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Chronic intermittent ethanol exposure selectively alters the expression of Gα subunit isoforms and RGS subtypes in rat prefrontal cortex

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Abstract

Chronic alcohol exposure induces pronounced changes in GPCR-mediated G-protein signaling. Recent microarray and RNA-seq analyses suggest associations between alcohol abuse and the expression of genes involved in G-protein signaling. The activity of G-proteins (e.g. Gαi/o and Gαq) is negatively modulated by regulator of G-protein signaling (RGS) proteins which are implicated in drugs of abuse including alcohol. The present study used 7 days of chronic intermittent ethanol exposure followed by 24 hour withdrawal (CIE) to investigate changes in mRNA and protein levels of G-protein subunit isoforms and RGS protein subtypes in rat prefrontal cortex, a region associated with cognitive deficit attributed to excessive alcohol drinking. We found that this ethanol paradigm induced differential expression of Gα subunits and RGS subtypes. For example, there were increased mRNA and protein levels of Gαi1/3 subunits and no changes in the expression of Gαs and Gαq subunits in ethanol-treated animals. Moreover, CIE increased the mRNA but not the protein levels of Gαo. Additionally, a modest increase in Gαi2 mRNA level by CIE was accompanied by a pronounced increase in its protein level. Interestingly, we found that CIE increased mRNA and protein levels of RGS2, RGS4, RGS7 and RGS19 but had no effect on the expression of RGS5, RGS6, RGS8, RGS12 or RGS17. Changes in the expression of Gα subunits and RGS subtypes could contribute to the functional alterations of certain GPCRs following chronic ethanol exposure. The present study suggests that RGS proteins may be potential new targets for intervention of alcohol abuse via modification of Gα-mediated GPCR function.

Keywords

chronic intermittent ethanol; Gαi; Gαo; RGS proteins

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The authors declare no conflict of interest

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1. Introduction

Alcohol use disorder is characterized by repeated episodes of excessive alcohol consumption and withdrawal in parallel with profound neuroadaptations in many brain regions including the prefrontal cortex (PFC) (Mcewen, 2013). Dysregulation of neurotransmission in the PFC is associated with learning and memory deficits and subsequent loss of control of drinking behavior in alcoholics (Moselhy *et al.*, 2001). Microarray and RNA-seq studies indicate that the expression of numerous genes involved in receptor function and receptor-mediated G-protein signaling are altered in the frontal cortex of human alcoholics (Lewohl *et al.*, 2000, Mayfield *et al.*, 2002, Warden & Mayfield, 2017). These changes likely mediate adaptive changes in neuronal function in response to alcohol exposure. Animal models of alcohol abuse have shown complex changes in the PFC, including functions of many G-protein coupled receptors (GPCRs) and voltage-gated ion channels (Johnson & Lovinger, 2016, Nimitvilai *et al.*, 2017), which interact with modulatory, heterotrimeric G-protein signaling pathways. G-proteins are divided into four major subfamilies based on Gα-subunit sequence homology and their specific interactions with downstream effector proteins: the Gαi/o family inhibits adenylyl cyclase, the Gαs family stimulates adenylyl cyclase, the Gαq/11 family stimulates phospholipase Cβ, and the Gα12/13 family activates rhoGEP and other effectors (Milligan, 1993, Nurnberg *et al.*, 1995). Neurotransmitter receptors are functionally associated with Gαi/o (e.g. dopamine D2/D3 receptors, serotonin 5-HT1A/1B receptors and cannabinoid 1B receptors), Gαs (dopamine D1/D5 receptors and adrenergic β1/β2 receptors) or Gαq (serotonin 5-HT2 receptors, adrenergic α1 receptors and metabotropic glutamate receptors group 1). Changes in the expression of Gα subunit isoforms likely impact the function of GPCRs coupled to these G-proteins. Thus, one goal of this study was to investigate whether chronic and intermittent ethanol exposure followed by 24 hours of withdrawal (CIE) had differential effects on the gene and protein expression of Gα subunit isoforms. Information learned would help understand the molecular mechanisms underlying altered functions of GPCRs coupled to different isoforms of G-proteins in animal models of chronic ethanol exposure.

Receptor-mediated G-protein signaling is regulated by regulators of G-protein signaling (RGS) proteins. RGS proteins directly bind to activated Gα subunits to accelerate GTP hydrolysis and subsequently terminate receptor's response to stimuli (Berman *et al.*, 1996). There are at least 20 identified subtypes of RGS proteins in the mammalian brain (Siderovski & Willard, 2005). The mRNAs of RGS subtypes are distributed in a brain-region and neuron-type dependent manner (Gold *et al.*, 1997, Han *et al.*, 2006), suggesting that subtypes of RGS proteins may confer specificity of actions to selective GPCRs. Thus, changes in the expression of RGS proteins would influence the duration and strength of receptor-mediated G-protein signaling. It has been shown that the mRNA levels of many RGS subtypes are sensitive to acute and chronic psychostimulant exposure (Sun *et al.*, 2015, Chen Hemby, 2014). Altered levels of RGS proteins are associated with changes in receptor signal transduction and addiction behavior [see review in (Chen & Hemby 2014)]. To date, limited reports indicate that certain RGS subtypes are also sensitive to alcohol exposure in a brain-region dependent manner. For example, RGS4 mRNA level is significantly lower in the superior frontal gyrus and the primary motor cortex of human alcoholic brains (Ho *et al.*,

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2010). Two RGS17 single nucleotide polymorphisms identified in drug addicts were associated with a pronounced reduction in RGS17 mRNA level and dependence on multiple substances including alcohol (Zhang *et al.*, 2012). Moreover, RGS6 knockout mice exhibit reduced ethanol drinking behavior (Stewart *et al.*, 2015). Investigation of ethanol-induced changes in RGS protein expression will not only help understand alterations in GPCR function but also provide potential new avenues for pharmacological interventions for alcohol abuse. Thus, the second goal of the present study was to examine the expression of RGS subtypes in rat PFC in response to CIE treatment.

The chronic intermittent ethanol vapor model has been widely used in rodent studies as a tool to approximate the repeated cycles of heavy consumption and withdrawal which are common characteristics of alcoholics (Becker & Hale, 1993; Budygin et *al.*, 2007; Morales et *al.*, 2016). Typically, the chronic intermittent ethanol treatment involves prolonged ethanol exposure (e.g. 4–6 weeks) in rodents; however, shorter length of repeated cycle of ethanol intoxication and withdrawal have also been frequently used to produce a marked behavioral phenotype that parallels human alcohol use disorder and is characterized by pronounced negative affect, seizure sensitization, and increased reward seeking behavior (Becker & Hale, 1993, Mccown & Breese, 1990, Roberts *et al.*, 1996, Valdez *et al.*, 2002). Further, this shorter duration of intermittent ethanol treatment is sufficient to alter signaling of various types of GPCRs (e.g. α1 adrenergic receptors, cannabinoid 1B receptors, dopamine D2/D3 receptors) in the CNS (Karkhanis et *al.*, 2015; McElligott et *al.*, 2010; Varodayan et *al.*, 2016; Robinson *et al.*, 2016). Thus, it is useful for studying the effect of ethanol on expression of molecular components involved in signaling of various GPCRs. In the present study, we adopted 7 days of CIE followed by 24 hrs of withdrawal because our unpublished data showed that this ethanol exposure produces increased anxiety-like behavior and presynaptic glutamate release on inputs arriving from the stria terminalis that are characteristic of animals following chronic and intermittent ethanol exposure (Morales et *al.*, 2015; McGinnis et *al.*, 2017). Using this ethanol exposure paradigm, we found that it differentially regulated the gene and protein expression of Gα subunits and RGS proteins in a subtype-dependent manner in rat PFC.

2. Results

2.1. CIE differentially regulates the gene and protein expression of G-protein alpha subunit isoforms in the PFC

To determine the effect of CIE on gene expression of Gα subunits, we performed qPCR to assess the mRNA levels of G-protein subunits including Gαi1, Gαi2, Gαi3, Gαo, Gαs and Gαq (Fig. 1). Data were normalized to the mRNA level of β-actin, which was not altered by ethanol treatment. A two-way ANOVA revealed significant main effects of ethanol treatment ($F_{1,81}$= 27.11, $p<0.01$), Gα subunit isoform ($F_{5,81}$ = 2.951, $p=0.017$) and interaction ($F_{5,81}$ =3.00, $p=0.02$). A posthoc Bonferroni test showed that CIE significantly increased the mRNA levels of Gαi1 ($p<0.01$), Gαi3 ($p<0.01$) and Gαo ($p<0.05$) when compared to air-exposed tissue.

We further examine whether the increased mRNA levels of Gα subunits translated into the protein levels (Fig. 2). The intensity of individual Gα subunit bands was normalized by the

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β-actin band which did not differ between the air and CIE groups. Because the antibodies against Gαi1 and Gαi3, respectively, recognize both proteins, we measured the levels of Gαi1/3 together instead of their individual levels. A two-way ANOVA indicated significant main effects of ethanol treatment ($F_{1,53} = 17.08$, $p<0.01$), Gα subunit isoform ($F_{4,53} = 4.853$, $p<0.01$) and interaction ($F_{4,53} = 4.911$, $p<0.01$). A posthoc Bonferroni test showed that CIE significantly increased Gαi1/3 levels in the PFC ($p<0.01$), which is consistent with their mRNA levels. Moreover, although CIE caused a slight, but not significant increase in Gαi2 mRNA levels, Gαi2 protein levels were significantly enhanced in these animals ($p<0.01$). However, the increased mRNA levels of Gαo did not result in an increase in its protein level. There was no change in protein levels of Gβ subunits in the PFC (data not shown).

2.2. CIE differentially regulates the gene and protein expression of RGS subtypes in the PFC

We also examined the impact of CIE on gene expression of RGS subtypes in the PFC including RGS2, RGS4, RGS5, RGS6, RGS7, RGS8, RGS12, RGS17 and RGS19 (Fig. 3). A two-way ANOVA indicated significant main effects of ethanol treatment ($F_{1,119} = 32.31$, $p<0.01$), RGS subtype ($F_{8,119} = 2.51$, $p=0.01$) and interaction ($F_{8,119} = 2.66$, $p=0.01$). A posthoc Bonferroni test showed that CIE significantly increased the mRNA levels of RGS2 ($p<0.05$), RGS4 ($p<0.01$), RGS7 ($p<0.05$) and RGS19 ($p<0.01$) when compared to the air-exposed tissue.

Because there is a lack of validated antibodies for many RGS subtypes, it is not feasible to examine the protein expression of every RGS subtype in the PFC. We used four validated RGS antibodies against RGS2 (Luessen *et al.*, 2016), RGS4 (Schwendt *et al.*, 2006), RGS7 (Orlandi et al., 2015) and RGS19 (Ji et al., 2010) to assess their protein levels (Fig. 4). A two-way ANOVA revealed significant main effects of ethanol treatment ($F_{1,40} = 75.78$, $p<0.01$), RGS subtype ($F_{3,40} = 5.88$, $p=0.01$) and interaction ($F_{3,40}) = 5.94$, $p<0.01$). A posthoc Bonferroni test showed that CIE significantly increased the protein levels of RGS2 ($p<0.01$), RGS4 ($p<0.01$), RGS7 ($p<0.01$) and RGS19 ($p<0.01$) in rat PFC when compared to the air-exposed tissue.

3. Discussion

In the present study, we showed that repeated and intermittent ethanol exposure followed by 24 hrs withdrawal differentially regulated the gene and protein expression of G-protein subunit isoform and RGS subtypes in rat PFC. Because Gα subunits and RGS proteins are integral part of GPCR signaling, our findings may help understand molecular mechanisms that underlie altered GPCR function and GPCR-mediated behavioral response to alcohol.

Chronic ethanol exposure alters the function of many GPCRs in the brain including receptors for dopamine, serotonin, glutamate, GABA, acetylcholine, cannabinoid and opioid systems, and these changes are often associated with relapse [see review in (Haass-Koffler *et al.*, 2014)]. These receptors are coupled to different Gα subunit isoforms which play differential and specific roles in regulation of receptors and channels involved in ethanol effects. For example, Gαo binds to neuronal G-protein-gated inwardly rectifying potassium

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(GIRK) channel (Clancy *et al.*, 2005), which is implicated in ethanol reward. Moreover, μ-opioid receptor-mediated Gαi/o activation is significantly reduced in the prefrontal cortex of rats after chronic ethanol consumption (Sim-Selley et *al.*, 2002). Additionally, LTD induction by Gαq-coupled mGluR1/5 receptors is disrupted in mouse hippocampus after CIE exposure (Wills *et al.*, 2017). A potential mechanism underlying dysregulated GPCR function in response to ethanol exposure is altered expression of Gα subunits that are coupled to GPCRs. For example, increased Gαo and Gαs protein levels in rat cortex are associated with decreased adenylyl cyclase activity after chronic ethanol exposure (Wenrich et *al.*, 1998). Moreover, chronic ethanol consumption increased Gαi2 and Gαi/3 protein expression which was paralleled by decreased adenylyl cyclase activity in mouse cerebellum (Wand et al., 1993). However, the reported effects of ethanol on G-protein expression in the literature have not been consistent due to variations in the length and dose of ethanol treatment, duration of withdrawal and brain regions (see Table 1). We found that 7 days of CIE treatment selectively altered the gene and/or protein expression of Gαi1/2/3 and Gαo expression in the PFC and had no effect on Gαs and Gαq, suggesting that Gαi/o subunits are more susceptible to ethanol treatment and may contribute to altered functions of certain GPCRs coupled to Gαi/o proteins following ethanol exposure. It is important to note that a proper ratio of Gα to Gβγ subunit is necessary for receptor and receptor-mediated channel activity. For example, overexpression of various types of Gα subunits alone can sequester Gβγ subunits and diminish interactions between G-protein subunits and their signaling pathways (Jeong & Ikeda, 1999). The present study showed that CIE increased the protein expression of Gαi/o subunits without altering Gβ subunit in the present study. Thus, the differential regulation of the expression of Gαi/o and Gβγ subunits in the CIE paradigm could be a potential mechanism underlying reduced signaling by GPCRs following chronic ethanol. On the other hand, changes in GPCR function following ethanol exposure can also be independent of Gα subunit expression. For example, CIE profoundly reduces dopamine D2 receptor-mediated activation of GIRK channels in the absence of significant alterations in Gαi/o subunit expression in mouse orbitofrontal cortex (Nimitvilai et *al.*, 2017). In this later instance, altered GPCR function following CIE could be due to changes in Gα-interacting proteins such as RGS proteins; this mechanism has not been extensively investigated in the alcohol field.

RGS proteins are negative modulators of GPCRs and comprise more than 20 members in the CNS. Changes in RGS protein levels are thus an important molecular mechanism underlying altered Gα-mediated GPCR signaling. We have reported previously that the gene expression of RGS subtypes is sensitive to psychostimulant exposure (Sun *et al.*, 2015). The present study demonstrates that ethanol exposure also significantly influences the expression of RGS proteins in the PFC. CIE increased the mRNA and protein levels of RGS2, RGS4, RGS7 and RGS19. RGS2 is known to modulate the signaling of D2 receptor-mediated Gαi/o protein (Luessen *et al.*, 2016) and the coupling between GABA receptors and GIRK channels (Labouebe *et al.*, 2007). Thus, increased RGS2 protein level may contribute to ethanol-induced dysregulation of GPCR and GIRK channel activity by rapidly uncoupling of activated Gα subunits and GPCR/channel. Moreover, it is well established that RGS4 and RGS19 negatively regulate the signaling of numerous GPCRs including mu opioid receptors (MORs), serotonin 5-HT1A receptors(5-HT1ARs), and dopamine D2 receptors (D2Rs) in a

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variety of experimental systems (Wang et *al*., 2013, Gu et *al*., 2007; Jeanneteau et *al*., 2004). Notably, chronic ethanol exposure alters the signaling of MORs, 5-HT1AR and D2Rs (Sim-Selley *et al*., 2002; Kelai et *al*., 2008; Martinez et *al*., 2005). RGS4 and RGS19 up-regulation following CIE may play a significant role in mediating the effects of chronic ethanol exposure on signaling by these receptors. Although the role of RGS7 in the pathophysiology of alcohol abuse is unclear, RGS7 is critical in mediating sensitivity to cocaine-induced locomotor activity in rodents (Anderson et *al*., 2010) and Gα-mediated GIRK activation (Saitoh et al., 1999). Thus, increased RGS7 expression in the PFC could also play an important role in regulating neuronal excitability and receptor-mediated signaling after chronic ethanol exposure. In addition to their established role in Gα-mediated signaling pathways, RGS proteins also negatively regulate Gβγ-mediated modulation of GIRK channel and N-type voltage-dependent calcium channel activity (Ikeda & Dunlap, 1999, Wickman & Clapham, 1995). Therefore, overexpression of RGS proteins not only accelerates termination of Gα-mediated GPCR activity but also regulates signaling by Gβγ. Future studies will investigate the effects of altered RGS proteins on specific interactions of Gα/βγ-RGS proteins which may underlie ethanol-induced alterations in GPCR/channel activity.

We are aware that PFC tissue samples used for qPCR and Western blot in the present study contained both neurons and glial cells. Neuronal and glial cells, such as astrocytes, express different RGS and G-protein subtypes and the function of these proteins are sensitive to changes under various physiological conditions (Endale et al., 2008; Pedram et al., 2000). Future studies should investigate changes of these proteins in a cell-type dependent manner. Additionally, the present study employs a 7-day CIE paradigm followed by 24 hr withdrawal, which does not allow us to distinguish the effect of repeated and intermittent ethanol treatment from 24 hr withdrawal. Notably, many biochemical effects of CIE, such as decreased expression of the α1 or γ2 subunits of $GABA_A$ receptors in rat basolateral amygdala and hippocampus are persist for some time during withdrawal (Cagetti et *al*., 2003; Diaz et *al*., 2011). However, other effects, like alterations in GABAA receptor δ subunit mRNA, return quickly back to baseline (Follesa et *al*., 2015). Therefore, it is necessary to determine both the individual contributions of ethanol exposure and withdrawal and how persistent these effects may be during protracted withdrawal periods.

In conclusion, CIE/withdrawal differentially regulates the expression of Gα subunit isoforms and RGS subtypes. In the PFC, expression of Gαi and Gαo subunits are more sensitive to ethanol treatment compared to Gαs and Gαq. Ethanol treatment also has pronounced effects on the expression of several RGS proteins. These findings suggest a molecular mechanism for literature showing that chronic ethanol dramatically alters GPCR function. Specifically, increased Gαi/o subunit and/or RGS protein levels could uncouple G-protein pathways resulting in decreased receptor activity. Whether changes in RGS proteins occur as a direct consequence of ethanol exposure or as a compensatory response to ethanol-mediated modulation of neurotransmitter systems remains to be determined.

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4. Experimental procedures

4.1. Animals

Male Sprague Dawley rats obtained from Envigo (Indianapolis, IN) were pair-housed and maintained on a 12:12 h light/dark cycle (lights on at 9 PM) with food and water *ad libitum*. At the time of tissue preparation, animals were approximately 10–11 weeks of age (300–350 g). All animal care procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Wake Forest Animal Care and Use Committee.

4.2. Ethanol exposure

Ethanol exposure was carried out by ethanol inhalation using a method similar to that used in other studies (Lack *et al.*, 2007, Morales *et al.*, 2015). Animals were placed into air-tight, Plexiglas chambers (Triad Plastics, Winston-Salem, NC) in their home cages and exposed to either ethanol vapor (CIE) or room air (Air) during the light cycle (12 h/day) for 7 consecutive days. Ethanol vapor, produced by submerging an air stone in 95% ethanol, was mixed with room air and was pumped into the chambers at a rate of 16 L/min. Ethanol levels were checked daily and were maintained at ~35 mg/L within the chamber. All CIE animals were euthanized following a 24 hr withdrawal period after the last ethanol exposure.

4.3. BEC measurement

Tail blood samples were taken periodically throughout the exposure to monitor blood ethanol concentrations (BECs) and allow for adjustment of ethanol vapor as necessary. BECs were determined using a commercially available assay kit (Carolina Liquids Chemistries, Winston-Salem, NC). Mean BECs from tail blood were 196.92 ± 48.31 mg/dL.

4.4. RNA extraction

Brains were rapidly removed after sacrifice and stored at −80°C. Bilateral PFC was hand-dissected using a rat brain block from the slice ~2.5 – 4.5 mm anterior to bregma and included the whole prelimbic cortex and part of the infralimbic and cingulate cortices as previously described (Reichel et al., 204) (see Supplemental Fig. 1). The total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA). Contaminating genomic DNA was removed by DNase I digestion using DNA-free RNA kit (ZYMO Research, Irvine, CA, USA). Quality and concentrations of RNA were checked and measured using NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific).

4.5. Reverse transcription

Reverse transcription was performed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Total RNA (100–500 ng) was converted to single stranded cDNA. Reverse transcription without reverse transcriptase was also performed to assess genomic DNA contamination.

4.6. Real-time polymerase chain reaction (qPCR)

Primers were designed using qPCR primer design software from Integrated DNA Technology and reported previously (Sun *et al.*, 2015). The qPCR was performed using All-

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in-One qPCR SYBR Green Master Mix (GeneCopoeia Inc., Rockville, MD, USA) in a 96-well format on an ABI PRISM 7500 Fast real-time PCR System (Applied Biosystems, Forester City, CA, USA). PCR reactions contained 0.2 μM of primers and 20 ng of reverse transcribed total RNA in 20 μL. PCR was performed with an initial 3-min denaturation at 95°C followed by 40 cycles of PCR (15 s at 95°C, 20 s at 60°C and 15 s at 72°C). Under the condition of qPCR, melt curve analysis performed at the end of qPCR reproducibly showed a single peak for each gene in each sample. The relative change in the target gene expression was analyzed using 2 CT method as described previously (Livak & Schmittgen, 2001). Samples containing no cDNA template and no reverse transcriptase were run as negative controls for contamination and amplification of genomic DNA, respectively. All samples were run in triplicate. For each gene, qPCR reactions for the air- and ethanol-exposed groups were run concurrently on the same 96-well plate. The mRNA levels were normalized to the average of the housekeeping gene actin. Data were represented relative to the air-exposed animals.

4.7. Western blot

The levels of Gαi/o, Gαq and Gαs subunits were measured on membranes prepared from the PFC tissue of air- and ethanol-exposed rats. Briefly, tissue was homogenized in cold hypotonic buffer (20 mM HEPES, pH 7.4, 10 mM KCl, 2 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and Protease Inhibitor Cocktail) followed by centrifugation at 16,000 × g for 15 min at 4°C. Supernatants were collected and centrifuged at 80,000 × g for 1 hr at 4°C. The pellet was washed in homogenization buffer and resuspended in TBS with 0.1% SDS. Western blotting was performed to assess the levels of specific Gα subunits using the following antibodies from Santa Cruz: mouse anti-Gαi2 (1:250, sc-13534), mouse anti-Gαi1/3 (1:250, sc-365422), Rabbit anti-Gαo (1:500, sc-387), rabbit anti-Gαq (1:250, sc-365906) and mouse anti-Gαs (1:250, sc-823). After incubation with mouse IgGκ BP-HRP secondary (1:5000, sc-516102). Immunoreactive bands were revealed by chemiluminescent substrate. Densitometric analysis was conducted using ImageJ (NIH). The immunoreactive bands were normalized to β-actin which was probed by goat anti-β-actin (1:1000, sc-1615), and expressed as relative to air-exposed samples.

Western blotting was also performed to measure the levels of RGS2, RGS4, RGS7 and RGS19 in total lysate from the PFC tissue of air- and ethanol-exposed rats using the following primary antibodies: mouse anti-RGS2 (1:500, SAB1406388, Sigma-Aldrich), rabbit anti-RGS4 (1:500, #15129, Cell Signaling Technology), rabbit anti-RGS19 (1:100, ab173878, Abcam) and rabbit anti-RGS7 (1:1000, a generous gift from Dr. William Simonds, NIH/NIDDK). Data were normalized to β-actin and expressed as relative to air-exposed samples.

4.8. Statistical analysis

Graph Pad Prism (version 6, La Jolla, CA, USA) was used for statistical analyses. A two-way analysis of variance (ANOVA) was performed followed by a posthoc Bonferroni's multiple comparison tests to examine the effect of CIE on the targeted gene and protein expression. Statistical significance was set at $p < 0.05$.

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Supplementary Material

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Abbreviations

GPCR	G-protein coupled receptor
RGS	Regulator of G-protein signaling
GIRK	G protein-coupled inwardly-rectifying potassium channel
CIE	Chronic intermittent ethanol/24 hr withdrawal
PFC	Prefrontal cortex
LTD	long-term depression

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Highlights

- Seven days of intermittent ethanol exposure followed by 24 hrs withdrawal (CIE) increased the mRNA levels of Gαi1, Gαi3 and Gαo in rat PFC
- CIE increased the protein levels of Gαi1/3 and Gαi2 and had no effect on Gαs, Gαq and Gαo in rat PFC
- CIE increased the mRNA and protein levels of RGS2, RGS4, RGS7 and RGS19 and had no effect on RGS5, RGS8, RGS12 and RGS17 in rat PFC

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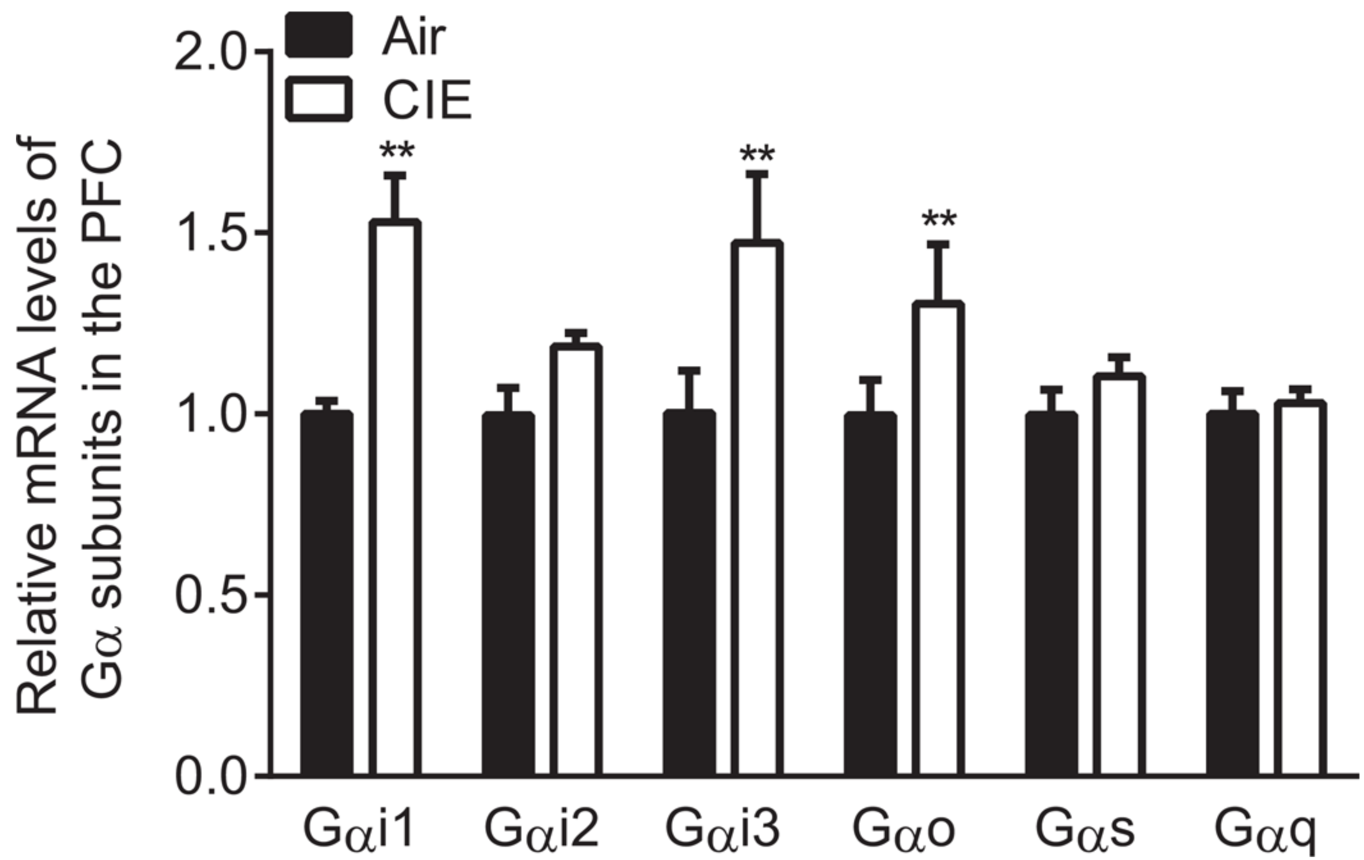

Figure 1.

The differential effects of CIE on the mRNA levels of Gα subunits in rat PFC. CIE significantly increased the mRNA levels of Gαi1, Gαi3 and Gαo and had no significant effect on Gαs and Gαq. Data were normalized to the actin gene and are presented as mean ± SEM relative to air-exposed animals. *$p<0.05$, **$p<0.01$ vs. the air-exposed group. N=6-7/group

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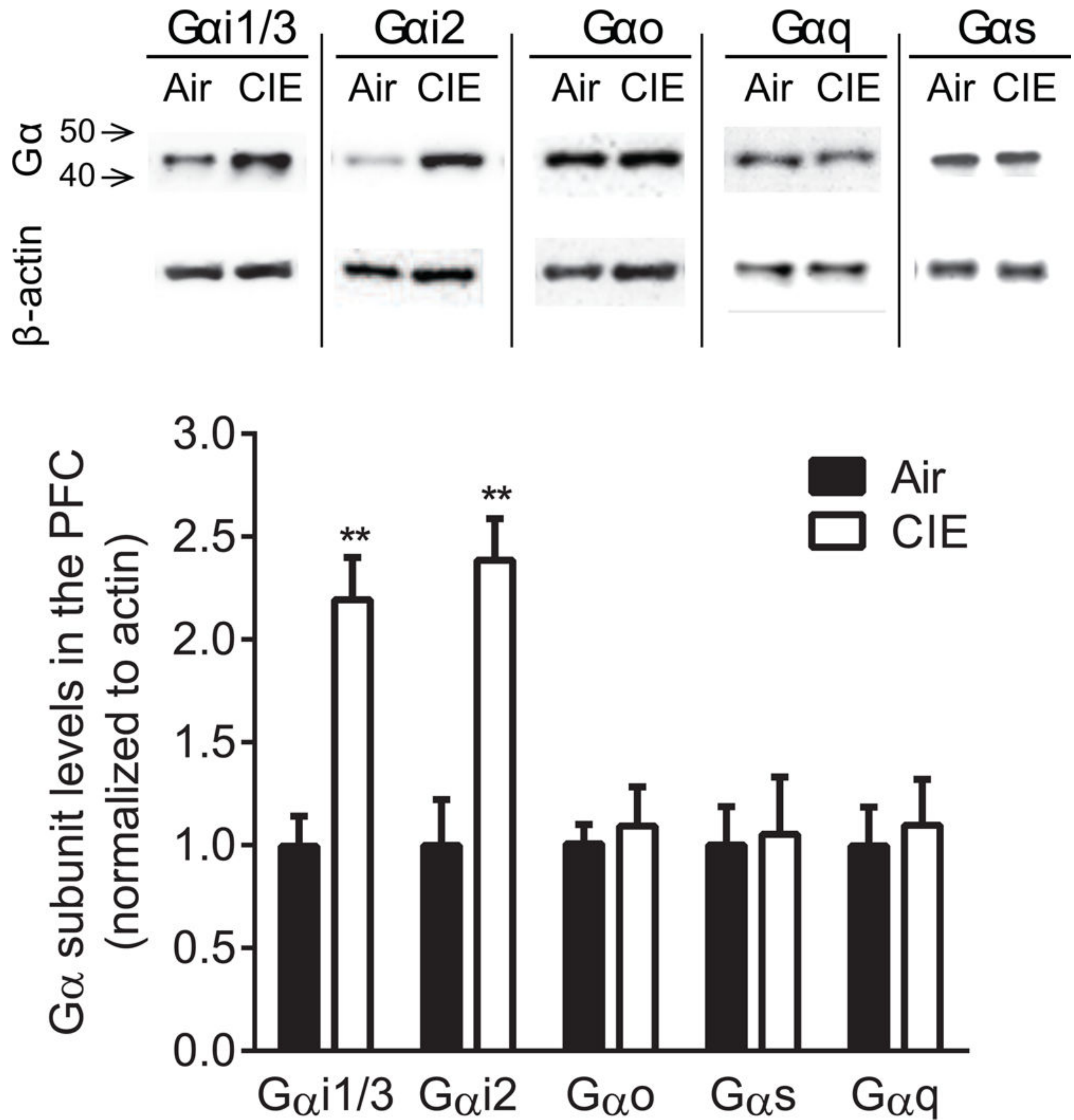

Figure 2.

The differential effects of CIE on the protein levels of Gα subunits in rat PFC. CIE significantly increased the protein levels of Gαi1/3 and Gαi2. Representative blots are shown. Data were normalized to actin and are presented as mean ± SEM relative to air-exposed animals. **$p<0.01$ vs. the air-exposed group. N=6/group

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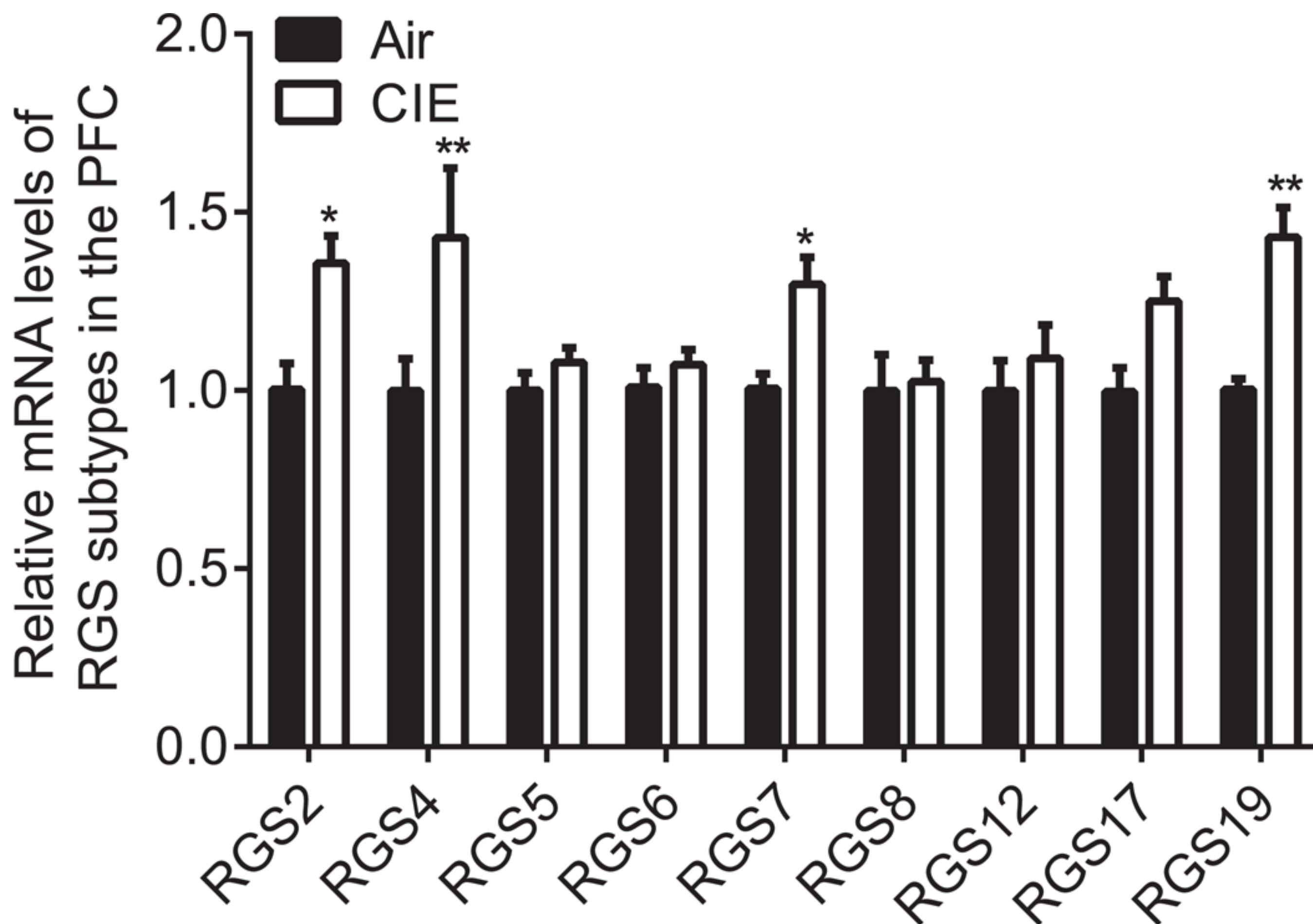

Figure 3.

The differential effects of CIE on the mRNA levels of RGS subtypes in rat PFC. CIE significantly increased the mRNA levels of RGS2, RGS4, RGS7 and RGS19. Data were normalized to the actin gene and are presented as mean ± SEM relative to air-exposed animals. *$p<0.05$, **$p<0.01$ vs. the air-exposed group. N=6–7/group

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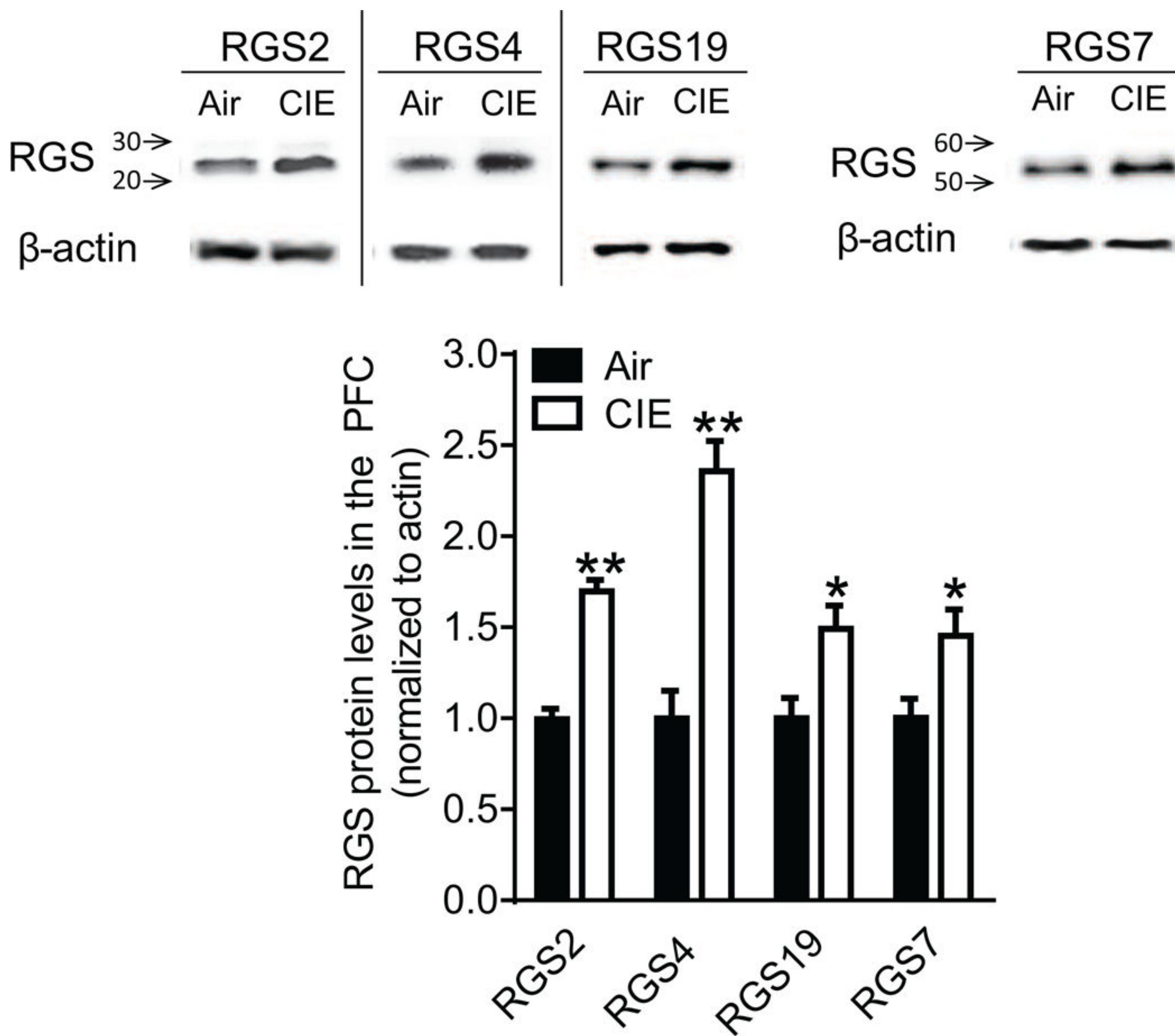

Figure 4.

The differential effects of CIE on the protein levels of RGS subtypes in rat PFC. CIE significantly increased the protein levels of RGS2, RGS4, RGS7 and RGS19. Representative blots are shown. Data were normalized to actin and are presented as mean ± SEM relative to air-exposed animals. $^{**}p<0.01$ vs. the air-exposed group. N=6/group

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Table 1

Animal	Ethanol Exposure	Withdrawal	Changes in G protein expression	References
Female Sprague-Dawley	25% (v/v) for 3 weeks	No	No change in Gαi1 and Gαi2 protein levels in frontoparietal cortex	Barrios et al., 2005
Male C57Bl/6J mice	17–21 mg/L CIE for 4 weeks	3, 7 or 14 days	No change in Gαi/o protein levels in OFC	Nimitvilai et al., 2017
Male Sprague-Dawley	9% (v/v) for 15 days	24 hours	↓Gαq/11 protein levels in cortex after 15 days of EtOH but returned to normal after 24 hr withdrawal.	Pandey et al., 1996
Male Fischer rats	6.6% (v/v) for 4 weeks	No	↑ Gαi3 mRNA levels in FCX, VTA and VP ↓Gαo mRNA levels in SN	Pellegrino et al., 1993
Female Lewis rats	12% (v/v) for 15 months	No	↓ $G\beta_1$ mRNA levels in the hippocampus	Saito et al., 2002
Male C57Bl/6J mice	7% (v/v) for 7 days	No	No change in protein levels of Gαs, Gαi1/2/3 or Gαo in cortex	Tabakoff et al., 1995
*LS mice	10.3% (w/v; i.p.) for 7 days	No	↑ Gαi1 and Gαi2 protein levels in cerebellum and pons	Wand et al., 1992
Male Wistar rats	5 or 20% (v/v) for 40 weeks	8 days	↑ Gαs and Gαo protein level in cortex	Wenrich et al., 1998
Male ICR mice	5 or 10% (2-bottle choice) for 55 days	No	↓Gαs mRNA levels in hypothalamus	Zou et al., 2014

CIE, chronic intermittent ethanol; FCX, frontal cortex; OFC, orbitofrontal cortex; SN, substania nigra; VP, ventral pallidum; VTA, ventral tegmental area

*Gender not reported