studies have analyzed the role of glucocorticoid signaling in behavioral responses to acute ethanol. Such work could be significant, given the predictive value for level of response to acute ethanol in the risk for alcoholism.

Methods—We studied whether the glucocorticoid receptor (GR) antagonist, RU-486, or adrenalectomy (ADX) altered male D2 mouse behavioral responses to acute (locomotor activation, anxiolysis or loss-of-righting reflex (LORR)) or repeated (sensitization) ethanol treatment. Whole genome microarray analysis and bioinformatics approaches were used to identify PFC candidate genes possibly responsible for altered behavioral responses to ethanol following ADX.

Results—ADX and RU-486 both impaired acute ethanol (2 g/kg) induced locomotor activation in D2 mice without affecting basal locomotor activity. However, neither ADX nor RU-486 altered initiation of ethanol sensitization (locomotor activation or jump counts), ethanol-induced anxiolysis or LORR. ADX mice showed microarray gene expression changes in PFC that significantly overlapped with acute ethanol-responsive gene sets derived by our prior microarray studies. Q-rtPCR analysis verified that ADX decreased PFC expression of *Fkbp5* while significantly increasing *Gpr6* expression. In addition, high dose RU-486 pre-treatment blunted ethanol-induced *Fkbp5* expression.

Conclusions—Our studies suggest that ethanol's activation of adrenal glucocorticoid release and subsequent GR activation may partially modulate ethanol's acute locomotor activation in male D2 mice. Furthermore, since adrenal glucocorticoid basal tone regulated PFC gene expression, including a significant set of acute ethanol-responsive genes, this suggests that glucocorticoid regulated PFC gene expression may be an important factor modulating acute behavioral responses to ethanol.

Keywords

Ethanol; Glucocorticoids; Acute Locomotor Activation; Gene Expression; Prefrontal Cortex

INTRODUCTION

Ethanol and other drugs of abuse acutely activate the hypothalamic pituitary adrenal (HPA) axis leading to glucocorticoid release (Piechota et al., 2010b), and alcoholics show a blunted HPA axis while drinking and upon withdrawal (Costa et al., 1996, Wand and Dobs, 1991). Glucocorticoid hormones are the final step in activation of the HPA axis and are known to function in the biological response to stress and circadian activity (Marinelli et al., 1997, De Kloet et al., 1998). Not only do glucocorticoids regulate the stress response, but their feedback regulation helps to terminate HPA activation (De Kloet et al., 1998).

Drug-induced locomotor stimulation is a common acute response to drugs of abuse, including ethanol, in rodents (Boehm et al., 2002, Phillips et al., 1992). It is suggested that ethanol's locomotor stimulation in rodents serves as a model of human ethanol-induced euphoria and arousal (Boehm et al., 2002, Palmer et al., 2002, Phillips et al., 1992). Behavioral sensitization, a long-lasting augmentation of the locomotor activating effects of a drug with repeated exposure, also occurs across different classes of addictive drugs, and represents a form of neural plasticity affecting chronic behavioral responses to ethanol and other drugs (Phillips et al., 1997b). Sensitization has been observed for psychostimulants, opioids, and ethanol, suggesting that drugs of abuse may exert effects on neural plasticity through shared mechanisms (Lessov and Phillips, 2003).

It has been suggested that HPA axis activation is a common pathway by which abused drugs induce neuroadaptations leading to sensitization (Deroche et al., 1995, Roberts et al., 1995, Piechota et al., 2010b). Studies have shown that the HPA axis plays a role in the sensitized response as stress can substitute for drug administration in cross sensitization studies and adrenalectomy (ADX) blocks the induction of psychostimulant locomotor sensitization (Roberts et al., 1995, Deroche et al., 1995). However, not all studies with ADX animals have reached the same conclusion. Badiani et al. found that ADX does not alter amphetamine sensitization (Badiani et al., 1995) and Prasad et al. showed that corticosterone does not mediate long term cocaine sensitization (Prasad et al., 1998).

Our laboratory and others have used genome-wide expression profiling to identify gene networks functioning in acute and chronic behavioral responses to ethanol (Kerns et al., 2005b, Treadwell and Singh, 2004, Wolstenholme et al., 2011). We previously identified a group of genes prominently regulated by acute ethanol in prefrontal cortex (PFC) of DBA2/J (D2) mice, but not C57BL/6 mice (Kerns et al., 2005a). Contained in this group were well-characterized glucocorticoid responsive genes such as *Fkbp5* and *Sgk1*. D2 mice show a robust locomotor activation response following acute ethanol administration, and reliably show locomotor sensitization upon repeated ethanol (Phillips et al., 1997b), whereas B6 mice do neither. Taken together, these results and prior work by Phillips and colleagues (Phillips et al., 1997a) suggests that glucocorticoid signaling might play a role in modulating acute ethanol locomotor activation and the initiation of locomotor sensitization. Here we have further studied the relationship between glucocorticoid signaling and acute ethanol locomotor activation. Our results show that glucocorticoid signaling and glucocorticoid-responsive genes may represent an important endophenotype modulating the acute response to ethanol.

MATERIALS AND METHODS

Animals

Mice were maintained in a temperature controlled room (23±1) with 12 h light/dark cycles and free access to standard chow (Harlan Teklad #7912, Madison, WI) and water. Cages and bedding (Harlan Sani-chips, #7090A, Harlan, Teklad, Madison, WI) were changed weekly. All tests were carried out between 0900 and 1200 h. Procedures were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the NIH Guide for the Care and Use of Laboratory Animals. All mice were DBA2/J mice from Jackson Laboratories (Bar Harbor, Maine) purchased at 10-13 weeks of age. ADX and sham mice were adrenalectomized or sham treated at Jackson and delivered following recovery from surgery. ADX mice were supplemented with 1% w/v saline solution in their drinking water. All mice were allowed to habituate to the animal facility for at least 1 week prior to testing.

Drugs

All drugs were administered intraperitoneally (i.p.). Saline solutions were 0.9% w/v sterile saline. Ethanol solutions were prepared from 200-proof absolute anhydrous ethanol (Pharmco-Aaper brand, Brookfield, CT). Ethanol was administered at 20% v/v in 0.9% saline. RU-486 (Sigma, St. Louis, MO), was dissolved by sonication in 20% w/v beta-cyclodextrin (Sigma, St. Louis, MO).

Behavioral Testing

Mice were habituated to injections with saline in their home cage for 2 days prior to experiments and allowed a 1-hour acclimation period to the behavioral room prior to testing. Locomotor activity was measured immediately following injection with either saline or ethanol during a 10-minute session in locomotor activity chambers (Med-Associates, model ENV-515; St. Albans, VT). Loss-of-righting assays were done following 3.8 g/kg ethanol administration. After ethanol injection, mice were returned to their home cage until they exhibited LORR as defined by the inability to right themselves three times in 30 seconds after placement in the supine position in a V-shaped trough. The duration of LORR was calculated by subtracting time of onset of LORR from the time at recovery from LORR. Anxiety testing was done using the light-dark transition model (Crawley and Goodwin, 1980). Five behavioral experiments were performed:

Exp. 1 -- Glucocorticoid receptor blockade by RU-486 and acute locomotor responses to ethanol—Six groups of mice (n = 7-9) were treated as follows: vehicle + saline (VS), vehicle + 2 g/kg ethanol (VE), 20 mg/kg RU + saline (RU20 S), 20 mg/kg RU + 2 g/kg ethanol (RU20 E), 35 mg/kg RU + saline (RU35 S), and 35 mg/kg RU + 2 g/kg ethanol (RU35 E). On test day 3, RU-486 was administered 30 minutes prior to ethanol or saline. Control mice received vehicle followed by saline or ethanol. Measurement of locomotor activity was performed as described above.

Exp. 2 -- Ethanol acute locomotor activation and sensitization following ADX—ADX and control animals (n=14-30) received one of three treatments: saline-saline (SS), saline-ethanol (SE) or ethanol-ethanol (EE) (Table 1). Animals were tested on day 3 for acute responses to either saline (SS, SE) or ethanol (EE). The dose of ethanol used for test days (days 3 and 14) was 2.0 g/kg. On days 4-13, animals received daily injections in their home cages of either saline (SS, SE) or 2.5 g/kg ethanol (EE). On test day 14 the SS group received saline, the SE group received ethanol and the EE group received ethanol as on day 3.

Exp. 3 -- Effects of RU-486 on initiation of ethanol sensitization—Four groups of D2 mice (n = 9) were treated according to the sensitization protocol and either vehicle or RU-486 pretreatment was administered prior to drug: vehicle + saline, RU + saline, vehicle + ethanol and RU + ethanol. On test day 3, vehicle or 25 mg/kg of RU-486 was administered 30 minutes prior to 2.0 g/kg ethanol or saline. Days 4-13 mice received vehicle + saline (VS) or 2.5 g/kg ethanol (VE), or 25 mg/kg RU-486 + saline (RS) or ethanol (RE). On day 14, only vehicle pretreatment was administered for all groups and locomotor activation was measured as described above in response to saline (VS, RS) or 2 g/kg ethanol (VE, RE).

Exp. 4 -- Anxiolytic-like response to ethanol following ADX—ADX and SHAM mice were tested in the light-dark transition model of anxiety. Testing took place in locomotor activity chambers described above with a black plastic partition inserted, dividing the chamber in half. SHAM and ADX mice (n=12-14) were restrained in a conical tube for 15 minutes, removed, injected with either saline or ethanol and placed into activity chambers facing the dark compartment.

Exp. 5 -- The Effect of ADX or RU-486 on Ethanol-Induced Loss of Righting Reflex (LORR)—Mice (n = 6-7) were either adrenalectomized or injected with 35 mg/kg RU-486 prior to injection with ethanol and LORR measurement. Treatment groups include: ADX, 3.8 g/kg ethanol; SHAM, 3.8 g/kg ethanol; RU-486, 3.8 g/kg ethanol; VEH, 3.8 g/kg ethanol. RU-486 and VEH were administered 30 minutes prior to ethanol.

Radioimmunoassay (RIA)

Four hours following ethanol or saline administration in behavioral Exp. 1, trunk blood was collected from individual mice. Serum was isolated by centrifugation at $2500 \times g$ for 15 minutes and stored at -80°C until RIA assay. A RIA containing I^{125} labeled corticosterone (MP Biomedicals, Cleveland, Ohio) was performed according to the manufacturer's instructions.

Brain Micropunch Dissection

Four hours following ethanol or saline administration in Exp. 1 at the time of blood collection, brain tissue was also collected. In a separate experiment, ADX and SHAM D2 mice (n = 4-5) were habituated to handling for two days, on day 3 they were sacrificed and brains were extracted. Collection of brain tissue occurred exactly as described in Kerns et al. (Kerns et al., 2005b). The medial prefrontal cortex (PFC) dissection contained tissue from dorsal and ventral anterior cingulate and some secondary motor cortex.

RNA Isolation and Microarray Hybridization

RNA was isolated from PFC tissue samples using the RNeasy Protect Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA concentration and quality was assessed by Experion automated electrophoresis (BioRad, Hercules, CA). 100ng total RNA was transcribed into double-stranded cDNA using the GeneChip® 3' IVT Express Kit (Affymetrix, Santa Clara, CA). Biotin-labeled cRNA was synthesized from cDNA, purified and fragmented according to the manufacturer's instructions. Labeled cRNA from individual animals (n=3/group) was hybridized to a single microarray. Samples were analyzed with oligonucleotide arrays (Affymetrix Mouse Genome 430A 2.0 array) with array hybridization and scanning performed according to the manufacturer.

Microarray and Bioinformatics Analysis

Microarray data were processed using GeneChip Operating Software v4.1 (GCOS, Affymetrix) and normalized to a mean total hybridization intensity of 190. Array quality was assessed by accepting arrays with scaling factor < 3 , 3'-5' actin ratio < 2 , and by examining chip validity and linearity. Chip-chip correlations all exceeded 0.98 and percent present calls by the Affymetrix GCOS software were greater than 58% for all arrays. Probesets with RMA expression values < 3.5 consistently across all microarrays were filtered to reduce variance from low expressing genes. Differential gene expression between ADX and SHAM treated mice was assessed using the R (R Development Core Team, 2011) implementation of the S-score algorithm (Kennedy et al., 2006). For a single gene, an $|S\text{-score}| > 2$ corresponds to a $p\text{-value} < 0.05$. We used the Benjamini-Hochberg false discovery rate (FDR) method to calculate $q\text{-values}$ for all $S\text{-scores}$. The differential expression of genes with $q\text{-values} < 0.05$ were considered statistically significant. Additional bioinformatics analyses were performed using Toppgene (<http://toppgene.cchmc.org/>) with a Bonferroni correction and $p\text{-value}$ cutoff of 0.05.

Quantitative Real Time Polymerase Chain Reaction (Q-rtPCR)

cDNA was generated from 1 μg total RNA by reverse transcription with the iScript CDNA kit (Bio-Rad, Hercules, CA) following the manufacturer's instructions. Q-rtPCR was performed using the iCycler iQTM system (Bio-Rad) according to the manufacturer's instructions for SYBR Green I-based detection. Quantification of gene expression levels was determined based on the threshold cycle for each well using the provided software and all results were normalized to multiple reference genes using Genorm as described in Vandesompele et al. (Vandesompele et al., 2002). Primers used were as follows, *Fkbp5* (Forward-*GCCGACTGTGTGTGTAATGC* and Reverse-*CACAATACGCACCTTGGGAGA*), *Gpr6* (Forward-*CCTCATCTTACACTTCGTGTTCC* and Reverse-*GGTCCACTGTGATAGCAAGCA*), *Gapdh* (Forward-*TTCCAGTATGACTCCACTCACGG* and Reverse-*TGAAGACACCAGTAGACTCCACGAC*), *Ppp2r2a* (Forward-*ATCTCTCACCCCTTGCCCTTT* and Reverse-*CCCATTTTGTGTGCTTTCGT*), *Ublcp1* (Forward-*ATGACAGGGACAGGACAAGC* and Reverse-*TACAATGACACCCGACTGGA*), and *Ndufv1* (Forward-*GACCGTGCTAATGGACTTCG* and Reverse-*GGCATCTCCCTTCACAAATC*).

Statistics

Data were expressed as mean $\pm$ SEM and analyzed parametrically. Data were analyzed with analysis of variance (ANOVA) or Kruskal-Wallis One Way Analysis of Variance on Ranks (jump counts) using appropriate between-and within subject factors. All post hoc comparisons were made using Student Newman-Kuel's test or Dunn's test (rank transformed, uneven sample number analysis). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Ethanol-induced acute locomotor response following RU-486

The effect of two different doses of RU-486 on ethanol-induced locomotor activation was tested in Exp. 1. A one-way ANOVA showed a significant effect of treatment ($F_{5,42} = 20.88$, $p < 0.01$). Post-hoc analysis identified no difference between VS, RU20 S or RU35 S groups showing that RU-486 had no effect on basal locomotor activity (Fig. 1A). A significant increase in locomotor activity compared to saline controls was found with VE, RU20 E and RU35 E treatments, confirming locomotor activation following acute ethanol.

VE and RU20 E groups showed significantly greater locomotor activity compared to RU35 E treated animals indicating that higher doses of RU-486 blunted the locomotor activating effect of ethanol while having no effect on basal activity. In examining corticosterone levels in mice following RU treatment, a one-way ANOVA showed a significant effect of treatment ($F_{5,26} = 13.42$, $p < 0.01$) and post-hoc analysis revealed significant increases in corticosterone levels in animals treated with R20 E, R35 S and R35 E compared to those treated with VS, VE and R20 S (Fig. 1B).

Acute locomotor activation and sensitization following adrenalectomy

Acute and repeated ethanol exposure was studied in a sensitization protocol (Table 1) with SHAM and ADX treated D2 mice. A mixed model repeated measures ANOVA (phenotype $\times$ treatment $\times$ time) showed an overall effect of time ($F_{1,112} = 212.14$, $p < 0.01$) indicating that both SHAM and ADX treated mice sensitized to the locomotor activating effects of ethanol (Fig. 2A.). A two-way ANOVA (phenotype $\times$ treatment) evaluating animals on just day 3 of sensitization studies showed a significant effect of phenotype ($F_{1,115} = 6.20$, $p < 0.01$), treatment ($F_{1,115} = 167.61$, $p < 0.01$) and a significant interaction ($F_{1,115} = 4.87$, $p < 0.03$). Both ADX and control animals showed locomotor activation following ethanol treatment. Post-hoc analysis identified significant differences between ethanol treatment in ADX vs. SHAM, with ADX animals exhibiting a blunted locomotor activation response. This suggests that ADX, like treatment with RU-486, blunted the ethanol-induced acute locomotor activation response while having no effect on basal locomotor activity. A two-way ANOVA (phenotype $\times$ treatment) evaluating animals on day 14 of sensitization studies showed a significant effect of phenotype ($F_{1,112} = 5.40$, $p < 0.05$) and treatment ($F_{1,112} = 153.45$, $p < 0.01$), but no significant interaction. Post-hoc analysis showed that while both SHAM and ADX animals sensitized to the locomotor activating effects of ethanol, chronically ethanol treated ADX mice showed a blunted locomotor activation response on day 14 of sensitization, similar to day 3. These results suggest that ADX blunted the locomotor activating properties of ethanol in naïve and sensitized animals, but had no effect on sensitization per se.

SHAM and ADX mice treated with repeated ethanol also sensitized to jump counts (Fig. 2B.). Both groups of mice showed significant increases in jump counts on day 14 of sensitization studies compared to day 3. Acute ethanol (day 3 or day 14) completely abrogated jumping, whereas chronically ethanol treated mice showed jump counts similar to saline controls.

Initiation of ethanol sensitization following RU-486 treatment

To determine whether direct glucocorticoid receptor blockade altered the initiation of ethanol sensitization, we pre-treated mice with RU-486 (25 mg/kg) or vehicle prior to saline or ethanol injections during the initiation phase of sensitization (Table 1). From our earlier acute studies (Fig. 1), we hypothesized 25 mg/kg RU-486 was a dose of drug that would antagonize the GR without producing the effects on acute locomotor activity seen at 35 mg/kg in Fig. 1. A mixed model repeated measures ANOVA showed a significant effect of treatment ($F_{3,32} = 182.02$, $p < 0.01$), day ($F_{1,32} = 47.98$, $p < 0.01$) and a significant interaction (treatment $\times$ day) ($F_{3,32} = 20.42$, $p < 0.01$) (Fig. 3A.). Post-hoc analysis revealed that both vehicle and RU pre-treated mice showed significant increases in locomotor activation following ethanol treatment on day 3 of sensitization studies. Both groups of mice also showed significant increases in locomotor activation on day 14 of sensitization studies compared to day 3, indicating both groups of mice sensitized to the locomotor activating effects of ethanol. There were no differences between RU and VEH pre-treated animals on day 14 of sensitization studies, indicating that RU-486 had no effect on the initiation of the sensitized response. Like SHAM and ADX treated animals, RU pre-treated animals also

sensitized to jump counts (Fig. 3B.). Both groups of mice showed significant increases in jump counts on day 14 compared to day 3.

Ethanol-induced anxiolysis and LORR responses to ADX or RU-486

To determine if glucocorticoid receptor blockade or adrenalectomy affected acute responses to ethanol other than locomotor activity, we tested anxiolytic-like responses to ethanol (2g/kg) or incoordinating responses to ethanol (3.8 g/kg). A two-way ANOVA (phenotype $\times$ treatment) revealed an overall effect of treatment for % distance traveled in the light ($F_{1,49} = 17.80$, $p < 0.01$) and % time spent in the light ($F_{1,49} = 24.46$, $p < 0.01$), but no differences between phenotypes and no interaction (Figs. 4A-B). Post-hoc analysis showed that both ethanol-treated ADX and SHAM animals spent a significantly greater percentage of time and traveled a larger distance in the light compared to saline treated control animals. A student's t-test between ADX vs. SHAM mice and RU-486 vs. VEH pre-treated mice revealed no significant differences in the time taken to recover the righting reflex (Fig. 4C., D.).

Microarray analysis of ADX-induced changes in PFC gene expression

Data presented above suggests that glucocorticoid signaling modulates acute locomotor activation by ethanol. To initiate studies on the brain mechanisms that might underlie this action, we performed a study of gene expression in PFC regulated by ADX. We reasoned that ADX-responsive genes might be involved in the mechanism of glucocorticoid modulation of acute ethanol locomotor activation. We chose PFC for expression studies due to the known feedback interaction between PFC and dopamine signaling (Durstewitz et al., 2000); because PFC is well characterized as a glucocorticoid-responsive brain region (Mizoguchi et al., 2003); and our prior microarray studies showed induction of a number of glucocorticoid-responsive genes by acute ethanol in PFC (Kerns et al., 2005b).

Microarray analysis of PFC identified 269 probesets (255 genes) with significant basal differences between SHAM and ADX mice. These included 75 probesets up-regulated and 194 down-regulated by adrenalectomy (Supplementary Table 1). Q-rtPCR was used to validate select genes from the microarray results. G protein-coupled receptor 6 (*Gpr6*) was significantly increased in ADX mice (Fig. 5A) while FK506 binding protein 5 (*Fkbp5*) expression was decreased by adrenalectomy (Fig. 5B) (student's t-test, $p < 0.05$). Bioinformatics functional over-representation analysis revealed significant differences between SHAM and ADX animals in gene groups involved in nerve impulse transmission and genes regulated by the synthetic glucocorticoid, dexamethasone (Table 2). Of particular interest, the entire ADX regulated set of genes had a highly significant overlap ($p = 1.8 \times 10^{-8}$) with those identified in our prior microarray studies on acute ethanol with D2 mice (Kerns et al., 2005b). These overlapping genes included: heat shock 70kDa protein (*Hspa8*), potassium large conductance calcium-activated channel, subfamily M, alpha member 1 (*Kcnma1*), phosphatase and tensin homolog (*Pten*), proteolipid protein 1 (*Plp1*), early growth response 2 (*Egr2*), early growth response 1 (*Egr1*), and *Fkbp5* (Table 2, *). Of note, all these genes showed significant decreased expression in PFC of ADX mice, except for *Plp1*, which had increased expression. Acute ethanol increased the expression of all these genes in our prior studies. Thus, except for *Plp1*, there was the expected inverse relationship between our prior microarray results on ethanol responses and results from ADX mice. These results not only demonstrate that ADX alters PFC gene expression, but that there is a potentially causal relationship between ethanol-evoked glucocorticoid release and at least a subset of acute ethanol-responsive genes in PFC.

To provide further initial evidence for the role of glucocorticoids in acute ethanol-responsive PFC gene expression, we determined whether RU-486 would block acute ethanol induction

of *Fkbp5*, one of the genes mentioned above as overlapping between our microarray studies on acute ethanol and ADX, and which functions as a critical regulator of glucocorticoid receptor function (Sinars et al., 2003). Q-rtPCR analysis of *Fkbp5* mRNA levels in PFC four hours following ethanol or saline administration (+/- RU-486) was done with animals from behavioral Exp. 1. One-way ANOVA showed an overall significant effect of treatment ($F_{5,22} = 8.99$, $p < 0.01$) (Fig. 5C.). Post-hoc analysis showed that RU pre-treatment had no effect on basal *Fkbp5* levels. Ethanol significantly increased *Fkbp5* levels compared to VS and RU-486 at the higher dose (RU35) blocked this response. These results indicated that higher doses of RU-486 blunted both the acute locomotor activating effects of ethanol and ethanol-induced *Fkbp5* expression.

DISCUSSION

Defining the mechanisms underlying acute behavioral responses to ethanol has significant biomedical implications since acute responses to ethanol serve as a predictor of risk for alcoholism or excessive ethanol intake (Schuckit, 1994, Metten et al., 1998). Our prior genomic studies (Kerns et al., 2005b) suggest that glucocorticoid signaling in the PFC might play an important role in brain gene expression responses to acute ethanol. Here we characterized the role of glucocorticoid signaling in responses to acute ethanol, ethanol sensitization, and in regulating PFC gene expression. We found that ADX and RU-486 both impair acute ethanol induced locomotor activation, but do not alter the induction of ethanol sensitization or basal locomotor activity. Further, ADX altered expression of genes in PFC that include a significant number of previously identified ethanol responsive genes. Overall, this work points to a previously uncharacterized mechanism modifying acute locomotor responses to ethanol and identifies an important role for glucocorticoid signaling in ethanol-responsive gene expression.

One prior study showed that ADX reduces ethanol stimulated locomotor activity in female C3H mice (Wallis et al., 1984). However, neither the effects of GR antagonists nor adrenalectomy on the acute locomotor activating effects of ethanol have been investigated in D2 mice, the strain most widely studied for motor responses to ethanol. We found that both higher doses of RU-486 and ADX blunted the acute locomotor activating effects of ethanol. In addition, acute ethanol activated the HPA axis leading to significant increases in corticosterone as soon as 5 minutes following ethanol administration (Supplementary Fig. 3a.). Thus, ethanol-induced glucocorticoid release could partially modulate ethanol-induced locomotor activation. The work presented here does not differentiate whether glucocorticoids mediate a portion of ethanol-induced locomotor activation, or simply provide a permissive effect in such a response to ethanol. However, since both RU and ADX treatments only affected a portion of the ethanol locomotor activation response, this clearly suggests a permissive effect. Future work will be needed to directly test this hypothesis.

Our results are consistent with prior studies on other drugs of abuse. ADX reduces the acute psychomotor stimulant effects of cocaine (Marinelli et al., 1997, Deroche et al., 1997) and amphetamine (Cador et al., 1993) in the rat and the locomotor response to psychomotor stimulants in ADX animals can be restored by corticosterone administration (Cador et al., 1993, Marinelli et al., 1997). Discrepancies in the psychostimulant literature do exist, as a more recent study found that GR inactivation in the nervous system did not affect acute cocaine locomotor activation in mice, suggesting that the acute locomotor effects of cocaine do not require GR signaling (Deroche-Gamonet et al., 2003). But it has been suggested that these divergent findings are due to methodological differences (Marinelli and Piazza, 2002).

Both RU-486 and ADX would affect more than just the glucocorticoid system as RU-486 is an antagonist of both the progesterone receptor (PR) and GR (Chrousos et al., 1988).

Further, ADX alters the synthesis of numerous steroid hormones, in particular corticosterone and aldosterone, which both interact with the mineralocorticoid receptor (Piechota et al.). ADX also removes adrenal medullary catecholamines such as epinephrine. However, the adrenal medulla does not provide the body's only source of catecholamines and RU-486 presumably has no effect on catecholamine action and only minimal binding affinity for the MR (Ricordi et al., 1988). Thus, the combined use of both ADX and RU-486 in our studies increases the likelihood that the alterations in ethanol-induced locomotor activation with these treatments were due to GR actions. Additional evidence for a role of the GR comes from our studies of RU-486 and ethanol effects on circulating corticosterone levels. Acting through hypothalamic and pituitary GR, RU-486 disrupts negative feedback loops responsible for returning corticosterone levels to normal following HPA axis activation (De Kloet et al., 1998). We only found significant basal RU-486 induced increases in circulating corticosterone at the higher dose of drug (35 mg/kg), which was also the only dose inhibiting ethanol-induced locomotor activation.

Studies on other drugs of abuse also support GR rather than MR as a mediator of the locomotor response. Blockade of MRs by spironolactone had no significant effects on locomotion induced by systemic morphine (Marinelli et al., 1998). Still, the possibility that other steroid hormones play a role in ethanol-induced locomotor activation cannot be ignored.

ADX or RU-486 effects on ethanol pharmacokinetics could be an alternative explanation for our results with ethanol-induced locomotor activation. Gililand and Finn showed that ADX in D2 mice produced slightly increased ethanol clearance rates following 4 g/kg ethanol administration, but had no effect on peak BEC (Gililand and Finn, 2007). We found slight decreases in peak BEC following 2 g/kg ethanol in ADX D2 mice (Supplementary Fig. 1). However, decreased ethanol locomotor activation was seen with both ADX and RU-486 treatment and RU-486 pre-treatment did not alter ethanol metabolism (Supplementary Fig. 2). We also found that ADX and RU pre-treatment had no effect on ethanol-induced LORR or anxiolysis (Fig. 4), indicating that ADX and RU selectively altered acute ethanol-induced locomotor activation. This combination of data strongly suggests that pharmacokinetic factors do not totally explain ethanol locomotor activation behavioral differences in ADX animals.

Our finding that RU-486 and ADX did not block initiation of behavioral sensitization (Figs. 2-3), were contrary to previously published findings showing that RU-486 blunted initiation of ethanol behavioral sensitization (Roberts et al., 1995). Some of the discrepancies could be due to differences in experimental design. Roberts et. al. used female D2 mice, a shorter protocol with lower doses of ethanol and lower doses of RU-486 (Roberts et al., 1995). Interestingly, we found that not only did all groups of mice sensitize to the locomotor activating effects of ethanol, but they also sensitized to jump counts. To our knowledge, this is the first report of ethanol sensitization to jumping behavior.

We hypothesized that basal differences in gene expression may be partially responsible for the observed behavioral differences between SHAM and ADX animals following acute ethanol administration. Our microarray studies showed prominent changes in basal gene expression of ADX animals in PFC, a brain region known to be sensitive to glucocorticoids (Mizoguchi et al., 2003), and a significant proportion of these were identified as ethanol-responsive genes in PFC in our prior studies. For example, *Fkbp5* was decreased basally in ADX mice whereas previously it was found to be induced by ethanol in D2 PFC (Kerns et al., 2005b). Our studies do not address the potential contribution of gene expression changes in other brain regions to the behavioral differences found in ADX mice. Of note, *Fkbp5* regulates glucocorticoid receptor sensitivity and polymorphisms in *Fkbp5* lead to a

dysregulated stress response and have been found in individuals with depression, bipolar disorder and post-traumatic stress disorder (Binder, 2009).

In addition, pre-treatment with doses of RU-486 that blunted ethanol locomotor activation also diminished ethanol induced *Fkbp5* expression (Fig. 5C). This overlap between ADX, RU-486 and ethanol-sensitive gene expression is consistent with our prior hypothesis that a subset of acute ethanol-responsive genes in PFC were possibly due to involvement of GR signaling. Our results also suggest that such GR and ethanol-responsive genes in PFC may play a role in modulating aspects of basal acute sensitivity to ethanol.

Chronic ethanol actions on the HPA axis, producing a dysregulated stress response, could contribute to long-term aberrant behavior in alcoholism via modulation of brain gene expression, particularly in PFC. Alcohol dependent individuals do show stress hypo-responsiveness (Lovallo et al., 2000). Additionally, HPA axis dysregulation exists in alcohol dependent patients and individuals with a familial history of alcoholism. Individuals who are low in sociability with a familial history of alcohol dependence show blunted cortisol responses (Sorocco et al., 2006). Individuals at high risk for alcoholism also show lower basal ACTH levels, lower stress-induced increases in plasma ACTH concentration, and delayed post-stress recovery of plasma ACTH and cortisol (Dai et al., 2002). Thus, disrupted stress responses may contribute to the risk for development of alcohol dependence, and our findings suggest that altered basal gene expression in PFC could be a mediating factor. Our work here clearly indicates that future studies are needed to further characterize the inter-relations of glucocorticoid signaling, prefrontal gene expression networks, acute responses to ethanol and mechanisms underlying risk for alcohol dependence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Badiani A, Morano MI, Akil H, Robinson TE. Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. *Brain Res.* 1995; 673:13–24. [PubMed: 7757465]
- Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology.* 2009; 34(Suppl 1):S186–195. [PubMed: 19560279]
- Boehm SL 2nd, Reed CL, McKinnon CS, Phillips TJ. Shared genes influence sensitivity to the effects of ethanol on locomotor and anxiety-like behaviors, and the stress axis. *Psychopharmacology (Berl).* 2002; 161:54–63. [PubMed: 11967631]
- Cador M, Dulluc J, Mormede P. Modulation of the locomotor response to amphetamine by corticosterone. *Neuroscience.* 1993; 56:981–988. [PubMed: 8284048]
- Chrousos GP, Laue L, Nieman LK, Kawai S, Udelsman RU, Brandon DD, Loriaux DL. Glucocorticoids and glucocorticoid antagonists: lessons from RU 486. *Kidney Int.* 1988; (Suppl 26):S18–23.

- Costa A, Bono G, Martignoni E, Merlo P, Sances G, Nappi G. An assessment of hypothalamo-pituitary-adrenal axis functioning in non-depressed, early abstinent alcoholics. *Psychoneuroendocrinology*. 1996; 21:263–275. [PubMed: 8817725]
- Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav*. 1980; 13:167–170. [PubMed: 6106204]
- Dai X, Thavundayil J, Gianoulakis C. Response of the hypothalamic-pituitary-adrenal axis to stress in the absence and presence of ethanol in subjects at high and low risk of alcoholism. *Neuropsychopharmacology*. 2002; 27:442–452. [PubMed: 12225701]
- De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocr Rev*. 1998; 19:269–301. [PubMed: 9626555]
- Deroche V, Marinelli M, Le Moal M, Piazza PV. Glucocorticoids and behavioral effects of psychostimulants. II: cocaine intravenous self-administration and reinstatement depend on glucocorticoid levels. *J Pharmacol Exp Ther*. 1997; 281:1401–1407. [PubMed: 9190876]
- Deroche V, Marinelli M, Maccari S, Le Moal M, Simon H, Piazza PV. Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. *J Neurosci*. 1995; 15:7181–7188. [PubMed: 7472472]
- Deroche-Gamonet V, Sillaber I, Aouizerate B, Izawa R, Jaber M, Ghazland S, Kellendonk C, Le Moal M, Spanagel R, Schutz G, Tronche F, Piazza PV. The glucocorticoid receptor as a potential target to reduce cocaine abuse. *J Neurosci*. 2003; 23:4785–4790. [PubMed: 12805318]
- Durstewitz D, Seamans JK, Sejnowski TJ. Dopamine-mediated stabilization of delay period activity in a network model of prefrontal cortex. *J Neurophysiol*. 2000; 83:1733–1750. [PubMed: 10712493]
- Gililand KR, Finn DA. The impact of gonadectomy and adrenalectomy on acute withdrawal severity in male and female C57BL/6J and DBA/2J mice following a single high dose of ethanol. *Alcohol Clin Exp Res*. 2007; 31:1846–1857. [PubMed: 17850218]
- Kennedy RE, Kerns RT, Kong X, Archer KJ, Miles MF. SScore: an R package for detecting differential gene expression without gene expression summaries. *Bioinformatics*. 2006; 22:1272–1274. [PubMed: 16574698]
- Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, Williams RW, Miles MF. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci*. 2005a; 25:2255–2266. [PubMed: 15745951]
- Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, Williams RW, Miles MF. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci*. 2005b; 25:2255–2266. [PubMed: 15745951]
- Lesso CN, Phillips TJ. Cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine and cocaine in mice. *Alcohol Clin Exp Res*. 2003; 27:616–627. [PubMed: 12711924]
- Lovallo WR, Dickensheets SL, Myers DA, Thomas TL, Nixon SJ. Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcohol Clin Exp Res*. 2000; 24:651–658. [PubMed: 10832906]
- Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. *Eur J Neurosci*. 2002; 16:387–394. [PubMed: 12193179]
- Marinelli M, Rouge-Pont F, Deroche V, Barrot M, De Jesus-Oliveira C, Le Moal M, Piazza PV. Glucocorticoids and behavioral effects of psychostimulants. I: locomotor response to cocaine depends on basal levels of glucocorticoids. *J Pharmacol Exp Ther*. 1997; 281:1392–1400. [PubMed: 9190875]
- Metten P, Phillips TJ, Crabbe JC, Tarantino LM, McClearn GE, Plomin R, Erwin VG, Belknap JK. High genetic susceptibility to ethanol withdrawal predicts low ethanol consumption. *Mamm Genome*. 1998; 9:983–990. [PubMed: 9880664]

- Mizoguchi K, Ishige A, Aburada M, Tabira T. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience*. 2003; 119:887–897. [PubMed: 12809708]
- Palmer AA, Miller MN, McKinnon CS, Phillips TJ. Sensitivity to the locomotor stimulant effects of ethanol and allopregnanolone is influenced by common genes. *Behav Neurosci*. 2002; 116:126–137. [PubMed: 11895174]
- Phillips TJ, Burkhart-Kasch S, Gwiazdon CC, Crabbe JC. Acute sensitivity of FAST and SLOW mice to the effects of abused drugs on locomotor activity. *J Pharmacol Exp Ther*. 1992; 261:525–533. [PubMed: 1578369]
- Phillips TJ, Roberts AJ, Lessov CN. Behavioral sensitization to ethanol: genetics and the effects of stress. *Pharmacol Biochem Behav*. 1997a; 57:487–493. [PubMed: 9218273]
- Phillips TJ, Roberts AJ, Lessov CN. Behavioral sensitization to ethanol: genetics and the effects of stress. *Pharmacol Biochem Behav*. 1997b; 57:487–493. [PubMed: 9218273]
- Piechota A, Lipinska S, Szymraj J, Goraca A. Long-term melatonin administration enhances the antioxidant potential of human plasma maintained after discontinuation of the treatment. *Gen Physiol Biophys*. 2010a; 29:144–150. [PubMed: 20577026]
- Piechota M, Korostynski M, Solecki W, Gieryk A, Slezak M, Bilecki W, Ziolkowska B, Kostrzewa E, Cymerman I, Swiech L, Jaworski J, Przewlocki R. The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. *Genome Biol*. 2010b; 11:R48. [PubMed: 20459597]
- Prasad BM, Ulibarri C, Sorg BA. Stress-induced cross-sensitization to cocaine: effect of adrenalectomy and corticosterone after short- and long-term withdrawal. *Psychopharmacology (Berl)*. 1998; 136:24–33. [PubMed: 9537679]
- Ricordi C, Shah SD, Lacy PE, Clutter WE, Cryer PE. Delayed extra-adrenal epinephrine secretion after bilateral adrenalectomy in rats. *Am J Physiol*. 1988; 254:E52–53. [PubMed: 3257359]
- Roberts AJ, Lessov CN, Phillips TJ. Critical role for glucocorticoid receptors in stress⁵ and ethanol-induced locomotor sensitization. *J Pharmacol Exp Ther*. 1995; 275:790–797. [PubMed: 7473168]
- Schuckit MA. Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry*. 1994; 151:184–189. [PubMed: 8296886]
- Sinars CR, Cheung-Flynn J, Rimerman RA, Scammell JG, Smith DF, Clardy J. Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes. *Proc Natl Acad Sci U S A*. 2003; 100:868–873. [PubMed: 12538866]
- Sorocco KH, Lovallo WR, Vincent AS, Collins FL. Blunted hypothalamic-pituitary¹² adrenocortical axis responsivity to stress in persons with a family history of alcoholism. *Int J Psychophysiol*. 2006; 59:210–217. [PubMed: 16360227]
- Treadwell JA, Singh SM. Microarray analysis of mouse brain gene expression following acute ethanol treatment. *Neurochem Res*. 2004; 29:357–369. [PubMed: 15002731]
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome biology*. 2002; 3 RESEARCH0034.
- Wallis CJ, Anton RF, Randall CL. Adrenalectomy reduces alcohol-stimulated activity: blood and brain alcohol content. *Pharmacol Biochem Behav*. 1984; 20:883–886. [PubMed: 6540448]
- Wand GS, Dobs AS. Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. *Journal of Clinical Endocrinology and Metabolism*. 1991; 72:1290–1295. [PubMed: 2026749]
- Wolstenholme JT, Warner JA, Capparuccini MI, Archer KJ, Shelton KL, Miles MF. Genomic analysis of individual differences in ethanol drinking: evidence for non³ genetic factors in C57BL/6 mice. *PloS one*. 2011; 6:e21100. [PubMed: 21698166]

Fig. 1.

Antagonism of glucocorticoid receptor function by RU-486 alters acute locomotor responses to ethanol and produces compensatory changes in serum corticosterone. Panel A: RU-486 at 35 mg/kg (RU 35), but not 20 mg/kg (RU 20), blunted the acute locomotor activating effects of ethanol in D2 mice. * $p < 0.05$ vs. all saline-treated animals; # $p < 0.05$ vs. VEH ethanol and RU 20 ethanol. Panel B: Corticosterone levels were determined 4 hours after behavioral testing. Corticosterone levels were significantly elevated in animals treated with RU 35 followed by saline or animals treated with RU 20 or RU35 following by ethanol. * $p < 0.05$ vs. VEH treated groups and RU 20 S; # $p < 0.05$ vs. RU 35 S.

Fig. 2.

Adrenalectomy (ADX) alters acute ethanol locomotor activation but not ethanol sensitization. Panel A: ADX significantly blunted the acute locomotor activating response to ethanol, but not locomotor sensitization. On day 3, locomotor responses were reduced in the ADX ethanol-treated group compared to sham controls. * $p < 0.05$ vs. day 3 Saline-Saline treated mice; † $p < 0.05$ vs. Day 3 Ethanol-Ethanol treated SHAM mice; § $p < 0.05$ vs. day 14 Saline-Saline treated mice; # $p < 0.05$ vs. day 3 Ethanol-Ethanol treated mice, Saline-Saline treated mice and Saline-Ethanol treated mice; § $p < 0.05$ vs. Day 14 Ethanol-Ethanol treated SHAM mice. Panel B: SHAM and ADX mice also sensitized to Jump Counts. * $p < 0.05$ vs. SHAM Saline-Ethanol Day 14, ADX Saline-Ethanol Day 14, SHAM Ethanol-Ethanol Day 3, ADX Ethanol-Ethanol Day 3; § $p < 0.05$ vs. SHAM Saline-Ethanol Day 14, ADX Saline-Ethanol Day 14, SHAM Ethanol-Ethanol Day 3, ADX Ethanol-Ethanol Day 3; # $p < 0.05$ vs. SHAM Saline-Ethanol Day 14, ADX Saline-Ethanol Day 14, SHAM Ethanol-Ethanol Day 3, ADX Ethanol-Ethanol Day 3; § $p < 0.05$ vs. SHAM Saline-Ethanol Day 14, ADX Saline-Ethanol Day 14, SHAM Ethanol-Ethanol Day 3; † $p < 0.05$ vs. SHAM Saline-Ethanol Day 14, ADX Saline-Ethanol Day 14, SHAM Ethanol-Ethanol Day 3; ^ $p < 0.05$ vs. SHAM Saline-Ethanol Day 14.

Fig. 3.

Pretreatment with 25 mg/kg RU-486 had no effect on the initiation of ethanol sensitization as measured via locomotor activity and jump counts. Panel A: Pretreatment with 25 mg/kg RU-486 had no effect on the initiation of ethanol sensitization as measured via locomotor activation. * $p < 0.05$ vs. Day 3 Saline-Saline treated mice; # $p < 0.05$ vs. Day 3 Ethanol-Ethanol treated mice. Panel B: VEH and RU-486 Ethanol-Ethanol pretreated mice also sensitized to Jump Counts. * $p < 0.05$ vs. VEH Ethanol-Ethanol Day 3, RU Ethanol-Ethanol Day 3; \$ $p < 0.05$ vs. VEH Ethanol-Ethanol Day 3 and RU Ethanol-Ethanol Day 3; # $p < 0.05$ vs. VEH Ethanol-Ethanol Day 3, RU Ethanol-Ethanol Day 3; § $p < 0.05$ vs. VEH Ethanol-Ethanol Day 3, RU Ethanol-Ethanol Day 3; † $p < 0.05$ vs. VEH Ethanol-Ethanol Day 3, RU Ethanol-Ethanol Day 3; ^ $p < 0.05$ vs. VEH Ethanol-Ethanol Day 3, RU Ethanol-Ethanol Day 3.

Fig. 4.

Acute ethanol-responsive behaviors that do not differ between VEH vs. RU or ADX vs. SHAM treated mice. Panel A: Both SHAM and ADX ethanol-treated mice spent significantly greater time in the light than their respective saline-treated counterparts. There were no differences in the % Time Spent in the Light between SHAM and ADX treated mice. Panel B: Both SHAM and ADX ethanol-treated mice traveled significantly greater distance in the light than their respective saline-treated counterparts. There were no differences in the % Distance Traveled in the Light between SHAM and ADX treated mice. Panel C: Pretreatment with 35 mg/kg RU-486 (RU 35) had no effect on ethanol-induced loss of righting reflex (LORR). Panel D: ADX had no effect on ethanol-induced loss of righting reflex (LORR). * $p < 0.05$ vs. respective saline-treated mice.

Fig. 5. Genes identified through microarray and S-score analysis to be significantly different in SHAM vs. ADX treated D2 mice and RU-486 pre-treated D2 mice. Panel A: *Gpr6* was identified and confirmed as significantly increased in ADX vs. SHAM treated mice. * $p < 0.05$ vs. SHAM treated mice. Panel B: *Fkbp5* was identified and confirmed as significantly decreased in ADX vs. SHAM treated mice. * $p < 0.05$ vs. SHAM treated mice. Panel C: Pre-treatment with higher doses of the GR antagonist RU-486 blunted ethanol-induced *Fkbp5* expression. RU pre-treatment had no effect on basal *Fkbp5* levels. * $p < 0.05$ vs. VS, RU20 S, RU35 S, RU35 E. # $p < 0.05$ vs. VS and RU20 S.

Table 1

Outline of experimental design for ethanol sensitization

Phase of sensitization		Initiation		Expression
Group	Days 1-2	Day 3	Days 4-13	Day 14
	Habituation	Activity test	Conditioning	Activity Test
SS	Saline	Saline	Saline	Saline
SE	Saline	Saline	Saline	Ethanol 2.0 g/kg
EE	Saline	Ethanol 2.0 g/kg	Ethanol 2.5 g/kg	Ethanol 2.0 g/kg

Table 2

Gene ontology over-representation analysis for ADX microarray results

Ontological Category	Gene Symbol	Gene Name	Entrez Gene ID	q-value; s-score
Nerve Impulse Transmission	HRH1	histamine receptor H1	3269	0.0169; 4.01
	HSPA8*	heat shock 70kDa protein 8	3312	0.0030; -4.52
	SLC6A13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	6540	< 0.001; 5.30
	NTRK2	neurotrophic tyrosine kinase, receptor, type 2	4915	0.0500; -3.62
	KCNMA1*	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	3778	0.0257; -3.87
	KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	3791	0.0022; 4.62
	CLDN5	claudin 5	7122	< 0.001; 7.24
	SOD1	superoxide dismutase 1, soluble	6647	0.0126; -4.11
	CACNG3	calcium channel, voltage-dependent, gamma subunit 3	10368	0.0257; 3.88
	RAB11A	RAB11A, member RAS oncogene family	8766	0.0013; -4.75
	RAB14	RAB14, member RAS oncogene family	51552	0.0080; -4.26
	CNP1	2',3'-cyclic nucleotide 3' phosphodiesterase	1267	0.0482; 3.63
	CPLX2	complexin 2	10814	0.0318; -3.79
	SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	6507	0.0259; 3.87
	SLC1A2	solute carrier family 1 (glial high affinity glutamate transporter), member 2	6506	0.0009; -4.87
	HOMER1	homer homolog 1 (Drosophila)	9456	0.0004; -5.03
	ATXN1	ataxin 1	6310	0.0109; -4.15
	KIF1B	kinesin family member 1B	23095	< 0.001; -6.42
	PTEN*	phosphatase and tensin homolog	5728	0.0453; -3.66
	CACNA1A	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	773	0.0061; -4.34
	CREB1	cAMP responsive element binding protein 1	1385	0.0075; -4.28
	SYN2	synapsin II	6854	0.0274; -3.84
	PLP1*	proteolipid protein 1	5354	0.0244; 3.89

Ontological Category	Gene Symbol	Gene Name	Entrez Gene ID	q-value; s-score
	EGR2	early growth response 2	1959	< 0.001; -5.81
	EGR1*	early growth response 1	1958	0.0014; -4.74
	HTR2C	5-hydroxytryptamine (serotonin) receptor 2C	3358	0.0441; -3.68
	ARC	activity-regulated cytoskeleton-associated protein	23237	0.0005; -5.01
	GRIA1	glutamate receptor, ionotropic, AMPA 1	2890	0.0036; -4.48
Dexamethasone	DCN	decorin	1634	0.0001; 5.30
	HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	3290	< 0.001; 5.65
	HRH1	histamine receptor H1	3269	0.0169; 4.01
	HSPA8*	heat shock 70kDa protein 8	3312	0.0030; -4.52
	KCNMA1*	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	3778	0.0257; -3.83
	KCNB1	potassium voltage-gated channel, Shab-related subfamily, member 1	3745	< 0.001; -6.35
	SOD1	superoxide dismutase 1, soluble	6647	0.0126; -4.11
	FKBP5*	FK506 binding protein 5	2289	0.0380; -4.12
	PLIN2	perilipin 2	123	0.0480; 3.64
	APCDD1	adenomatosis polyposis coli down-regulated 1	147495	0.0316; 3.80
	EPHX2	epoxide hydrolase 2, cytoplasmic	2053	0.0274; 3.84
	EDNRB	endothelin receptor type B	1910	0.0200; 3.95
	S100A9	S100 calcium binding protein A9	6280	0.0005; -5.01
	CACNA2D1	calcium channel, voltage-dependent, alpha 2/delta subunit 1	781	0.0009; -4.84
	SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	6817	< 0.001; -7.05
	CXCL12	chemokine (C-X-C motif) ligand 12	6387	0.0365; 3.74
	TTR	transthyretin	7276	< 0.001; 9.17
	FOS	FBJ murine osteosarcoma viral oncogene homolog	2353	< 0.001; -8.72
	EGR1	early growth response 1	1958	0.0014; -4.74
	NR2C2	nuclear receptor subfamily 2, group C,	7182	< 0.001; -6.11

Ontological Category	Gene Symbol	Gene Name	Entrez Gene ID	q-value; s-score
		member 2		
	TPM1	tropomyosin 1 (alpha)	7168	0.0244; -3.90