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Long-term effects of alcohol drinking on cerebral glucose utilization in alcohol-preferring rats

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Abstract

The 2-[^{14}C]deoxyglucose (2-DG) quantitative autoradiography technique was used to determine rates of local cerebral glucose utilization (LCGU) in discrete brain regions in alcohol-chronic (A-C), alcohol-deprived (A-D) and alcohol-naïve (A-N) adult, male alcohol-preferring (P) rats. The hypothesis to be tested is that neuronal alterations occur as a result of chronic alcohol drinking and some of these alterations persist for long periods in the absence of alcohol. Following 6 weeks of daily 4-h scheduled access sessions to 15% (v/v) ethanol and water, group A-D received only water during the sessions over the next 2 weeks, whereas groups A-C and A-N continued to receive ethanol–water and water–water, respectively. On the 14th day of the deprivation interval, LCGU rates were measured 1 h prior to the scheduled access period. Mean ethanol intake for the A-D and A-C groups was 1.5 ± 0.1 g ethanol/kg body weight per 4 h. LCGU rates were significantly decreased in 49 of 57 regions or subregions examined in the A-C group compared to the A-N group, including subregions of the cerebral cortex, hippocampus and structures in the mesocorticolimbic and nigrostriatal systems. Following alcohol deprivation, LCGU values in the A-D group were partially or completely returned to A-N levels in many, but not all, regions. In several limbic regions (e.g., ventral tegmental area, olfactory tubercle, medial prefrontal cortex, ventral pallidum and lateral septum), no recovery of LCGU rates was observed after 2 weeks of alcohol deprivation. This study demonstrates that chronic alcohol consumption produces significant reductions in functional neuronal activity in P rats, some of which persist in the absence of ethanol. The extent to which LCGU rates returned to normal levels following 2 weeks of alcohol deprivation varied among brain regions, suggesting that there are imbalanced interactions among and within several CNS sites, which do not reflect either the alcohol-naïve or chronic alcohol-exposed state. Such neuronal imbalances may underlie relapse of alcohol drinking following prolonged abstinence.

Keywords: Local cerebral glucose utilization; Chronic alcohol drinking; Alcohol deprivation; Alcohol-preferring rats

1. Introduction

THE 2-[^{14}C]deoxyglucose (2-DG) technique has been used in alcohol research to determine local cerebral glucose utilization (LCGU) rates immediately following alcohol administration or consumption. Previous research has shown that the acute administration of alcohol produces anatomically distinct changes in rates of LCGU that are time-, dose- and route of administration-dependent (Lyons et al., 1998; Porrino et al., 1998a; Williams-Hemby and Porrino, 1994, 1997; Williams-Hemby et al., 1996). The intraperitoneal injection of 1.0 g/kg of ethanol given 10 min prior to 2-DG injection produced a 10–30% reduction in LCGU rates in 17 of 54 regions examined, including CA3 and dentate gyrus subregions of the hippocampus, anterior cingulate, motor and auditory cortices, lateral, medial and lateral dorsal thalamic nuclei, and the locus coeruleus

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(Williams-Hemby and Porrino, 1994). In contrast, the intragastric administration of 1.0 g/kg ethanol 3 min before 2-DG injection produced an increase of about 15% in LCGU rates in the prefrontal cortex, nucleus accumbens core and shell, olfactory tubercle and medial septum (Williams-Hemby and Porrino, 1997). In another study, rats orally self-administered alcohol for 70 days under a schedule-induced polydipsia paradigm, and consumed an average of 1.5 g/kg ethanol during the drinking session on test day (Williams-Hemby et al., 1996). LCGU rates were measured immediately after the 50-min drinking session and reductions were observed in the posterior nucleus accumbens, CA1, CA3 and dentate gyrus subregions of the hippocampus, and the locus coeruleus.

It has also been shown that doses of ethanol lower than 1.0 g/kg produce increases in LCGU rates. The intragastric administration of 0.25 g/kg ethanol 3 min before 2-DG injection significantly elevated LCGU values in the ventral tegmental area, medial prefrontal cortex, nucleus accumbens, olfactory tubercle, septum, hippocampus and basal ganglia structures (Williams-Hemby and Porrino, 1997). Similar changes were observed after an intraperitoneal dose of 0.25 g/kg ethanol injected 10 min prior to 2-DG administration, whereas 0.5 g/kg produced increased LCGU values in only three regions (Williams-Hemby and Porrino, 1994). Overall, these studies indicate that low doses of ethanol increase LCGU rates in multiple brain regions, whereas doses higher than 1.0 g/kg suppress functional neuronal activity. In addition, the time between ethanol administration and the initiation of the 2-DG procedure may be an important influence on LCGU rates.

The long-lasting effects of free-choice chronic alcohol drinking on LCGU rates in rats selectively bred for high-alcohol drinking behavior have not been examined. To improve our understanding of the effects of chronic alcohol drinking on CNS function, it is important to use animal models that exhibit voluntary oral self-administration of alcohol (McBride and Li, 1998). The selectively bred alcohol-preferring (P) rat line satisfies the perceived criteria (Cicero, 1979; McMillen, 1997) for an animal model of alcoholism, and will voluntarily consume unadulterated alcohol without the use of conditioning paradigms.

The P line of rats will voluntarily consume 5–8 g ethanol/kg body weight per day, attain blood ethanol concentrations of 50–200 mg% in free-choice drinking paradigms (Murphy et al., 1986), and maintain ethanol consumption in the presence of other palatable solutions (Lankford et al., 1991). It has been shown that ethanol has CNS reinforcing actions in P rats because they will self-administer ethanol intragastrically (Murphy et al., 1988; Waller et al., 1984), and directly into the ventral tegmental area (Gatto et al., 1994). P rats will also work by bar-pressing to obtain oral ethanol when food and water are available ad libitum (Murphy et al., 1989). Furthermore, with chronic free-choice ethanol consumption, P rats develop metabolic (Lumeng and Li, 1986) and functional tolerance (Gatto et al., 1987), as well as physical dependence (Waller et al., 1982). Moreover, behavioral data indicate that tolerance to ethanol persisted for at least 10 days following a single intraperitoneal injection in the P line of rats (Gatto et al., 1987), suggesting that long-lasting changes occurred as a result of exposure to ethanol. A microdialysis study (Smith and Weiss, 1999) also indicates that prior treatment with 1 g/kg ethanol for five consecutive days produced neuronal alterations in monoamine systems within the nucleus accumbens in the absence of ethanol.

Several studies (McKinzie et al., 1998; Rodd-Henricks et al., 2000b,c; Sinclair and Li, 1989) have shown that P rats readily resume oral alcohol self-administration following long-term forced abstinence and demonstrate a robust alcohol deprivation effect. The alcohol deprivation effect is a temporary increase in alcohol drinking upon re-presentation after a period of abstinence, and has been suggested to reflect craving (Sinclair and Li, 1989). Relapse drinking among rehabilitated alcoholics is a major medical problem (Mello and Mendelson, 1972; Miller et al., 1996) and suggests that chronic alcohol drinking may produce long-lasting neuronal changes in the CNS in the absence of ethanol.

The objectives of this study were to determine LCGU rates in alcohol-naïve, chronic alcohol drinking and alcohol-deprived rats. The hypothesis to be tested is that neuronal alterations occur as a result of chronic alcohol drinking and some of these changes persist in the absence of alcohol.

2. Methods

2.1. Animals

Adult male P rats, weighing 450–525 g at the time of 2-DG injection, were used. Animals were housed individually and maintained on a 12-h reversed light/dark cycle (lights off at 0900 h) in a temperature- and humidity-controlled environment. All animals were handled daily and weighed once per week. Food and water were available ad libitum in the home cages. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute of Drug Abuse, National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, 1996.

2.2. Alcohol-drinking procedure

Following 2 weeks of acclimation to the reverse light–dark cycle, animals in the alcohol-chronic (A-C) and the

alcohol-deprived (A-D) groups were given continuous free-choice access in the home cage to 15% (v/v) ethanol and water for 2 weeks. The left–right positions of the two bottles were alternated daily and fluid intake was determined by weighing the bottles at 24-h intervals. Intake was measured to the nearest 1.0 g and converted into grams ethanol per kilogram body weight. At the end of the continuous access stage, all animals were transferred at the onset of the dark phase to test cages in a separate environmentally controlled room with water access and no food. Three hours later (1200 h), a 4-h free-choice scheduled access period to ethanol (15% v/v) was conducted. Animals were returned to their home cages after the scheduled access period each day. Following 6 weeks of daily scheduled access sessions, the alcohol-deprived group (A-D; $n=8$) received only water during the 4-h sessions over the next 2 weeks (deprivation interval). Alcohol-naive animals (A-N; $n=6$) and the A-C group (A-C; $n=6$) continued on their respective scheduled access regimens. The volumes of 15% ethanol and water consumed were measured at the end of each session. Baseline drinking values for each animal were the average of the 5 days prior to deprivation for the A-D group and prior to 2-DG injection for the A-C group.

2.3. Surgical procedure

Following the scheduled access session on the 12th deprivation day, animals were anesthetized with 1–3% isoflurane gas for catheterization surgery. A 3- to 5-cm unilateral incision was made to expose the femoral vein and artery. Each vessel wall was pierced and 3–4 cm of Tygon (Norton Performance Plastics, Akron, OH) polyethylene catheter (0.01-in. ID, 0.03-in. OD) inserted into the portion of each vessel that is aligned dorsal to the stomach. Both catheters were then flushed with heparinized saline and secured with surgical silk sutures. The open ends of the catheters were subcutaneously guided to the nape of the neck, where they were anchored to a Bard polyethylene mesh (Davol, Cranston, RI) implant and exited the skin. The catheters were glued (silicone glue) to the implant, which was sutured to the underlying muscle in order to absorb any tension on the catheters during the test session. The external portions (4–5 cm) of the catheters were capped with sewing pins. The incisions exposing the femoral vessels and at the nape of the neck were closed with surgical steel staples. Immediately following surgery and once each day for the next 2–3 days, incision sites were examined and Bacitracin Zinc Ointment USP (Fougera, Melville, NY) was applied if necessary. All animals were catheterized 2–3 days before LCGU measurement to establish that normal eating and drinking behavior returned.

2.4. Measurement of local cerebral glucose utilization rates

LCGU rates were measured in accordance with the method described by (Sokoloff et al., 1977) and (Crane and Porrino, 1989). On the day of 2-DG injection, a pulse injection of [^{14}C]2-DG (125 μCi/kg, specific activity 55 mCi/mmol) was administered into the venous catheter 1 h prior to the onset of the ethanol-access period. Arterial blood samples (between 60 and 100 μl) were collected 5 min before and 0, 0.5, 0.75, 1.0, 1.5, 3.5, 7, 15, 30 and 45 min after the 2-DG injection, using 1-ml syringes with needle tips inserted into the open end of the arterial catheter. Samples were immediately centrifuged for 1 min at 16,000 rpm, and the plasma was assayed for ^{14}C concentration using liquid scintillation spectrometry (Beckman LS 7500 scintillation counter, Irvine, CA) and for glucose levels using an Analox GL5 (Analox Instruments, London).

Immediately following the last blood sample at 45 min, animals were decapitated, the brains were rapidly extracted, frozen in isopentane chilled in dry ice and stored at -70°C until sectioning. Brains were blotted with embedding matrix and sliced into 20-μm coronal sections in a cryostat (Cambridge Instruments, Buffalo, NY) set at -25°C. The brain regions containing the structures selected for LCGU determination were identified according to the rat brain atlas of Paxinos and Watson (1986). Sections were mounted onto glass cover slips and dried on a hot plate set to 60°C. The sections and methyl methacrylate standards (Amersham, Arlington Heights, IL) were then secured in X-ray cassettes and apposed to Kodak SB-40 X-ray film for 48 h. The films were developed by hand, dried and analyzed by quantitative densitometry with a computerized image processing system (Scion Image 1.59, NIH). Several bilateral measurements were taken for each region and the average constituted the value for that region for one animal. Tissue [^{14}C]2-DG concentrations were determined using a calibration curve obtained with the ^{14}C standards. LCGU rates were derived from the ^{14}C tissue concentrations, plasma glucose and ^{14}C concentrations, and the "lump" constant according to the operational equation developed by Sokoloff et al. (1977).

2.5. Statistical analysis

An unpaired Student's t test was used to compare alcohol consumption between the A-D and A-C groups. Drinking levels were the average of the intake values from the 5 days prior to deprivation for the A-D group and for the 5 days prior to 2-DG injection for the A-C group. Brain regions were separated according to general neuroanatomical groups (e.g., all layers within a specific cerebral cortical region were grouped together, thalamic nuclei were a separate group, hippocampal regions were another group, etc.) and analyzed with a Group (A-N, A-C, A-D) × Subregion mixed ANOVA with repeated measures on subregion. Significant Group × Subregion interactions were further analyzed with post hoc Tukey's HSD test for multiple comparisons. The significance level was set at $P<.01$ to reduce the chance for Type I errors with multiple comparisons. An additional Group × Subregion mixed ANOVA with repeated measures on subregion and the significance level set at $P<.05$, was

performed on regions clustered according to the a priori hypothesis that LCGU rates in functionally related, reward-relevant structures would be altered by ethanol exposure. Based on the literature on metabolic mapping in rodents (Porrino et al., 1998a,b; Williams-Hemby and Porrino, 1994, 1997) and the neurobiological substrates of alcohol drinking in rodents (Koob and Bloom, 1988; McBride and Li, 1998), the ventral tegmental area, nucleus accumbens core and shell (collapsed across anterior, middle and posterior subregions), olfactory tubercle (collapsed across anterior, middle and posterior subregions), medial prefrontal cortex, ventral pallidum, lateral hypothalamus, lateral septum, central and basolateral amygdaloid nuclei and the bed nucleus of the stria terminalis were analyzed as a group. Brain regions that could not be subdivided or grouped with other regions were analyzed with one-way ANOVA followed by Tukey's HSD post hoc test for multiple comparisons when appropriate.

3. Results

Animals behaved normally following surgery. During the [^{14}C]2-DG injection procedure, all animals moved freely and displayed normal grooming behavior. Mean ethanol consumption was derived from the last 5 days of the predeprivation phase for the A-D group and the 5 days prior to 2-DG injection for the A-C group. Intake did not significantly differ between the A-D group (1.5 ± 0.3 g/kg per 4 h) and A-C group (1.3 ± 0.1 g/kg per 4 h), $F(1,12) = 0.18$, $P = .68$.

3.1. Reward-relevant brain regions

Due to the high anatomical resolution allowed by the 2-DG technique, the nucleus accumbens core, nucleus accumbens shell and olfactory tubercle were initially subdivided into anterior, middle and posterior subregions. However, Group × Subregion mixed ANOVAs performed separately on each structure revealed that the effects of group on LCGU rates were the same across the anterior, middle and posterior subregions within each of these structures. The data showed that LCGU values were significantly higher in the A-N group compared to the A-C group, whereas rates in the A-D group were not different from either the A-N or A-C groups. Moreover, there was no apparent gradient in LCGU rates from one end of the anterior–posterior axis to the other end. For instance, mean rates in the anterior, middle and posterior nucleus accumbens core in the A-N group were 159, 161 and 152 μmol/100 g per minute, respectively. Therefore, LCGU values from the nucleus accumbens core and shell and the olfactory tubercle represent the pooled rates from anterior, middle and posterior subregions.

The overall Group × Region mixed ANOVA for the 11 reward-relevant brain areas revealed a significant effect of group, $F(2,17) = 20.82$, $P < .001$ and region, $F(10,170) = 207.7$, $P < .001$, and a significant Group × Region interaction, $F(20,170) = 3.2$, $P < .001$ (Fig. 1). Post hoc Tukey's test for multiple comparisons indicated that LCGU rates were 15–40% lower in the A-C compared to the A-N group in the ventral tegmental area, nucleus accumbens core, nucleus accumbens shell, olfactory tubercle, medial prefrontal cortex, central amygdaloid nucleus, basolateral amygdaloid nucleus, ventral pallidum, lateral hypothalamus and lateral septum.

Post hoc Tukey's test revealed that LCGU rates in the nucleus accumbens core and shell and the lateral hypothalamus were 10–20% higher in the A-D than A-C group, but significantly lower than the A-N group (Fig. 1). LCGU values in the basolateral amygdaloid nucleus of the A-D group were also higher than values in the A-C rats, but there was no difference in this region between the A-D and A-N groups. A trend toward higher LCGU rates in the A-D group than the A-C group was observed in the ventral tegmental

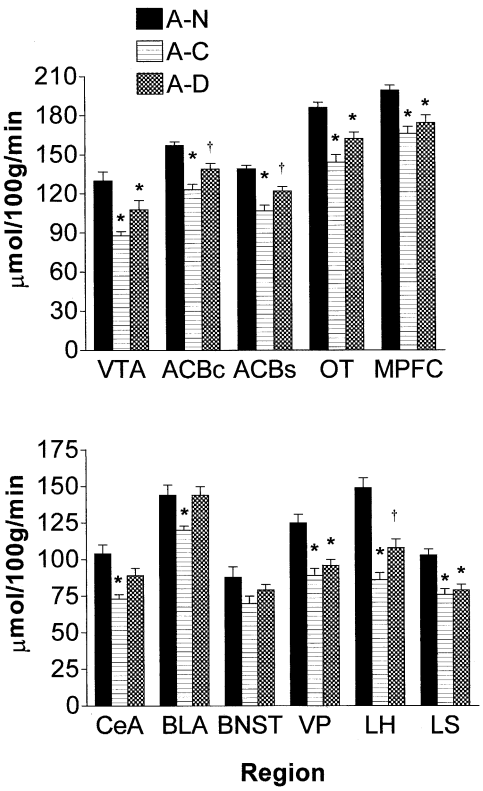

Fig. 1. LCGU rates (mean ± S.E.M.) in subregions of the mesocorticolimbic system and other reward-relevant structures in the CNS of alcohol-naïve (A-N), alcohol-chronic (A-C) and alcohol-deprived (A-D) groups of P rats. * $P < .05$ versus A-N group; † $P < .05$ versus A-N and A-C groups with Group × Subregion mixed ANOVA with repeated measures on subregion and Tukey's post hoc test for multiple comparisons. Abbreviations: VTA, ventral tegmental area; ACBc, ACBs, nucleus accumbens core and shell; OT, olfactory tubercle; MPFC, medial prefrontal cortex; CeA, central amygdaloid nucleus; BLA, basolateral amygdaloid nucleus; BNST, bed nucleus of the stria terminalis; VP, ventral pallidum; LH, lateral hypothalamus; LS, lateral septum.

area, olfactory tubercle and central amygdaloid nucleus, but the differences were not statistically significant and values for both groups were lower than rates for A-N rats. LCGU values for the medial prefrontal cortex, lateral septum and ventral pallidum of the A-D group were similar to A-C levels, and both were significantly lower than the A-N group. LCGU rates in the bed nucleus of the stria terminalis were not significantly different among the three groups, although there was a trend for lower values in the A-C and A-D groups compared with A-N values.

3.2. Cerebral cortex

The cerebral cortex was divided into the frontal, parietal, temporal and occipital areas, which were further subdivided into Layers 1–3, Layer 4 and Layers 5–6 (Table 1). Each area was analyzed separately with a Group × Layer mixed ANOVA with repeated measures on layer. The analyses revealed a significant main effect of group in all areas [frontal: $F(2,17)$; = 13.88, $P < .001$; parietal: $F(2,17) = 11.30$, $P = .001$; temporal: $F(2,17) = 7.45$, $P = .005$; occipital: $F(2,17) = 9.55$, $P = .002$], a significant main effect of layer in all areas [frontal: $F(2,34) = 96.62$, $P < .001$; parietal: $F(2,34) = 85.84$, $P < .001$; temporal: $F(2,34) = 216.72$, $P < .001$; occipital: $F(2,34) = 75.04$, $P < .001$], and a significant Group × Layer interaction in the occipital area, $F(4,34) = 4.54$, $P = .005$, but not in the frontal, $F(4,34) = 2.24$, $P = .09$, parietal, $F(4,34) = 0.63$, $P = .63$ or temporal, $F(4,34) = 2.16$, $P = .09$, areas. Turkey's post hoc test indicated that LCGU rates in Layers 1–3 of the occipital cortex were significantly lower in the A-C group compared to the A-N and A-D groups. Rates in occipital cortical layers 4 and 5–6 were lower in the A-C group than in the A-N group; LCGU rates in the A-D group were intermediate between the A-N and A-C groups and were not significantly different from either.

Table 1
LCGU rates in cerebral cortical regions in alcohol-naïve, alcohol-chronic and alcohol-deprived P rats

Region	A-N	A-C	A-D
Frontal (all layers)	183 ± 5	147 ± 4 *	154 ± 6 *
Layers 1–3	182 ± 4	143 ± 4	147 ± 8
Layer 4	202 ± 5	161 ± 5	171 ± 6
Layers 5–6	167 ± 5	137 ± 3	145 ± 3
Parietal (all layers)	186 ± 5	152 ± 3 *	167 ± 6
Layers 1–3	180 ± 5	150 ± 3	162 ± 8
Layer 4	208 ± 6	170 ± 4	191 ± 7
Layers 5–6	169 ± 4	136 ± 5	148 ± 3
Temporal (all layers)	195 ± 8	156 ± 5 *	189 ± 8
Layers 1–3	203 ± 9	155 ± 5	195 ± 10
Layer 4	217 ± 8	181 ± 6	215 ± 9
Layers 5–6	165 ± 9	131 ± 3	156 ± 6
Occipital (all layers)	181 ± 6	137 ± 5 *	167 ± 8
Layers 1–3	193 ± 4	133 ± 7 *	169 ± 8
Layer 4	190 ± 8	153 ± 5 *	185 ± 8
Layers 5–6	160 ± 9	125 ± 3 *	146 ± 9
Cingulate (total)	211 ± 8	164 ± 4 *	176 ± 7 *
Anterior	205 ± 8	164 ± 5	178 ± 8
Posterior	216 ± 10	165 ± 6	173 ± 7
Piriform	223 ± 6	190 ± 5 *	206 ± 6
Entorhinal	129 ± 7	105 ± 2	121 ± 7

Values are the means ± S.E.M. (μmol/100 g per minute).

* $P < .01$ vs. A-N with Group × Subregion mixed ANOVA and Tukey's post hoc test for multiple comparisons or one-way ANOVA (collapsed data for layers; piriform and entorhinal cortices).

Because the frontal, parietal and temporal areas exhibited nonsignificant Group × Layer interactions, data were collapsed across layers and each area was analyzed individually with one-way ANOVAs (Table 1). Significant group differences were found in the frontal, $F(2,19) = 13.88$, $P < .001$, parietal, $F(2,19) = 11.3$, $P = .001$, and temporal, $F(2,19) = 7.5$, $P = .005$, cortices. LCGU rates in these regions were 20% lower in the A-C group compared with the A-N group. Within the frontal cortex, rates in the A-D group were also lower than rates in the A-N group. LCGU values in the parietal cortex of the A-D group were not significantly different from A-N or A-C rats, whereas rates in the temporal cortex of the A-D group were significantly higher than the A-C group but were not different than the A-N group. Collapsing the data for the layers in the occipital cortex for the three treatment groups revealed lower values in A-C versus A-N rats and a value for the A-D group between the A-N and A-C groups, which was not significantly different than either group.

The cingulate cortex was subdivided into anterior (bregma +2.2 to +1.7 mm) and posterior (bregma +1.6 to +1.2 mm) subregions and analyzed with a Group × Subregion mixed ANOVA. The analysis indicated a significant main effect of group, $F(2,17) = 11.71$, $P < .001$, a nonsignificant effect of subregion, $F(1,17) = 0.48$, $P = .50$, and a nonsignificant Group × Subregion interaction, $F(2,17) = 1.56$, $P = .24$. Because the Group × Subregion interaction was not significant, the data for the cingulate cortex were collapsed across subregions and analyzed with a one-way ANOVA as a single region. The test indicated that LCGU rates were 15–20% lower in the A-C and A-D groups compared to the A-N group (Table 1).

The piriform and entorhinal cortical regions were analyzed individually with one-way ANOVAs. The analyses revealed a significant effect in the piriform cortex, $F(2,17) = 7.15$, $P = .006$, and a nonsignificant effect in the entorhinal cortex, $F(2,17) = 3.34$, $P = .06$ (Table 1). LCGU rates within the piriform cortex were 15% lower in the A-C group than in the A-N group, and rates in the A-D group were not significantly different than either the A-N or A-C groups.

3.3. Hippocampus

LCGU values were obtained from the CA1, CA3 and dentate gyrus subregions in both the anterior (bregma −3.6 to −3.8 mm) and posterior (bregma −4.8 to −5.3 mm) hippocampus. Values for the posterior dentate gyrus were taken in the dorsal section. All hippocampal subregions were subjected to an overall Line × Subregion mixed

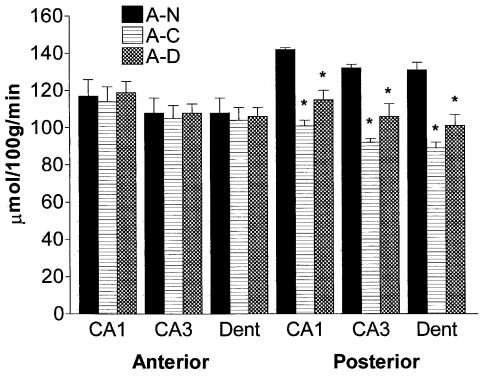

Fig. 2. LCGU rates (mean ± S.E.M.) in subregions of the anterior and posterior hippocampus of alcohol-naïve (A-N), alcohol-chronic (A-C) and alcohol-deprived (A-D) groups of P rats. LCGU values for the posterior dentate gyrus are from the dorsal arm. * $P<.01$ versus A-N group with Group × Subregion mixed ANOVA with repeated measures on subregion and Tukey's post hoc test for multiple comparisons. Abbreviations: CA1 and CA3, corpus ammons areas 1 and 3; Dent, dentate gyrus.

ANOVA (Fig. 2). The analysis revealed significant effects of group, $F(2,17)=6.65$, $P=.007$, and subregion, $F(5,85)=5.08$, $P<.001$, and a significant Group × Subre-Subregion interaction, $F(10,85)=5.78$, $P<.001$. Post hoc Tukey's tests indicated that LCGU rates in the anterior subregions did not differ significantly across groups. In contrast, LCGU values within the posterior CA1, CA3 and dentate gyrus subregions were significantly lower in the A-C and A-D groups compared with the A-N group. LCGU levels in the posterior subregions were approximately 30% lower in the A-C group and 20% lower in the A-D group.

3.4. Basal ganglia

The striatum was divided into ventral, dorsomedial and dorsolateral subregions. The subregions were analyzed together in a Group × Subregion mixed ANOVA. The analysis revealed significant effects of group, $F(2,17)=17.65$, $P<.001$, and subregion, $F(2,34)=53.23$, $P<.001$, but a nonsignificant Group × Subregion interaction, $F(4,34)=2.06$, $P=.12$. Because the interaction failed to reach significance, the data for the striatum were collapsed across subregions and analyzed with a one-way ANOVA. This test demonstrated that LCGU rates were 15–20% lower in the A-C and A-D groups than in the A-N group (Table 2).

The substantia nigra was divided into the pars compacta and pars reticulata. A Group × Subregion mixed ANOVA indicated significant effects of group, $F(2,17)=7.81$, $P=.004$, and subregion, $F(1,17)=499.16$, $P<.001$, and a nonsignificant Group × Subregion interaction, $F(2,17)=0.30$, $P=.75$ (Table 2). Because of the large subregional difference, the substantia nigra was not collapsed into one region. The main effect of group was the result of 20–25% lower values in both subregions of the A-C group compared with the A-N group. Values for the A-D group approached values for the A-N group in both subregions.

Table 2
LCGU rates in basal ganglia in alcohol-naïve, alcohol-chronic and alcohol-deprived P rats

Region	A-N	A-C	A-D
Striatum	164 ± 4	129 ± 5 *	142 ± 3 *
Dorsomedial	158 ± 3	123 ± 5	132 ± 3
Dorsolateral	174 ± 6	136 ± 5	150 ± 3
Ventral	161 ± 4	129 ± 5	143 ± 4
Substantia nigra			
Pars compacta	152 ± 5	124 ± 5 *	144 ± 5
Pars reticulata	93 ± 4	70 ± 1 *	87 ± 6
Globus pallidus	104 ± 5	85 ± 6	90 ± 4
Entopeduncular nucleus	93 ± 5	66 ± 2 *	86 ± 7

Values are the means ± S.E.M. (μmol/100 g per minute).
* $P<.01$ vs. A-N with Group × Subregion mixed ANOVA and Tukey's post hoc test for multiple comparisons or one-way ANOVA (striatum, Globus pallidus, Entopeduncular nucleus).

LCGU rates in the entopeduncular nucleus and the globus pallidus were analyzed with separate one-way ANOVAs (Table 2). A significant group effect was observed in the entopeduncular nucleus, $F(2,17)=5.76$, $P=.01$, whereas the difference did not quite reach significance in the globus pallidus, $F(2,17)=3.40$, $P=.06$. Post hoc Tukey's test indicated that the LCGU rate in the entopeduncular nucleus was 25–30% lower in the A-C group compared to the A-N or A-D groups.

3.5. Thalamus, habenula and hypothalamus

LCGU rates were measured in seven thalamic nuclei (mediodorsal, central medial, ventroposterior, medial geniculate, ventromedial, lateral dorsal and ventrolateral) and two subregions of the habenula (medial and lateral; Table 3). Values in the thalamic nuclei were compared in a Group -

Table 3
LCGU rates in thalamic, habenula and hypothalamic regions in alcohol-naïve, alcohol-chronic and alcohol-deprived P rats

Region	A-N	A-C	A-D
Thalamus			
Mediodorsal	196 ± 3	167 ± 8 *	177 ± 6
Central medial	160 ± 4	132 ± 7 *	142 ± 5
Ventroposterior	169 ± 6	141 ± 7 *	160 ± 5
Medial geniculate	185 ± 7	142 ± 2 *	162 ± 9
Ventromedial	193 ± 6 †	143 ± 6	168 ± 5
Lateral dorsal	198 ± 5	146 ± 3 *	161 ± 6 *
Ventrolateral	180 ± 6	148 ± 8 *	163 ± 7
Habenula			
Medial	127 ± 6	116 ± 7	114 ± 6
Lateral	186 ± 8	174 ± 5	182 ± 10
Hypothalamic preoptic area	116 ± 6 †	89 ± 4	91 ± 5
Medial	97 ± 5	75 ± 5	76 ± 4
Lateral	135 ± 7	103 ± 4	106 ± 6

Values are the means ± S.E.M. (μmol/100 g per minute).
* $P<.01$ vs. A-N.
† $P<.01$, all groups different from each other with Group × Subregion mixed ANOVA and Tukey's post hoc test for multiple comparisons.

× Nucleus mixed ANOVA. The analysis indicated significant main effects of group, $F(2,17) = 12.43$, $P < .001$, and nucleus, $F(6,102) = 26.80$, $P < .001$, and a significant Group × Nucleus interaction, $F(12,102) = 2.68$, $P = .004$. Post hoc Tukey's test revealed that in the mediodorsal, central medial, ventroposterior, medial geniculate and ventrolateral thalamic nuclei, LCGU values were 15–20% lower in the A-C than A-N group, and values in the A-D group were not different than values in the other two groups. In the ventromedial and lateral dorsal nuclei, LCGU values were 25% lower in the A-C versus the A-N group. In the ventromedial nucleus, LCGU rates in the A-D group returned partially toward baseline values and were significantly different than the other two groups. In the lateral dorsal nucleus, rates for the A-D group were 20% lower than the A-N group and were not different than values for the A-C group.

The habenula was divided into medial and lateral subregions and analyzed with a Group × Subregion mixed ANOVA (Table 3). A nonsignificant group effect, $F(2,17) = 0.66$, $P = .53$, a significant subregion effect, $F(1,17) = 385.51$, $P < .001$, and a nonsignificant Group × Subregion interaction, $F(2,17) = 0.97$, $P = .40$, were obtained. When pooled across groups, LCGU rates were 35% higher in the lateral than medial habenula.

The hypothalamic preoptic area was divided into medial and lateral subregions (Table 3). A group × subregion mixed ANOVA indicated significant main effects of group, $F(2,17) = 8.69$, $P = .003$, and region, $F(1,17) = 435.70$, $P < .001$, but a nonsignificant interaction, $F(2,17) = 3.16$, $P = .07$. Collapsing the data across subregions revealed that LCGU rates were 25% lower in the A-C and A-D groups compared with the A-N group.

4. Discussion

Results from the present study demonstrate that prolonged voluntary alcohol drinking, under scheduled access conditions, reduces functional neuronal activity in many of the CNS regions examined (A-N vs. A-C group). Furthermore, in areas that exhibited reduced LCGU rates following chronic alcohol drinking, after 2 weeks of alcohol deprivation (A-C vs. A-D group), values were either (a) completely restored to baseline levels, (b) partially restored toward baseline values, or (c) remained completely reduced. The degree of recovery was region specific and indicated that some of the neuronal changes caused by chronic alcohol drinking were long-lasting and persisted for at least 2 weeks in the absence of alcohol. Moreover, these changes in the rates of LCGU are attributable to the effects of extended ethanol drinking and forced abstinence, and not to the presence of ethanol per se in the CNS, because LCGU rates were measured prior to the daily alcohol access session. Taken together, the results indicate that chronic alcohol drinking produces widespread changes in functional neuronal activity throughout many CNS regions and that, following prolonged deprivation, some of these changes have recovered whereas many others have not, suggesting an imbalance in the interactions among several CNS regions during this deprivation period.

One pronounced effect observed in the present experiment was a general reduction in LCGU rates following extended, limited-access alcohol drinking. Functional neuronal activity was decreased by an average of 25% in reward-relevant regions and by 20% in all other areas, demonstrating that chronic intermittent alcohol exposure has a robust effect on most systems in the CNS. However, it is not likely that this effect is due to nonspecific factors, such as hypercapnia, hypoglycemia or decreased cerebral blood flow because the magnitude of the reduction varied across regions from a zero net effect (e.g., anterior dorsal hippocampus, Fig. 2; habenula, Table 3) to a 40% decrease in LCGU values (e.g., lateral hypothalamus, Fig. 1). The robust reduction in LCGU rates following chronic alcohol drinking points to widespread alterations in synaptic activity. Because neuronal terminals utilize the most energy in order to restore the resting membrane potential (Kurumaji et al., 1993), LCGU rates primarily reflect metabolic activity in terminal fields (Kadekaro et al., 1983; Schwartz et al., 1979). Thus, LCGU values represent the synaptic activity of afferent projections and interneuronal activity within a given region, rather than the neuronal activity of output neurons within that region. However, with the 2-DG technique, it is not possible to identify the neurotransmitter systems affected or distinguish between excitatory or inhibitory activity.

The robust effect of chronic alcohol drinking on LCGU rates in the A-C group is similar to that observed following the acute intraperitoneal injection of 1.0 g/kg ethanol 10 min prior to 2-DG injection in Sprague–Dawley rats (Williams-Hemby and Porrino, 1994); both reflect widespread suppression of functional activity. In addition, one study (Williams-Hemby et al., 1996) found that the oral self-administration of 1.5 g/kg ethanol also produced a reduction in LCGU rates when 2-DG was injected immediately after a 50-min drinking session in Long–Evans rats, although this effect occurred in only seven structures. In the current study, it is likely that animals were experiencing similar acute doses during the first 15 min of the 4-h drinking sessions because P rats have been shown to consume nearly 40% of the total 4-h ethanol intake within this time period (Murphy et al., 1986; Russell et al., 1996). These results suggest that acute exposure to 1.0–1.5 g/kg ethanol produces a reduction in LCGU rates; therefore, with repeated consumption of similar amounts of alcohol, it is possible that chronic suppression of functional activity has occurred, which persists for up to 24 h after the last drinking bout.

The second prominent result was that, after 2 weeks of alcohol deprivation, LCGU rates remained completely reduced, or recovered partially or completely to basal levels. The regions that showed little or no recovery to A-N LCGU

rates included the VTA, medial prefrontal cortex, olfactory tubercle, cingulate and frontal cortices, ventral pallidum, lateral septum, CA1, CA3 and dentate gyrus subregions of the posterior hippocampus, lateral dorsal thalamic nucleus, striatum and the hypothalamic preoptic area (see Figs. 1 and 2 and Tables 1–3). LCGU values in the parietal cortex demonstrated a trend ($P=.03$) toward recovery, but were not different from basal or chronically alcohol exposed levels after 2 weeks of deprivation (Table 1). LCGU rates within these regions indicate that the effect of prolonged alcohol drinking on functional neuronal activity persists over a 2-week period in the absence of alcohol. The long-lasting nature of these changes in LCGU values suggest that alterations in synaptic activity may be involved in aberrant behavior that characterizes alcohol relapse drinking and/or persistent tolerance. Further experimentation is required to determine the effects of longer alcohol deprivation intervals, alcohol reexposure and an ethanol challenge on LCGU rates in P rats.

Partial recovery to A-N LCGU levels was observed in the nucleus accumbens core and shell (Fig. 1), lateral hypothalamus (Fig. 1) and ventromedial thalamic nucleus (Table 3), whereas complete recovery was shown in the central and basolateral amygdaloid nuclei (Fig. 1), temporal, occipital and piriform cortices (Table 1), the entopeduncular nucleus, and substantia nigra (Table 2). Partial recovery may be a result of a uniform limited recovery of pathways affected by chronic ethanol drinking and/or a recovery of some pathways, but not others, which were reduced by chronic drinking. In either case, imbalances in neuronal circuitries, which represent neither the chronic alcohol-exposed state nor the basal state, may exist in regions where LCGU values partially recovered to A-N levels. This condition may reflect that various neuronal systems are slowly returning to a normal state and that a greater duration of alcohol deprivation may be required for complete recovery.

The third major finding of the present study was that chronic alcohol drinking and alcohol deprivation had no significant effect on LCGU values in the bed nucleus of the stria terminalis (Fig. 1), globus pallidus (Table 2), entorhinal cortex (Table 1), habenula (Table 3), or within the CA1, CA3 and dentate gyrus subregions of the anterior dorsal hippocampus (Fig. 2). However, these regions cannot be ruled out as being affected by alcohol drinking and forced abstinence. These regions may be components of neuronal pathways that were affected by alcohol, but the overall changes within the pathways were manifested as a zero net effect in certain regions. It may also be the case that distinct populations of projections into these regions were differentially activated and that by taking a composite LCGU value for each region, the effects were masked or too small to be measured with the 2-DG method. Other possibilities are that the duration of alcohol exposure was too short to alter functional neuronal activity in these regions, or that alterations did occur within these areas with chronic drinking, but that they did not persist for at least 24 h.

The 2-DG technique has been used in alcohol research primarily to characterize the effects of alcohol immediately following alcohol administration or consumption, when ethanol is present in the CNS (Porrino et al., 1998b; Williams-Hemby and Porrino, 1994, 1997). This is different than the present study in which LCGU rates were measured 1 h prior to the alcohol access session. The differences in the experimental paradigms make it difficult to draw comparisons between prior 2-DG experiments and the present study. Moreover, prior research examining the effects of self-administered unadulterated alcohol solutions on LCGU rates were done under operant conditions, used nonselected rats and were conducted during the animals light phase of the light/dark cycle (Porrino et al., 1998b; Williams-Hemby et al., 1996). However, a comparison of LCGU rates in A-N control groups across experiments revealed that some values from the present study were notably higher (up to 50%) than values from other studies (Porrino et al., 1998b; Williams-Hemby et al., 1996). A combination of methodological and rat strain differences are likely to account in large part for the disparity between studies, especially the fact that in the present study all procedures were carried out during the dark phase of the animal's light/dark cycle. LCGU rates have been shown to be 10–20% higher during the dark than light phase (Room and Tielemans, 1989). Differences in physiological factors such as body temperature, blood pressure and respiration may also contribute to the higher LCGU values obtained in P versus nonselected rats. In addition, intrinsic differences owing to selection for ethanol preference may also contribute to the higher values observed in the P line of rats.

The mesocorticolimbic dopamine pathway and associated limbic regions have been implicated in mediating the rewarding effects of alcohol and other drugs of abuse (Koob and Bloom, 1988; Koob et al., 1998; Wise, 1980). Acute ethanol administration can increase the firing rates of VTA dopaminergic neurons (Brodie et al., 1990; Gessa et al., 1985), and the extracellular levels of dopamine within the VTA (Campbell et al., 1996; Kohl et al., 1998) and nucleus accumbens (Imperato and DiChiara, 1986; Weiss et al., 1993). Findings from intracranial self-administration studies indicate that Wistar (Rodd-Henricks et al., 2000a) and P rats (Gatto et al., 1994) will self-administer ethanol directly into the VTA. In addition, the shell of the nucleus accumbens, central amygdaloid nucleus, bed nucleus of the stria terminalis and lateral hypothalamus form the "extended amygdala," which has been implicated in regulating alcohol drinking (Hyytia and Koob, 1995; Koob et al., 1998; McBride and Li, 1998). In the present study, LCGU rates in key limbic regions, including the VTA, nucleus accumbens core and shell, olfactory tubercle, medial prefrontal cortex, central and basolateral nuclei of the amygdala, ventral pallidum, lateral hypothalamus and lateral septum were significantly decreased in the A-C group compared to A-N rats (Fig. 1). In the A-D group, LCGU values were significantly higher than A-C rats but lower than A-N rats in the nucleus accumbens core and shell and lateral hypothal-

amus, whereas values completely recovered to A-N levels in the central and basolateral amygdala. These results indicate that synaptic activity in key limbic regions was reduced following chronic alcohol self-administration and may reflect the functional state of a system that adapted to the repeated presence of alcohol. Moreover, the pattern of LCGU rates in limbic structures in the A-D group was not representative of A-N or A-C levels. Instead rates in the A-D group showed that a return to A-N levels occurred to varying extents in different regions, suggesting the existence of an intermediate state of functional neuronal activity that is characterized by persistent neuronal changes observable up to 2 weeks after the cessation of alcohol intake. These changes are not likely to be attributable to an alcohol-withdrawal syndrome since withdrawal signs usually dissipate within 1 week (Cicero, 1979; Waller et al., 1982), and the length of alcohol exposure and amount of alcohol consumed in the present study with limited access are not sufficient to induce physical signs of withdrawal (Waller et al., 1982). It is possible that such imbalances within these limbic structures may contribute to relapse drinking and the expression of an alcohol deprivation effect.

In the present study, it was surprising to find distinct dissociations between LCGU rates in the anterior and posterior hippocampus (Fig. 2). First, in the A-N group, LCGU rates within the CA1, CA3 and dentate gyrus subregions were significantly higher in the posterior than anterior division. Secondly, LCGU values in the anterior subregions were not statistically different across groups, whereas rates in all posterior subregions were significantly reduced in the A-C and A-D groups compared to the A-N group. Differences in afferent projections to these regions may explain the variation in basal LCGU rates. There is a prominent serotonergic fiber tract projecting from the median raphe nucleus to the posterior ventral hippocampus (Azmitia and Segal, 1978) that is sensitive to ethanol administration (Bare et al., 1998; LeMarquand et al., 1994).

Despite the lack of observable changes in LCGU rates in the anterior hippocampus following chronic alcohol drinking and alcohol deprivation, it is well established that hippocampal structure and function are sensitive to the effects of alcohol. Previous work has shown that ethanol suppresses electrophysiological activity (Bloom and Siggins, 1987; Grupp, 1980) and reduces LCGU rates throughout the hippocampus (Williams-Hemby and Porrino, 1994; Williams-Hemby et al., 1996). In the present study, it is possible that alterations in LCGU values occurred within the anterior hippocampus with chronic alcohol drinking, but that these effects were short-lived and rates in the A-C group were restored to A-N levels by the time of testing. It may be the case that such changes indicate some neural substrates of tolerance to the effects of chronic alcohol drinking.

Regions comprising the basal ganglia were differentially affected by prolonged alcohol drinking and subsequent alcohol deprivation. LCGU rates in the substantia nigra pars compacta and reticulata, striatum, globus pallidus and entopeduncular nucleus were reduced in A-C rats compared to A-N rats. In the A-D group, LCGU values remained lower than in A-N rats in the striatum and globus pallidus, but were restored to A-N levels in the substantia nigra and entopeduncular nucleus. A complete return to basal rates in these regions indicates that 2 weeks of forced abstinence was sufficient to reverse the effects of chronic alcohol drinking. Because these brain areas are critically involved in motor behavior, the changes in functional activity may identify neuronal substrates involved in the development of tolerance to the motor-impairing effects of alcohol.

In contrast to the results of the present study, different and more discrete changes in LCGU rates were reported in a study that examined the effects of prolonged alcohol exposure over a similar duration (Williams-Hemby et al., 1996). Following 70 days of alcohol drinking under a 1-h limited-access schedule-induced polydipsia paradigm, it was demonstrated that decreases in LCGU rates occurred in only seven regions: CA1 (-18%) and CA2 (-16%) subfields and the dentate gyrus (-15%) of the anterior dorsal hippocampus, entorhinal cortex (-22%), anterior ventral thalamus (-12%), medial habenula (-13%) and mammillary bodies (-12%). Moreover, increases in LCGU rates were observed in the posterior nucleus accumbens ($+14\%$) and locus coeruleus ($+18\%$). The discrepancy between this study and the present study may be due to the fact that these investigators (Williams-Hemby et al., 1996) measured cerebral glucose utilization within 5 min after the animals consumed an average of 1.5 g/kg ethanol during a 60-min session, whereas the present study was designed to measure LCGU rates when alcohol was not present in the CNS. A second factor contributing to the different results between the two studies may be due to the use of different rat strains.

An alternative interpretation of the results of the present study is that changes in LCGU rates reflect conditioned responses to the pharmacological effects of alcohol and are not the result of chronic alcohol drinking or subsequent deprivation. It is well established that conditioned drug-opposite responses may develop when salient cues are repeatedly associated with systemic drug effects (Eikelboom and Stewart, 1982; Siegel, 1991). In the present study, there were several salient cues associated with alcohol presentation, including the act of being transported at the same time each day from the home room to the testing cages, the noise in the test room and the size and coloring of the testing chambers. These cues were always associated with alcohol presentation for the A-C group, whereas the alcohol-deprivation interval may have served to extinguish the alcohol-predicting nature of the cues for the A-D group. Accordingly, the meaningfulness of the cues may have contributed to the group differences. However, the decrease in functional neuronal activity observed in the A-C group was not consistent with compensatory response theory (Siegel, 1978). Moderate to high doses of ethanol (1–2 g/kg), similar to those consumed in the present study, produce reductions in functional neuronal activity (Williams-Hemby

and Porrino, 1994; Williams-Hemby et al., 1996). The compensatory response theory would predict increases in LCGU rates that serve to counteract the drug effect and are recruited immediately prior to and during the period when alcohol consumption would normally have occurred. Therefore, the results of alcohol drinking in the present study are not likely to be attributed to learned compensatory responses alone.

An advantage of the 2-DG technique is that it is analogous to PET imaging in humans, which potentially allows for the direct comparison of animal and human brain imaging research. In the only study to directly compare the two techniques, significant positive correlations were found between rats and humans in regional metabolic rates that were normalized to the whole brain metabolic rate (r=.72) and in the variation in LCGU values between brain regions (r=.59), suggesting that 2-DG data reliably predicts PET data in humans (Blin et al., 1991). Moreover, the changes in LCGU rates observed in the present study are similar to changes in cerebral metabolic rates observed in alcoholics after detoxification. First, it was shown that alcoholics had significantly lower whole brain glucose metabolism compared to age-matched controls, and that metabolic levels were significantly correlated (r=.73) with days since last alcohol use (Volkow et al., 1992). Significantly lower rates were observed in the frontal, parietal, temporal, and occipital cortices, the basal ganglia, thalamus and cerebellum, and these differences remained after correcting for age. The relationship between metabolic rates and days of abstinence suggests a cerebral response to abstinence, similar to the response observed in the A-D group in the current study. Secondly, global brain metabolic rates in alcoholics increased to normal levels during the first 31 days of detoxification (Volkow et al., 1994). Comparison of normal and alcoholic subjects after 8 days of detoxification revealed significantly lower metabolic rates in the frontal, parietal and orbitofrontal cortices and the basal ganglia of alcoholics, but after 31–60 days the only significantly lower value in alcoholics was in the basal ganglia. Similarly, it was shown that global cerebral blood flow improved significantly in severe alcoholics between an initial detoxification period and a retest after 3–13 weeks of abstinence (Ishikawa et al., 1986). Overall, the results from the present experiment are consistent with imaging data from human alcoholics, and suggest that prolonged alcohol drinking and alcohol deprivation produce parallel functional changes in the brains of human alcoholics and P rats.

In summary, this study demonstrates significant alterations in functional neuronal activity have occurred following prolonged alcohol consumption, and that some of these changes were long-lasting (e.g., VTA, medial prefrontal cortex, regions of the hippocampus, etc.). The effects of chronic alcohol consumption and the extent to which LCGU rates returned to normal levels following forced abstinence varied between regions and neural circuits, suggesting multiple imbalances within and among brain systems that represent neither the basal state nor the chronically alcohol-exposed state. This neuronal imbalance may be a major factor contributing to alcohol relapse and the expression of a robust alcohol-deprivation effect in the P line of rats (McKinzie et al., 1998; Rodd-Henricks et al., 2000b,c.

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