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Metabolic Mapping of the Effects of Chronic Voluntary Ethanol Consumption in Rats

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WILLIAMS-HEMBY, L., K. A. GRANT, G. J. GATTO AND L. J. PORRINO. *Metabolic mapping of the effects of voluntary ethanol consumption in rats.* PHARMACOL BIOCHEM BEHAV **54**(2) 415–423, 1996.—The 2-[^{14}C]deoxyglucose method was used to examine the effects of chronic, voluntary ethanol consumption on rates of local cerebral glucose utilization (LCGU). LCGU was measured in male Long-Evans rats immediately following the completion of a 60-min schedule-induced polydipsia drinking session. Three groups of animals were examined: animals with a history of ethanol consumption that received ethanol on the test day (ethanol-ethanol), animals with a similar ethanol history that were presented with water on the test day (ethanol-water), and a control group that received water throughout the experiment (water-water). Ethanol consumption on the test day resulted in a highly discrete pattern of metabolic changes, with significant decreases in glucose utilization in the hippocampal complex, habenula, anterior ventral thalamus, and mammillary bodies, whereas increases were observed in the nucleus accumbens and locus coeruleus. Rates of LCGU in the ethanol-water group were increased throughout all regions of the central nervous system examined, indicating that the long-term consumption of moderate ethanol doses that do not produce physical dependence can cause significant changes in functional brain activity.

Ethanol Cerebral metabolism Voluntary consumption Hippocampus Rat

CHRONIC ethanol administration has been shown to produce a wide variety of neurochemical (1,12,14,43,62,64), morphological (3,26,27,41,66,67), electrophysiological (13,36,50), and metabolic changes (8,15) in the central nervous system (CNS). To date, most studies have examined the effects of chronic ethanol administration following a period of abstinence. It is equally important, however, to understand how the functional consequences of ethanol in the CNS change with long-term ethanol use. The purpose of the present study was to examine the effect of ethanol consumption on functional brain activity in rats with a history of chronic ethanol intake. In addition, a separate group of animals with a similar ethanol history was examined 24 h after ethanol consumption to determine the effects of chronic consumption on CNS activity in the absence of ethanol.

Because ethanol has such diverse physiological and neurochemical effects in the CNS, it is difficult to identify the specific anatomical sites of its action. To determine the neuroanatomical substrates that mediate ethanol's effects, neural activity must be measured throughout a wide variety of circuits and pathways in the brain. The 2-[^{14}C]deoxyglucose (2-DG) method is one technique that allows the comprehensive evaluation of changes in local rates of cerebral glucose utilization (LCGU) throughout the central nervous system in response to pharmacological manipulations. Because LCGU in discrete brain regions has been shown to correlate with changes in functional activity in those regions (55,61), the 2-DG method is a useful tool for identification of the neuroanatomical substrates and circuits in which functional activity is altered by chronic ethanol consumption.

A number of studies have applied the 2-DG method to examine the functional correlates of chronic ethanol administration in rats (8,15). Eckhardt (8) reported significant decreases in cerebral metabolism in limbic regions, the extrapyramidal motor system, and the cerebellum in rats following the administration of high ethanol doses for 4 days. Moreover, Grünwald (15) reported a similar pattern of decreased metabolic activity in rats with a chronic history of ethanol intake (in drinking water) that received 3.2 g/kg ethanol by oral intubation just prior to the 2-DG experiment. In both of

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these studies, animals were administered high doses of ethanol for a relatively short period of time: 4 and 24 days, respectively. Additionally, it is important to note that the ethanol was not voluntarily consumed by animals in these studies; rather, the ethanol was administered noncontingently by the experimenter (8) or directly in drinking water (15). Studies of other drugs of abuse suggest that contingent and noncontingent drug administration have different neurochemical consequences (18,38,52–54). The short-term, noncontingent administration of ethanol used in previous 2-DG studies then may not provide a close approximation of chronic alcohol use in humans. To better understand the neural mechanisms of chronic ethanol consumption in humans it is important to utilize animal models that more closely model human abuse patterns. The schedule-induced polydipsia (SIP) method of ethanol consumption is one such model, characterized by chronic and excessive drinking, repeated elevation of blood ethanol levels, and elective overindulgence (10). Moreover, the SIP paradigm provides stable rates of ethanol consumption, making it possible to assess the effects of chronic ethanol intake with the 2-DG method. In this study, rates of LCGU were measured immediately after a SIP drinking session in three groups of animals: a) animals with a minimum 70-day history of drinking ethanol in the SIP paradigm that consumed ethanol on the test day (ethanol-ethanol), b) animals with a similar ethanol history presented with water on the test day (ethanol-water), and c) control animals that consumed water throughout the experiment and on the test day (water-water). By comparing the pattern of metabolic changes in rats with similar ethanol histories that consumed either ethanol or water on the test day, it was possible to attribute the metabolic changes observed to the action of ethanol in the CNS, whereas differences between the ethanol-water and water-water groups were attributed to chronic ethanol consumption.

METHOD

Animals

Experiments were performed on male Long–Evans rats (Harlan Industries, Indianapolis, IN). Rats were housed individually in standard plastic rodent cages with constant temperature (22–24°C) and humidity (60–75%). Water was available ad lib; food was restricted to maintain body weight at 350–360 g.

Schedule-Induced Polydipsia

Experimental sessions were conducted daily in computer-operated chambers (Coulbourn Instruments Inc., Lehigh Valley, PA). All chambers were equipped with a retractable lever, a fluid dipper, and a pellet trough. Each 60-min experimental session began with a 10-min time out period. Sucrose pellets (45 mg) were then delivered under a fixed-time 210-s interval (FT210) independent of responding, while responses on the lever resulted in a 3-s presentation of the dipper (0.1 ml) containing 10% w/v ethanol. Animals had a minimum of 70 days of ethanol or water consumption in the SIP paradigm before the 2-DG experiment. A full description of the procedures is presented elsewhere (Grant and Colombo, in preparation).

Experimental Groups

Prior to the 2-DG experiment, the animals consuming ethanol in the SIP paradigm were randomly divided into two groups: one group received ethanol on the test day (ethanol-ethanol; $n = 5$), while the other group received water on the test day (ethanol-water; $n = 3$). Prior to the day of the 2-DG experiment, the ethanol-ethanol and ethanol-water groups had consumed similar amounts of ethanol: the mean ($\pm$SE) daily intake 14 days prior to the experimental procedure was 3.1 $\pm$ 0.3 g/kg and 2.2 $\pm$ 1.0 g/kg, respectively. Finally, a third group consumed only water throughout all drinking sessions (water-water; $n = 4$).

Surgery

On the day prior to the 2-DG experiment, subjects were lightly anesthetized with sodium pentobarbital (40 mg/kg), and polyethylene catheters were inserted into one femoral vein and artery. The catheters were guided subcutaneously to exit at an incision at the nape of the neck, coiled, and secured with tape in an effort to keep the animal from disturbing the tubing. Catheters were gently flushed with heparinized saline (0.1 units/ml) on the morning after surgery to maintain patency. This catheter placement allowed the animal to move freely throughout the experimental session (6). A minimum of 18 h was allowed for anesthesia clearance and recovery from surgery before measurement of LCGU was begun.

Blood Alcohol Levels

Arterial blood ethanol levels were measured at the initiation of the 2-DG experiment. Levels were determined using the alcohol dehydrogenase assay (Sigma Chemical Co., St. Louis, MO). Blood plasma (10 μl) was added to a glycine buffer and incubated in a water bath for 10 min at 35°C. Samples were then measured on an Ultrospec II spectrophotometer at 340 nm.

Measurement of Cerebral Glucose Utilization

Rates of LCGU were measured by the methods described by Sokoloff et al. (56) as adapted for use in freely moving animals (6). Within 5 min of the end of the drinking session, rats were transferred from the experimental chambers to small shoe-box cages and 2-[^{14}C]deoxyglucose (125 μCi/kg, specific activity 55 mCi/mmol; New England Nuclear, Boston, MA) was injected via the femoral venous catheter. Timed arterial blood samples were collected over the course of the next 45 min. The blood samples were immediately centrifuged and plasma 2-[^{14}C]deoxyglucose concentrations were determined by liquid scintillation counting (Beckman Instruments, Fullerton, CA). The concentration of plasma glucose was measured by a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA).

Immediately following collection of the last sample at approximately 45 min, the animals were sacrificed by intravenous administration of sodium pentobarbital. Brains were rapidly extracted and frozen in isopentane at -45°C and stored at -80°C until sectioning. Brains were then coated with embedding matrix and coronal brain sections (20 μm) were cut in a cryostat maintained at -20°C. Sections were transferred to glass coverslips and dried on a hot plate at 60°C for 10–15 min. Coverslips were then pasted to poster board plates (19.05 $\times$ 11.43 cm) and exposed to Kodak EMC x-ray film along with a set of [^{14}C]methylmethacrylate standards (Amersham, Arlington Heights, IL) previously calibrated for their wet weight [^{14}C]concentrations in similarly cut brain sections. Films were exposed for 10–14 days and developed by hand in GBX developer (Kodak, Rochester, NY).

Autoradiograms were analyzed by quantitative densitometry with a computerized image processing system (MCID, Imaging Research, St. Catherines, Ontario). Optical density mea-

surements for each structure were made in a minimum of five brain sections. Tissue [^{14}C]concentrations were determined from optical densities and a calibration curve obtained by densometrical analysis of calibration standards from the standards on the autoradiograms. Glucose utilization was then calculated from the local [^{14}C]tissue concentrations, the time course of the plasma glucose, and the appropriate constants according to the operational equation of the method (56).

Statistics

Upon the completion the densometric analysis of autoradiograms, data were analyzed by a one-way ANOVA followed by the Student-Newman-Keul's test for multiple comparisons. Each brain structure was analyzed independently.

RESULTS

Behavior

On the test day, the water-water control animals drank a volume of 4.3 ± 2.2 ml (mean; SE), whereas animals drinking ethanol (ethanol-ethanol) consumed 5.2 ± 1.4 ml (mean; SE) or a dose of 1.5 ± 0.8 g/kg. Finally, only one of the ethanol-water animals drank on the test day, consuming 0.8 ml of water.

Blood Ethanol Levels

The ethanol-ethanol group had a mean blood ethanol level of 36.85 ± 2.9 mg/dl (mean; SE) at the initiation of the 2-DG procedure.

Rates of Local Cerebral Glucose Utilization

Rates of LCGU in the 53 structures examined are presented in Table 1. Each ethanol treatment group displayed a distinct pattern of change in rates of cerebral metabolism as compared to water-water controls.

Animals drinking ethanol (ethanol-ethanol) exhibited significantly decreased rates LCGU as compared to water-water controls in 7 of the 53 structures examined, indicating a highly discrete pattern of functional changes. Significant decreases in glucose utilization were particularly prominent within the hippocampal formation (Fig. 1). As compared to water-water controls, ethanol decreased LCGU in the CA1 (−18%) and CA2 (−16%) subfields, as well as the dentate gyrus (−15%) and entorhinal cortex (−22%). Ethanol consumption also decreased glucose metabolism in the anterior ventral thalamus (−12%), medial habenula (−13%), and in the mammillary bodies (−12%). In contrast, glucose utilization was significantly increased in the ethanol-ethanol animals in the posterior portion of the nucleus accumbens (+14%) and the locus coeruleus (+18%) as compared to water-water controls.

Animals with a history of ethanol consumption that received water on the test day (ethanol-water) exhibited significant increases in glucose utilization in all 53 of the brain regions measured as compared to either water-water controls or the ethanol-ethanol group. The most pronounced increases (>45%) in LCGU were observed in cortical regions including the lateral prefrontal cortex (+51%), somatosensory cortex (+55%), and anterior cingulate cortex (+48%). In addition, glucose utilization was significantly increased in anterior (+47%) and posterior (+49%) regions of the nucleus accumbens, posterior olfactory tubercle (+53%), lateral septum (+53%), and dorsolateral caudate nucleus (+50%). Other regions, including the substantia nigra (+49%), the locus coeruleus (+55%), dorsal raphe nucleus (+48%), and cerebellum (+67%), were profoundly affected by receiving water instead of ethanol. These changes in metabolic activity were both quantitatively and qualitatively different than those measured in rats ingesting ethanol (ethanol-ethanol) on the day of the experiment.

DISCUSSION

The present data demonstrate that ethanol consumption produces discrete, localized changes in functional activity following a long history of chronic ethanol intake in a schedule-induced polydipsia paradigm. To our knowledge, this is the first study examining the effects of chronic ethanol intake on cerebral metabolic activity in animals voluntarily consuming ethanol. Ethanol consumption on the test day significantly decreased cerebral glucose utilization in the hippocampus and entorhinal cortex, as well as the habenula, anterior ventral thalamus, and mammillary bodies. In contrast, voluntary ethanol consumption increased functional activity in the posterior portion of the nucleus accumbens and the locus coeruleus. These alterations in cerebral metabolism are likely due to the presence of ethanol in the CNS, because animals with a similar ethanol history that did not consume ethanol on the test day had a significantly different pattern of changes in LCGU. Moreover, the fact that both increases and decreases in LCGU were observed suggests that these changes in glucose utilization are due directly to alterations in functional activity rather than the result of generalized, nonspecific factors including changes in glucose transport, hypercapnia, or alterations in cerebral blood flow (19,28,65).

Although the images that result from the application of the 2-DG method appear anatomical in nature, it is important to point out that this technique is a dynamic biochemical measure of the rate of glucose utilization in anatomically distinct brain regions. These regional changes in glucose utilization in response to a pharmacological treatment, such as the ethanol consumption in the present study, reflect increases or decreases in neural activity in that region, as changes in rates of glucose utilization have been directly correlated with neuronal cell firing (55,61). Moreover, functional activation of glucose occurs primarily in the nerve terminals (23,44); thus, the regional changes in glucose utilization are thought to reflect changes in neuronal input to a particular region rather than to changes in the firing rate of cell bodies contained within that region. Although the 2-DG method cannot be used to distinguish between the direct site of ethanol's action in the brain and the secondary activation of other brain circuits, or the specific neurotransmitters involved, this technique can be used to comprehensively determine the circuits and pathways in which ethanol is acting following consumption.

Because restricted food intake can lead to hypoglycemia, one concern in the present study was that animals restricted to 80% body weight may have lower than normal plasma glucose levels, requiring the use of different constants for the calculation of accurate rates of CNS glucose utilization. The level of plasma glucose is an important issue when applying the 2-DG method because the transport of both glucose and 2-deoxyglucose across the blood-brain barrier is carried out by competitive substrates with different kinetic constants, thus any significant change in arterial blood glucose levels could lead to abnormal 2-DG incorporation. Specifically, hypoglycemia significantly alters the distribution of glucose to neural tissue, resulting in lower cerebral rates of glucose utilization (58,59). To this end, arterial blood glucose levels were evaluated on

TABLE 1

RATES OF LOCAL CEREBRAL GLUCOSE UTILIZATION (MEAN μmol/100 g/min ± SE) IN WATER-WATER, ETHANOL-ETHANOL, AND ETHANOL-WATER TREATMENT GROUPS

Structure	Water-Water $n = 4$	Ethanol-Ethanol $n = 5$	Ethanol-Water $n = 3$
Mesocorticolimbic system			
Prefrontal cortex			
Medial	76 ± 2	82 ± 4	108 ± 3*†
Lateral	73 ± 4	75 ± 4	111 ± 2*†
Nucleus accumbens			
Anterior	77 ± 2	70 ± 4	103 ± 3*†
Middle	83 ± 3	78 ± 5	119 ± 5*†
Posterior	78 ± 3	89 ± 3*	117 ± 3*†
Shell	78 ± 4	83 ± 4	109 ± 4*†
Olfactory tubercle			
Anterior	92 ± 4	83 ± 5	135 ± 7*†
Middle	85 ± 5	90 ± 3	109 ± 6*†
Posterior	74 ± 2	79 ± 3	114 ± 3*†
Lateral septum	65 ± 2	69 ± 4	97 ± 2*†
Ventral tegmental area	69 ± 4	62 ± 3	85 ± 5*†
Ventral pallidum	53 ± 2	55 ± 2	72 ± 3*†
Medial forebrain bundle	60 ± 1	61 ± 3	79 ± 1*†
Limbic system			
Amygdala			
Basolateral	75 ± 1	72 ± 4	102 ± 4*†
Central	51 ± 1	48 ± 2	67 ± 2*†
Anterior cingulate cortex	93 ± 6	93 ± 3	137 ± 3*†
Hippocampus			
CA1	65 ± 1	53 ± 2*	80 ± 2*†
CA2	63 ± 2	53 ± 2*	77 ± 5*†
CA3	64 ± 1	59 ± 2	82 ± 2*†
Dentate gyrus	67 ± 1	57 ± 2*	95 ± 6*†
Entorhinal cortex	68 ± 2	53 ± 2*	85 ± 2*†
Mammillary bodies	123 ± 5	107 ± 4*	150 ± 8*†
Extrapyramidal system			
Caudate			
Dorsomedial	80 ± 4	74 ± 3	116 ± 5*†
Dorsolateral	91 ± 6	91 ± 4	136 ± 12*†
Ventral	88 ± 5	89 ± 4	127 ± 9*†
Globus pallidus	52 ± 1	51 ± 3	71 ± 2*†
Entopeduncular nucleus	49 ± 1	45 ± 2	64 ± 1*†
Subthalamic nucleus	84 ± 3	75 ± 4	103 ± 6*†
Substantia nigra compacta	69 ± 2	68 ± 3	91 ± 4*†
Substantia nigra reticulata	55 ± 2	53 ± 4	82 ± 5*†
Neocortical areas			
Somatosensory	81 ± 5	90 ± 3	126 ± 6*†
Auditory cortex	120 ± 5	116 ± 7	154 ± 6*†
Motor cortex	81 ± 4	88 ± 3	114 ± 7*†
Thalamus			
Anterior ventral thalamus	102 ± 2	89 ± 2*	126 ± 2*†
Anterior medial thalamus	97 ± 1	92 ± 5	130 ± 4*†
Lateral dorsal thalamus	95 ± 2	99 ± 2	132 ± 5*†
Lateral thalamus	80 ± 1	82 ± 3	114 ± 3*†
Medial thalamus	95 ± 3	95 ± 3	137 ± 4*†
Medial habenula	70 ± 3	60 ± 1*	98 ± 5*†
Medial-lateral habenula	86 ± 3	77 ± 3	104 ± 4*†
Lateral habenula	90 ± 3	86 ± 5	122 ± 4*†
Medial geniculate nucleus	109 ± 3	112 ± 7	151 ± 7*†

(*continued*)

TABLE 1
(continued)

Structure	Water-Water $n = 4$	Ethanol-Ethanol $n = 5$	Ethanol-Water $n = 3$
Hypothalamus			
Medial preoptic nucleus	52 ± 2	48 ± 5	66 ± 1*†
Lateral preoptic nucleus	62 ± 2	56 ± 4	70 ± 1*†
Supraoptic nucleus	62 ± 1	61 ± 3	89 ± 6*†
Suprachiasmatic nucleus	55 ± 3	49 ± 3	82 ± 6*†
Periventricular nucleus	81 ± 3	77 ± 4	106 ± 4*†
Brainstem			
Superior colliculus	82 ± 3	77 ± 1	109 ± 3*†
Dorsal raphe nucleus	78 ± 4	85 ± 4	116 ± 4*†
Median raphe	84 ± 9	88 ± 7	116 ± 2*†
Locus coeruleus	56 ± 2	66 ± 3*	87 ± 3*†
Cerebellar white matter	50 ± 2	53 ± 2	83 ± 3*†

*$p < 0.05$ different from Water-Water group; Student–Newman–Keuls test.
†$p < 0.05$ different from Ethanol-Ethanol group.

the day of the experiment in the water-water, ethanol-ethanol, and ethanol-water groups, with the respective mean ± SEM glucose values of 8.42 ± 0.5 μmol/ml, 8.05 ± 0.4 μmol/ml, and 8.35 ± 0.5 μmol/ml. These values are within the normal physiological range (56), thus demonstrating that animals in this study were not hypoglycemic at the time of the experiment, and that the 2-DG method could be accurately applied for the calculation of CNS glucose utilization.

Compared to previous studies of the effects of acute ethanol administration on functional brain activity, chronic ethanol consumption produced a far more discrete pattern of metabolic changes. The acute administration of moderate doses of ethanol to ethanol-naive rats has been shown to decrease rates of cerebral metabolism in motor and sensory areas, as well as in portions of the hippocampal complex (9,68). A similar pattern of decreases was observed by Grünwald et al. (15), following the oral administration of a higher ethanol dose (3.2 g/kg) to naive rats. In these studies, significant decreases in functional activity were particularly prominent in the auditory system, including the auditory cortex, inferior colliculus, and medial geniculate nucleus. In the present study, changes in functional activity in either the auditory or motor systems were not observed in the ethanol-ethanol group, suggesting that metabolic tolerance may develop to the effects of ethanol in these brain regions with long-term ethanol use. Functional tolerance, however, appears to be regionally selective. Most importantly, the hippocampal formation and anatomically related regions are sensitive to the effects of both acute (68) and chronic ethanol administration, with both treatment regimens producing decreased rates of LCGU throughout the hippocampal formation. These findings are important because they demonstrate the continued sensitivity of the hippocampal formation to ethanol. Because the hippocampus is known to play a prominent role in memory processing (24,35), the alterations in functional activity within the hippocampus may provide an anatomical substrate for the effects of ethanol on memory. The present findings are consistent with animal and human studies that indicate the deleterious effects of ethanol on memory and cognitive performance persist, and even intensify with continued ethanol use (11,13,22,37,63,66).

In the present study, the most striking functional consequences of chronic ethanol consumption were observed in the hippocampus. Glucose utilization was significantly decreased in the entorhinal cortex, dentate gyrus, and CA1 and CA2 subfields of the hippocampal formation. These data are consistent with a number of in vivo and in vitro electrophysiological studies that indicate ethanol significantly decreases neuronal firing rates in these regions (5,16,17,57). The majority of these studies, however, have examined the effects of acute ethanol administration on neural activity, and have not explored the role of a prior history of ethanol consumption. Moreover, the studies that have examined the effects of chronic ethanol consumption on neuronal firing rates have focused on ethanol withdrawal rather than the direct effects of ethanol on neural activity in the chronically treated animal (66). Thus, one major finding of the present study is that ethanol, when consumed by animals with a history of chronic ethanol consumption, selectively decreases functional activity within the hippocampal complex. In addition, structures that receive projections from the hippocampus, including the mammillary bodies (60) and anterior thalamus (51), also show decreased rates of metabolic activity, suggesting that the hippocampus, and its efferent connections may be important in mediating the effects of chronic ethanol consumption.

One possible explanation for the decreased rates of metabolism in the hippocampus is that these changes result from neuronal loss consequent to chronic alcohol exposure. Chronic ethanol administration has been shown to produce selective changes in hippocampal cell morphology, the location of which closely parallels the regional changes in LCGU reported in this study (3,26,27,66,67). Although no formal morphological analysis was performed in this study, several factors cast doubt on whether the observed decreases in LCGU are due to neuronal loss. First, the daily amount of ethanol administered to animals in the morphological studies (9.0–24.0 g/kg/day) far exceeds the amount of ethanol voluntarily consumed by animals in the SIP paradigm in the present study. Secondly, animals with a similar ethanol history that received water on the test day exhibited a dramatic global increase in glucose utilization, suggesting that the observed decreased LCGU in the ethanol-ethanol animals is a pharmacological response to ethanol consumption and not due to neuropathological changes in these hippocampal regions. If cell loss or other neuronal damage had been present, similar

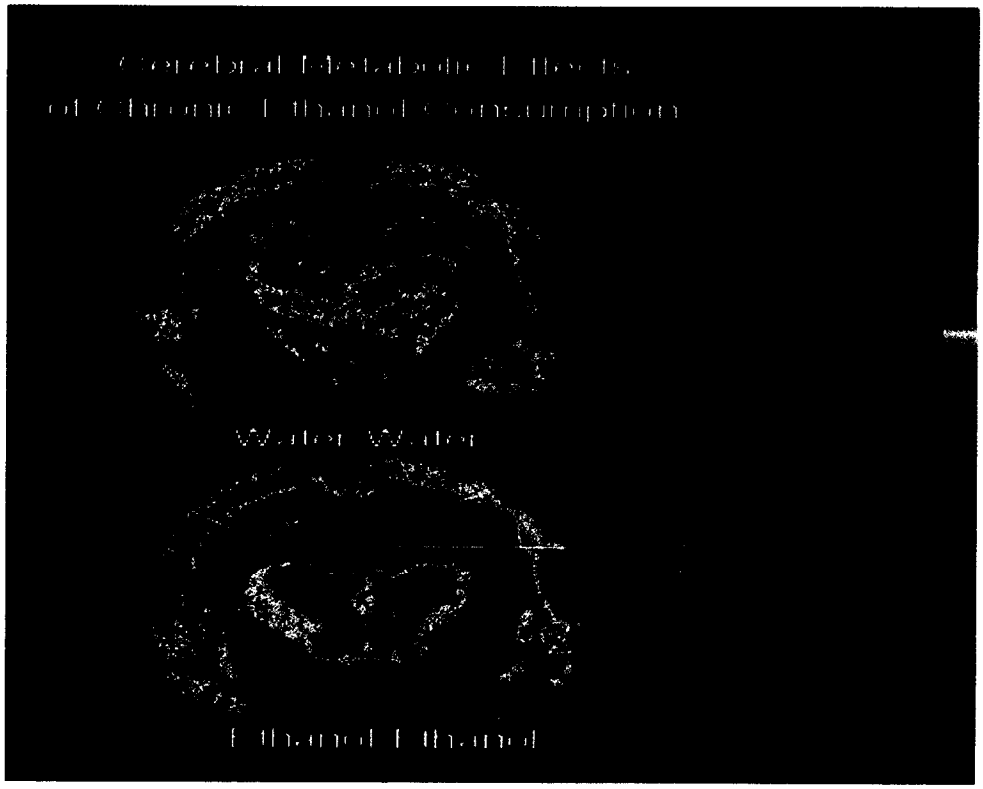

FIG. 1. Effects of chronic ethanol consumption on rates of local cerebral glucose utilization. Shown are color-coded transformations of autoradiograms of coronal sections of rat brain at the level of the hippocampus. Each color represents a range of rates of glucose utilization in μmol/100 g/min according to the calibration scale on the right. Note the decreased rates of glucose utilization in the CA1, CA2, and dentate gyrus of the hippocampus in the ethanol-ethanol animal (bottom) as compared to the water-water control animal.

decreases in metabolism would have been expected in both ethanol-exposed groups. Taken together, these findings indicate that relatively moderate doses of ethanol consumed chronically alter functional activity in the same regions of the hippocampus that are affected by higher, neurotoxic ethanol doses, suggesting that these regions in the hippocampal complex are among the most sensitive in the brain to ethanol.

Mounting evidence indicates that the nucleus accumbens is a critical substrate for oral ethanol consumption (20,39,40, 42). In the present study, ethanol consumption resulted in a significant increase in functional activity in the posterior nucleus accumbens as compared to the water control animals. This increase is in contrast to results from other studies in which ethanol was administered in different treatment regimens. Ethanol administered chronically by oral intubation does not produce a change in glucose utilization in the nucleus accumbens (8), nor do high doses of ethanol consumed chronically in drinking water (15). The increase in LCGU observed in the present study may be related to the dose of ethanol consumed, as these animals drank lower doses as compared to the doses administered to animals in the aforementioned studies. However, ethanol in the dose range consumed by animals in the present study does not alter functional activity in the nucleus accumbens when administered acutely (68), suggesting that the observed increase in LCGU is due to other factors. It is possible that the changes in functional activity in the nucleus accumbens are the result of voluntary ethanol consumption, as compared to noncontingent or forced administration used in previous studies.

The ethanol-water group was included in this study as a control to distinguish between the effects of ethanol consumption in an animal with a chronic ethanol history, and the effects of chronic ethanol history on CNS functional activity in the absence of ethanol, to specify that the effects seen in the ethanol-ethanol group were due to ethanol and not to history alone. It is important to point out the amount of ethanol consumed daily by animals in this study was not sufficient to produce any overt signs of physical dependence. Furthermore, animals in the ethanol-water group were not actively undergoing withdrawal on the test day. Animals in the ethanol-water group exhibited increases in glucose utilization in all structures examined when compared to either the ethanol-ethanol or water-water groups. Although the number of animals in this group ($n = 3$) was limited, the significant increases in glucose utilization (many greater that 45%), coupled with the low standard errors for most structures indicate that long-term ethanol consumption has profound effects on central nervous system activity.

One explanation for the pattern of cerebral metabolic activity observed in the ethanol-water group is the potential stress associated with receiving water on the test day when ethanol was expected. Although this factor cannot be ruled

out, the pattern of functional activity is not consistent with the effects of stress on CNS functional activity. Previous studies examining the effects of stress on rates of glucose utilization have established that both increases and decreases in glucose utilization occur in a limited number of brain nuclei in response to stress (45,46). In contrast, the global increases in metabolic activity observed in the ethanol-water group are consistent with previous studies examining the effect of ethanol withdrawal on functional brain activity (4,30,33). A large body of experimental evidence suggests that neuronal hyperexcitability develops throughout the CNS as early as 9–12 h after the cessation of ethanol intake (1,2,12,14,21,34,43,62,64); however, data from the present study suggests that physical withdrawal is not necessary for increased functional activity. Interestingly, increases in glucose utilization in the present study are more widespread than those reported by Eckhardt and colleagues (8) measured 12–18 h after the withdrawal of ethanol. The discrepancy in metabolic patterns may be due to differences in the length of ethanol exposure; the length of ethanol consumption in the present study was a minimum of 70 days, whereas rats in the Eckhardt study only had 4 days of alcohol exposure. Much attention in the alcohol field has been focused on the damaging effects of very high doses of ethanol; however, results from this study indicate that the long-term consumption of even moderate doses can produce significant functional changes in the brain without evidence of physical withdrawal.

On the day of the experiment, only one animal in the ethanol-water group actually consumed a measurable amount of water. One possible explanation is that animals did not respond when they determined that ethanol was not available, possibly by the absence of ethanol's olfactory cues. The ethanol-water animal that did consume a small amount of water on the test day had LCGU values that were very similar to the other rats in this group, as indicated by the low standard errors. Thus, the significant increases in glucose utilization exhibited by the ethanol-water group are likely due to the absence of ethanol on the test day rather than to the lack of schedule-induced consumption. This functional response to the absence of ethanol on the test day is similar in many ways to a drug-compensatory response. The increases in glucose utilization in the ethanol-water group, which are opposite in direction from those exhibited by the ethanol-ethanol group, are consistent with Siegel's (47–49) classical conditioned model of tolerance. This model proposes that environmental cues, repeatedly paired with drug administration, can elicit conditioned physiological responses opposite in nature from the drug effect. These compensatory responses act to counter the onset effect of the drug, thereby reducing the drug effect and resulting in the development of tolerance. Conditioned-compensatory responses to ethanol have been observed with other measures including core body temperature (7,25,29,31,32), skin temperature (31), heart rate (32), and pulse transit time (31); however, such responses have not been reported in previous studies of functional brain activity. While the development of drug-opposite metabolic responses in the ethanol-water group cannot be directly concluded from this study without the comparison of a group with the same ethanol history examined outside of the experimental environment, these results raise an interesting issue worthy of future investigation.

In summary, these data demonstrate that voluntary ethanol consumption does not result in generalized reductions in functional activity, but in a highly discrete constellation of changes focused within the limbic system. Ethanol consumption produced decreased LCGU throughout the hippocampal complex, anterior thalamus, and mammillary bodies, highlighting the importance of these regions in mediating the effects of chronic ethanol consumption. The fact that glucose utilization is altered in sensory and motor regions by acute ethanol administration but not following chronic consumption suggests that tolerance develops specifically in these brain regions with continued ethanol intake. Moreover, functional tolerance does not develop in the hippocampal formation where the effects of ethanol on memory are known to persist and intensify with chronic use. Finally, the increases in glucose utilization in the ethanol-water group indicate that the long-term consumption of even a moderate dose of ethanol can produce significant changes in functional brain activity in the absence of physical dependence.

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