Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1121–1127, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Microstructural Analysis of the Effects of THIP, a $GABA_A$ Agonist, on Voluntary Ethanol Intake in Laboratory Rats

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Received 18 December 1991

BOYLE, A. E., B. R. SMITH AND Z. AMIT. *Microstructural analysis of the effects of THIP, a $GABA_A$ agonist, on voluntary ethanol intake in laboratory rats.* PHARMACOL BIOCHEM BEHAV 43(4) 1121–1127, 1992.—The effects of $GABA_A$ agonist THIP on the acquisition of voluntary ethanol intake and the pattern of food and water consumption were examined through the use of a computer-controlled data acquisition system. Twenty male Long-Evans rats were randomly assigned to two groups, one of which received THIP (16 mg/kg, IP) and the other an equal volume of saline. Subjects were presented with a free choice of ethanol and water immediately following drug injections, which occurred every other day. The initial concentration of ethanol presented was 2% and was increased by increments of 2% following the second presentation of each concentration, up to a maximum concentration of 10%. Subjects treated with THIP consumed significantly greater amounts of ethanol than did saline controls. A microstructural analysis of bout patterns suggested that the increased consumption of ethanol was a function of an increase in the size, duration, and frequency of ethanol drinking bouts. Food intake was also attenuated by THIP treatment. The results indicated that the decrease in total food intake was a function of a decrease in the frequency of the food bouts. However, in contrast to that observed for ethanol intake, the size and duration of the food bouts were unchanged. The qualitatively different patterns in the microstructure of consummatory behavior for ethanol and food following THIP treatment would suggest that differential mechanisms may mediate the food and ethanol effects observed in the present study. In addition, the differential effects of THIP on ethanol consumption relative to water would suggest that $GABA_A$ manipulations may play a role in influencing the acquisition of voluntary ethanol drinking.

Ethanol $GABA_A$ THIP Microstructure

IT has been suggested that the major inhibitory neurotransmitter GABA may play a role in mediating some of the behavioral and pharmacological effects of ethanol (3,9,16,25,26).

Further, progress has also been made in identifying a putative role for specific GABA receptor subtypes in their relationship to the actions of ethanol (2,3,27). In general, two pharmacologically and functionally unique GABA receptor subsystems have been identified (15). The $GABA_A$ receptor subtype was found to be a complex unit in which a chloride (CL)-ionophore was controlled by the GABA receptor and interacted with binding sites for benzodiazapine, picrotoxin, and barbiturates. In contrast, the $GABA_B$ receptor was found to be associated with a pertusis toxin-sensitive G protein (27). The two receptor systems also differ from one another in that the $GABA_A$ system produces inhibition (pre- or postsynaptically) by modulating CL^- conductance while the $GABA_B$ receptors exert their action through coupling to Ca^{++} or K^+ channels, pre- or postsynaptic, respectively (17,26).

A body of data, which has accumulated recently, suggested that ethanol in particular interacts with the $GABA_A$ receptor system. Specifically, it has been demonstrated that ethanol interacts and enhances $GABA_A$ CL channel flux both directly and indirectly (22,23).

Further, recent studies demonstrated that the sensitivity of rodents to the behavioral effects of ethanol may be a function of the sensitivity of the specific $GABA_A$ receptor to ethanol. For example, it has been demonstrated (18) that in rats selectively outbred for differential sensitivity to the hypnotic effects of ethanol an acute administration of ethanol resulted in a downregulation of the $GABA_A$ receptor function in alcohol-sensitive rats. Similarly, in mice (2) selected for high and low sensitivity to the hypnotic effects (SS-LS) of ethanol differences in sensitivity were found to be related to the differential sensitivity of the $GABA_A$ CL channels to the effects of ethanol. Thus, studies on subjects selectively bred for ethanol sensitivity have suggested that the behavioral effects of ethanol administration may be a function of the relative sensitivity of the $GABA_A$ receptor system (2,18,28).

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Consistent with the notion that $GABA_A$ may mediate ethanol-induced behavioral responding, pharmacological manipulations of the $GABA_A$ receptor system were shown to produce changes in various measures of ethanol intoxication. For example, the application of $GABA_A$ agonists (20) and antagonists (20,21) resulted in the potentiation or attenuation of measures of ethanol intoxication, respectively. While there is also some evidence to suggest that the $GABA_B$ receptor may also play a role in the mediation of some of the physically intoxicating effects of ethanol (1,21), little additional data can be found at the moment to support the notion of a major contribution involving the $GABA_B$ receptor in the mediation of ethanol effects.

As in the case of ethanol-induced intoxication, voluntary ethanol consumption has also been shown to be influenced by manipulations of the GABAergic system (6,10). In our laboratory, the $GABA_A$ agonist THIP has been shown to enhance the acquisition of ethanol ingestion in a voluntary intake paradigm (24). In the same study, the $GABA_B$ agonist baclofen was found to have nonspecific effects in that its effects on ethanol were related to a generalized increase in fluid intake (24). While the literature is not entirely consistent concerning the role of the $GABA_A$ receptor in the mediation of ethanol consumption (7), findings from the present laboratory tend to support the notion that the $GABA_A$ receptor may specifically play a role in mediating the voluntary intake of ethanol (24).

Within the context of voluntary oral ethanol self-administration, the 24-h drinking paradigm within which the effects of GABA manipulations have been traditionally evaluated has proven somewhat limited in scope. This appears to be so because the assessment of changes in consummatory behaviors is strictly limited to the changes in gross fluid intake and body weight over a 24-h period. Thus, the 24-h paradigm prohibits the assessment of changes in the relationship between the food and fluid intake.

It has become evident from the literature (5) that a microstructural analyses of consumption in general could provide a more accurate and sensitive assessment of the types of changes observed in consummatory behaviors following pharmacological manipulations. In line with this notion, a drinkometer system that continuously and simultaneously monitors voluntary food, water, and ethanol intake has been successfully developed and employed in our laboratory (11,14). The system is uniquely capable of providing a detailed description of the changes in bout size, frequency, and duration of food, water, and ethanol intake.

To further expand our understanding of the role of the $GABA_A$ receptor in the acquisition of voluntary ethanol intake, the present experiment was designed to examine the influence of the $GABA_A$ agonist THIP on the microstructure of ethanol drinking behavior using the automated drinkometer system (14).

METHOD

Subjects

Twenty male Long-Evans rats (Charles Rivers Canada, Inc., Hull, Quebec) weighing 200–250 g at the start of the experiment were individually housed in operant chambers in a room controlled for temperature and humidity. Lighting was maintained on a 12 L : 12 D cycle. Food and fluids were available ad lib throughout the test period.

Apparatus

A microcomputer-controlled data acquisition system was utilized in the present experiment to dynamically monitor food and fluid intake. The system consisted of operant chambers (Grason-Stadler chamber, W. Concord, MA) equipped with feeders that dispensed 45-mg standard Bioserve pellets. The feeders were activated by the interruption of photobeams resulting from the placement of the animal's head into a food cup. Each photobeam interruption resulted in the delivery of a single pellet. In addition, each chamber was equipped with two plastic drinking tubes fitted with steel ball bearing spouts.

All operant feeding and drinking activity were monitored continuously over a 23-h period. During a daily 60-min computer shutdown period, the volume of each fluid type was recorded and incorporated into the subsequent data analysis. All accumulated raw data was processed to produce a detailed microanalysis of the bouts of feeding and drinking responses. A bout of activity was considered initiated when the rat activated one of the input devices, such as the food dispenser. On the other hand, the termination of a bout occurred when responding on any given input device was absent for 5 min or there was a transition to another input device. Subsequent data analysis yielded measurements of frequency, duration, and size of individual feeding and drinking bouts. The amount of fluid consumed during each bout was determined through the calculation of a volume/lick ratio.

To avoid the confounding influence of inadvertent contact by animals with the food cups and drinking spouts, only those bouts consisting of more than five consecutive events were included in the analysis. Complete design specifications for the computerized acquisition system have been previously detailed (14).

Procedure

Following a 5-day period of acclimatization to the operant boxes, acquisition of ethanol drinking was initiated through the presentation of a sequence of increasing concentrations of ethanol solutions in a free choice with water on an alternate day schedule. The position of the ethanol-filled tube was altered on successive ethanol presentation days to avoid a position bias. Both tubes were filled with water on intervening days.

Beginning with a 2% (v/v) ethanol solution, ethanol concentrations were increased by 2% following every second ethanol presentation day, until a final concentration of 10% ethanol was achieved. The final concentration of 10% was available for a total of four ethanol presentation sessions.

On those presentation days when ethanol was available, animals received either an injection of THIP [16 mg/kg, IP (saline vehicle); $n = 10$] or an equal volume of saline ($n = 10$). The concentration of drug utilized in the present study was selected on the basis of previous research that indicated 16 mg/kg THIP produced optimal increases in voluntary ethanol intake. Injections were administered during the daily 60-min computer shutdown, which occurred 1 h prior to the onset of animals' dark cycle.

RESULTS

The effects of THIP on total food, ethanol, and water intake, as well as duration, size, and frequency of individual consumption bouts, were examined. The data were analyzed by two-way analyses of variance (ANOVAs) (with repeated measure) across treatment days.

It was observed that THIP treated subjects maintained higher, $F(1, 18) = 13.06$, $p < 0.002$, overall levels of absolute ethanol intake (g/kg) across ethanol concentrations (Fig. 1). There was also a significant treatment × days interaction for the intake of ethanol following THIP administration, $F(11, 198) = 2.39$, $p < 0.01$. Similarly, the results of the analysis of the effects of THIP on ethanol preference indicated that THIP-treated animals maintained consistently higher preference levels for ethanol, $F(1, 18) = 14.99$, $p < 0.001$.

A microanalysis of the increased intake of ethanol reported above indicated significant THIP-induced increases in the size, $F(1, 18) = 7.30$, $p < 0.01$, of the ethanol bouts. Figure 2 shows the THIP-induced increase in mean ethanol (ml) bout size. Of the various bout parameters examined, the changes in mean ethanol bout size were the most consistent with the observed changes in total ethanol intake.

However, significant increases were also observed for the duration, $F(1, 18) = 6.72$, $p < 0.01$ (Fig. 3), and frequency, $F(1, 18) = 6.32$, $p < 0.02$, of mean ethanol bouts (Fig. 4).

While THIP treatment failed to significantly alter total fluid intake, food intake, as measured by total food pellets consumed, was significantly decreased in THIP-treated subjects, $F(1, 18) = 12.58$, $p < 0.002$. The microanalysis suggested that the decrease in total food intake induced by THIP treatment was a function of a significant decrease in the frequency of food bouts, $F(1, 18) = 5.7$, $p < 0.04$. The decreased food intake was further expressed by a slower rate of increase in body weight. Both the size and duration of the food bouts were not significantly influenced as a result of THIP treatment. The decreased frequency of food bouts in THIP-treated subjects can be observed in Fig. 5.

DISCUSSION

Results of the present experiment were consistent with previous reports (24) demonstrating an enhanced acquisition of voluntary ethanol intake and preference throughout the range of concentrations tested following administration of THIP. The effectiveness and specificity of the $GABA_A$ agonist THIP in enhancing the acquisition of voluntary ethanol intake would support the notion that the $GABA_A$ receptor may be involved in regulating the acquisition of ethanol intake.

The microanalysis of the pattern of ethanol drinking revealed that THIP-treated subjects exhibited significant increases in the size, frequency, and duration of ethanol drinking bouts. It is important to note that while all variables contributing to the pattern of ethanol drinking (e.g., size, duration, etc.) were seen to increase it was the size of ethanol bouts that appeared to be the major contributing factor in the overall observed increase in ethanol intake.

However, the concomitant reduction in total food intake induced by THIP suggests that THIP also induced a significant anorexic effect. It was evident, however, that the observed decrease in food intake was primarily a function of a reduction in the frequency of food bouts. The various treatment groups did not differ in terms of the amount consumed per bout or the duration of food bouts. This would suggest that it is unlikely that THIP acted to decrease total food intake through effects upon satiety processes that normally act to limit the size of meals rather than their frequency (4).

While it is evident that THIP influenced both food and ethanol intake, there nevertheless appears to be a relatively

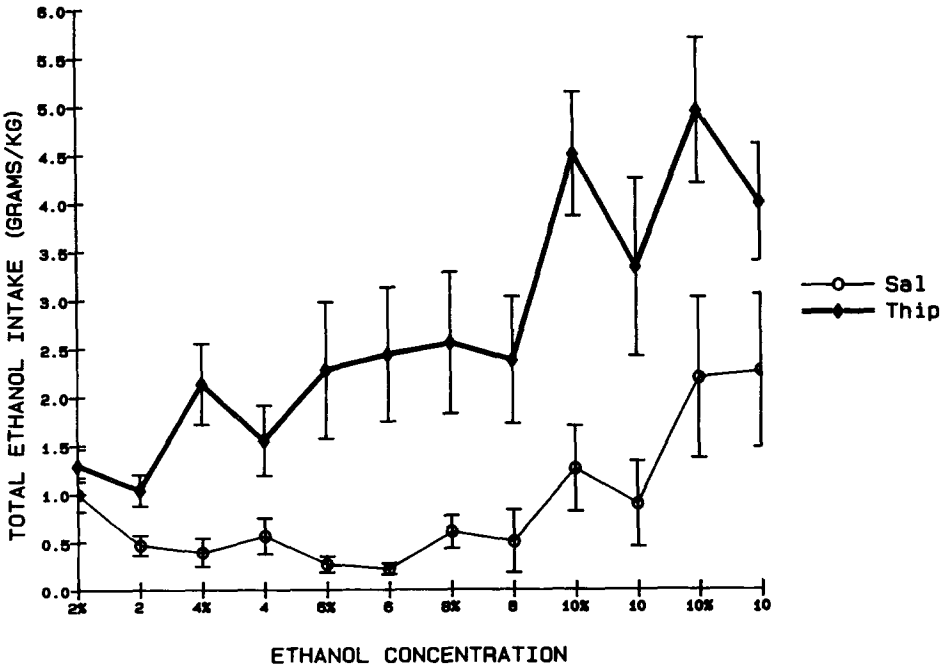

FIG. 1. Effect of THIP treatment on the overall levels of absolute ethanol intake across ethanol concentrations. Vertical lines represent the SEM.

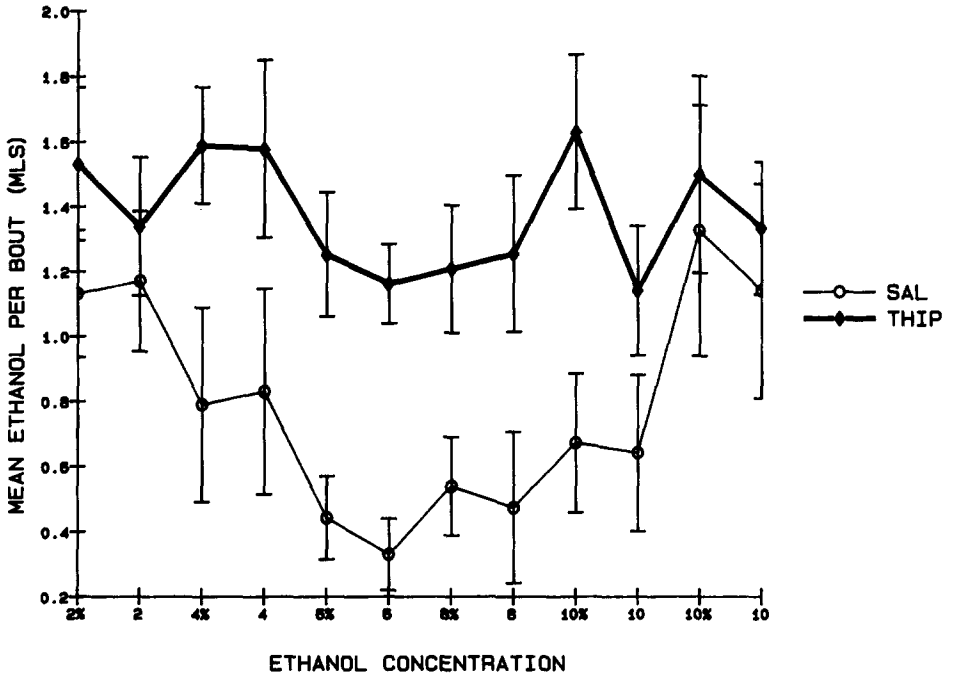

FIG. 2. Differential effect of THIP treatment on the amount of ethanol consumed (ml) per bout across concentrations of ethanol. Vertical lines represent the SEM.

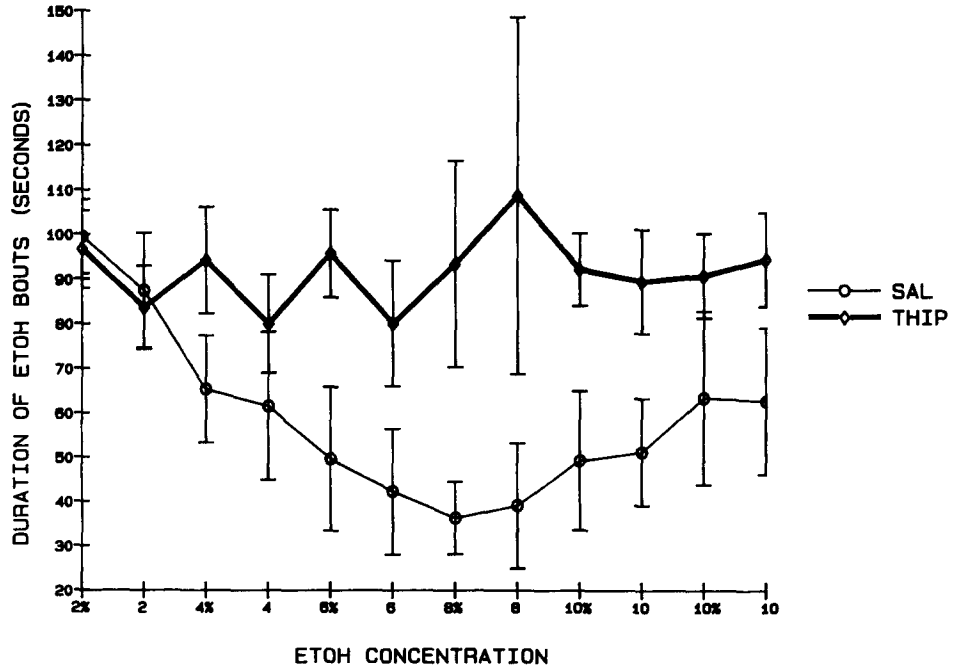

FIG. 3. Effect of THIP treatment on the duration of individual ethanol bouts across ethanol concentrations. Vertical lines represent the SEM, ETOH, ethanol.

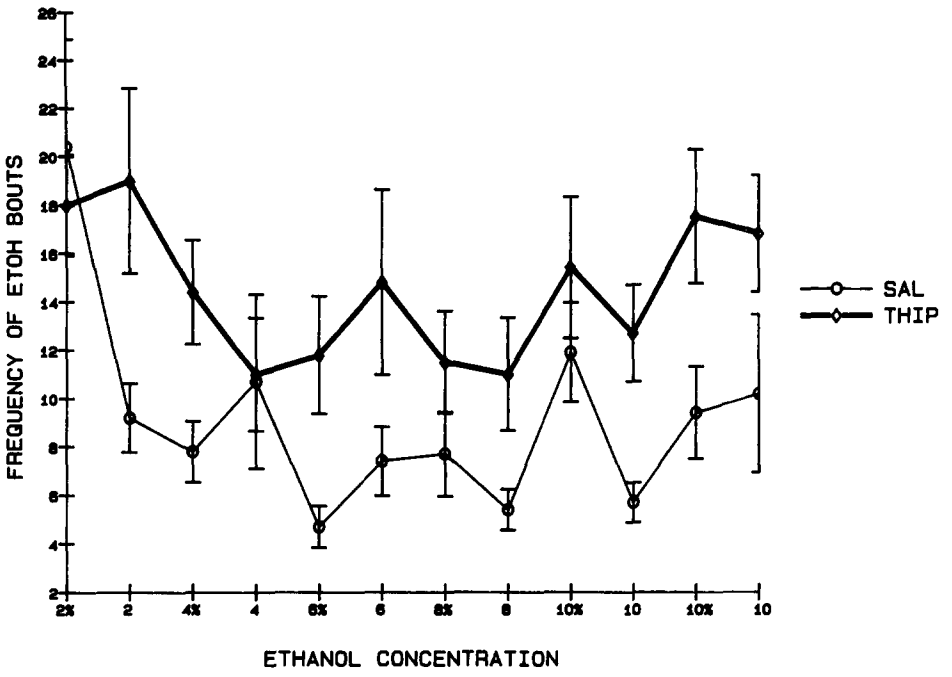

FIG. 4. Effect of THIP treatment on the frequency of individual ethanol bouts across ethanol concentrations. Vertical lines represent the SEM, ETOH, ethanol.

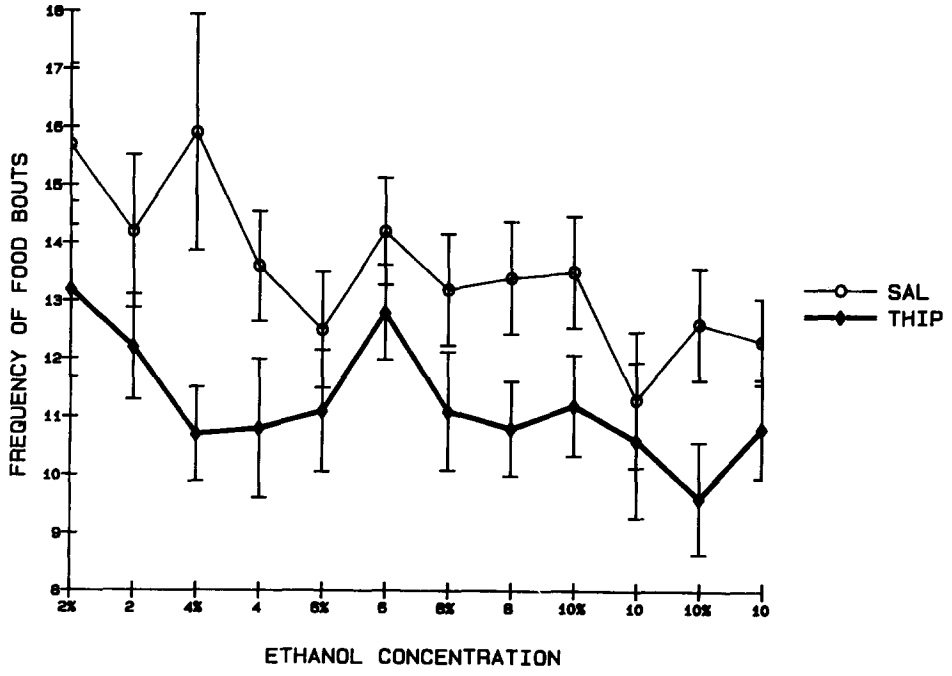

FIG. 5. Effect of THIP treatment on the frequency of food bouts across ethanol concentrations. Vertical lines represent the SEM.

clear dissociation between the effects of THIP on the microstructural pattern of ethanol and food intake. The result of the present study suggest that the observed increases in ethanol intake are a reflection of changes in mechanisms regulating both the initiation and termination of drinking bouts as reflected by decreases in the frequency and size of drinking bouts, respectively. This is in contrast to the observed pattern of food intake in which the initiation of feeding bouts alone appeared to be inhibited. The processes that mediate the initiation or termination of consummatory behaviors, such as those mentioned above, have been suggested to correspond to motivational states mediated by distinct physiological events (13). Thus, the qualitatively different patterns in the microstructure of the consummatory behavior for ethanol and food observed following THIP administration would suggest that differential mechanisms may underlie the effects on food and ethanol observed in the present study.

While it is argued by the present authors that the microstructural pattern of ethanol and food intake is differentially influenced by THIP, there is an alternative interpretation of the results based upon the somewhat popular notion that ethanol is consumed for its caloric value (8,19). This position suggests that in the context of the present findings the increase in ethanol intake could be interpreted in terms of a compensatory mechanism resulting from the loss of calories following a THIP-induced decrease in food intake. In essence, one source of food calories is substituted for another. This interpretation would by necessity suggest that the THIP manipulations result in a decrease in the perceived palatability or rewarding value of solid food, precipitating as a consequence an increase in preference for ethanol.

The literature suggests, however, that pharmacological manipulations that result in a change in the palatability or reinforcing qualities of food-related stimuli are observed to produce changes in the size of individual feeding bouts (5,13). In the present study, changes in the size of food bouts were not observed. Thus, one cannot readily assume that the reduction in food intake was a reflection of a change in the food's palatability or incentive value.

Further, the literature suggests that a manipulation that decreases food intake need not result in a compensatory increase in ethanol intake (13). Illustrative of this point, 5-hydroxytryptamine reuptake inhibitors, such as sertraline or zimeldine, have been demonstrated to produce a decrease in food intake, characterized by a decrease in the size of individual food bouts. However, both zimeldine and sertraline failed to produce a compensatory increase in ethanol intake and in point of fact reduced the intake of ethanol (12,13).

Thus, while one cannot rule out a role for caloric factors in the regulation of ethanol intake, due to alcohol's inherent value as a source of calories, it would be difficult to interpret the present results as supporting a role for calories in the regulation of ethanol intake.

Although the precise mechanism by which GABA may participate in the regulation of ethanol intake still remains uncertain, it has been proposed that GABA may act to facilitate the acquisition of voluntary ethanol intake through its mediation of ethanol-induced intoxication (3,25). Specifically, it is suggested that the rate and potentially the magnitude of ethanol acquisition is a function of the extent to which the rat is able to make an association between the drinking behavior and the reinforcing properties of ethanol. Further, ethanol may possess properties, such as its physical intoxicating effects, that are perceived as introceptive cues and function to enhance the association between ethanol consumption and subsequent reinforcement. Thus, it is proposed that the magnitude of these perceived cues would have an influence on the extent to which animals will acquire the ethanol drinking behavior. The GABAergic manipulations that result in a potentiation of ethanol intoxication would act to enhance the discriminative stimuli associated with ethanol, thereby facilitating the acquisition of ethanol drinking. The present study supported this interpretation (3,25) of the role of GABA in voluntary ethanol intake.

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