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A Descriptive Analysis of Voluntary Ethanol Consumption in Rats: Evidence for a Common Mechanism Mediating Differentially Induced Increases in Ethanol Intake

Alan Edmund Lawrence Boyle

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Abstract

A Descriptive Analysis of Voluntary Ethanol Consumption in Rats:
Evidence for a Common Mechanism Mediating Differentially Induced Increases in Ethanol Intake

Alan Edmund Lawrence Boyle Ph.D.
Concordia University, 1995

Use of the microstructural approach, to identify the mechanisms mediating voluntary ethanol consumption has been advocated as a high behavioral resolution alternative to traditional preference paradigms. In the present thesis, the microstructural approach was used to identify the processes which act to shape ethanol intake during both the normal acquisition of voluntary ethanol intake and following specific pharmacological manipulations.

Phase 1 of the present thesis examined the microstructure and temporal pattern of consumatory behavior in Long Evans rats, during the normal acquisition of voluntary ethanol intake and following an acute period of forced ethanol exposure. In Experiment 1, a microanalysis of consumatory behavior was carried out throughout an ethanol acquisition procedure, which consisted of the presentation of increasing concentrations of ethanol in a free choice with water. The results indicated the involvement of differential, concentration dependent, behavioral mechanisms in the mediation of ethanol intake. Unique to the presentation of ethanol concentrations above 6%, ethanol intake was consolidated into a smaller number of increasingly larger ethanol bouts. Addressing the low levels of ethanol intake characteristic of the microstructural paradigm, Experiment 2 demonstrated the potential for an acute period of forced ethanol exposure to increase ethanol intake in a preference paradigm. Experiment 3 confirmed that within the context of a microstructural paradigm an acute period of forced ethanol exposure also increased ethanol intake, primarily through an increase in the size of ethanol bouts.

In Phase 2, the utility of the microstructural approach in delineating the effects of manipulations of the GABAergic system on ethanol intake was demonstrated. In order to clarify the role of the GABA-A receptor system in the mediation of voluntary ethanol intake, Experiment 4 examined the influence of the GABA-A agonist THIP and the GABA-A CL- channel blocker (antagonist) picrotoxin on the maintenance of ethanol intake. The results demonstrated bi-directional effects of the agonist and antagonist on ethanol intake. A microanalysis of the effects of THIP performed in Experiment 5 indicated that THIP administration produced a specific increase in the self-administration of ethanol primarily through an increase in the size of ethanol bouts. However, Experiment 6 demonstrated that picrotoxin failed to induce a change in voluntary ethanol intake. An examination of the influence of the GABA-B agonist baclofen, in Experiment 7, indicated that baclofen increased ethanol self-administration through an increase in the frequency of ethanol bouts. However, baclofen's generalized effect on fluid intake, suggested that the GABA-B receptor system was unlikely to play a direct role in the mediation of voluntary ethanol intake.

The present thesis indicated that specific increases in ethanol intake, for both pharmacological and nonpharmacological manipulations alike, were primarily a function of an influence on the size of ethanol bouts.

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PHASE I - INTRODUCTION

During the past 60 years, ongoing research has been conducted to investigate the processes mediating drug addiction (e.g. Seevers and Deneau 1963; Jaffe 1977). In keeping with the medical-biological zeitgeist, this body of evidence has attempted to illuminate the essential biological nature of this behavioral phenomenon. More recently, the predominant notion that physical or psychological dependence may account for the voluntary intake of drugs has been eclipsed by the idea that self-administration is fundamentally a function of a drug's positive reinforcing properties (Deneau, Yanagita, & Seevers, 1965; Schuster & Thompson 1969; Deneau, Yanagita, & Seevers, 1969). Accordingly, initiatives directed towards both a basic understanding of the mechanisms mediating addiction and the development of pharmacological treatments have focused upon reinforcement processes. In particular, biobehavioral models have been developed which investigate the neurobiological substrates of drug reinforcement or motivation_.

Interestingly, while the development of models used to investigate the reinforcing properties of ethanol have occurred in parallel with those used for most other drugs of abuse, they have remained essentially distinct. In large part, this has been attributable to the fact that ethanol research has been burdened by the common perception that animals, rats in particular, do not like to orally consume ethanol, and as such must be induced to do so (Erikson 1968; Myers 1968). As a consequence, research in the field of ethanol self-administration has been distinguished by multiple independent research efforts whose focus has been the development of animal models with enhanced levels of ethanol intake. These research initiatives have been characterized by attempts to increase ethanol intake through the use of numerous induction techniques (Amit & Stern 1971; Samson 1986; Grant & Samson 1985; Gill, France & Amit., 1986; Spivak & Amit, 1987), and the use of inbred (e.g. Mclearn & Rodgers 1961; Elmer, Meisch, George 1988) or selected strains of animals (Mcbride, Murphy, Lumeng & Li 1990; Froehlich, Harts, Lumeng, & Li 1990; Froehlich, Harts, Lumeng, & Li 1991).

Unfortunately, the multitude of ethanol self-administration models or paradigms in use have resulted in the creation, or at least the perception, of abundant data essentially unique to particular models and of little general applicability (Deitrich 1992). This perception has been borne out to some extent by the inconsistencies and general confusion which is evident in the literature concerning the interpretation of pharmacological effects upon ethanol intake (e.g. Weiss, Mitchener, Bloom & Koob 1990; Pfeffer & Samson 1985).

Therefore, in the present thesis it was suggested that the multifaceted and fractured approach to the study of ethanol reinforcement has failed to provide a means for understanding the mechanisms mediating this phenomenon. A specific criticism of traditional ethanol intake models was presented which argued that these models lack the behavioral resolution necessary to differentiate the influence of different mechanisms on ethanol intake. Furthermore, it has been argued in the literature (e.g. Cicero 1980) that the deceptive nature of behavioral measures inherent in paradigms such as the two bottle preference paradigm is sufficient to prohibit their use. The use of a microstructural analysis was presented as a preferable alternative to traditional behavioral measures for the description of those factors which collectively act to regulate voluntary ethanol intake. Intrinsically, the microstructural approach allowed for the dissection of a complex behavior such as ethanol self-administration into discrete behavioral and temporal components, including the frequency, size and duration of food and fluid bouts (Gill, Mundl, Cabilio & Amit 1988). Furthermore, the enhanced behavioral resolution of the microstructural approach was used to demonstrate the role of differential behavioral mechanisms in mediating the acquisition of ethanol intake and the effects of pharmacological manipulations on voluntary ethanol intake.

Historical Perspective

A review of the literature would suggest that the differential development of animal models of ethanol intake and the absence of any standardized methodology could be argued to have stemmed from several factors. First, the development of the intravenous (IV)

catheterization process for rats (Weeks 1962) and the subsequent demonstration of IV self-administration of psychoactive drugs in monkeys (e.g. Weeks 1962, Deneau, Yanigata & Seevers 1969) initiated a sequence of events which resulted over time in a consolidation towards a standardized protocol for evaluating the reinforcing efficacy of drugs of abuse. With regard to the majority of drugs of abuse (i.e. psychomotor stimulants, opiates, sedative hypnotics), the protocol within both primates and rodents revolved around the IV self-administration paradigm (e.g. Deneau et al 1969; Yokel 1987) and its variants including intracranial administration (e.g. Amit & Smith 1985; Bozarth 1987).

While ethanol was shown to be self-administered intravenously (IV) in monkeys (Deneau et al., 1969; Winger and Woods 1973), its self-administration had proven to be unreliable in rats (e.g. Collins, Weeks, Cooper, Good, & Russell, 1984; Denoble, Mele & Porter 1985; Grupp 1981; Smith, Davis 1974; Numan 1981) . The unlikely use of primates, due to questions of cost and availability, coupled with the absence of a viable alternative species in which to utilize the IV paradigm resulted in ethanol self-administration research remaining focused on the traditional oral self-administration paradigm.

Additionally, the preferential use of the oral ethanol intake model may also have been a function of its superficial resemblance to the route of human administration (i.e. oral consumption). It has been in fact argued, specifically on this basis, that oral administration should form the foundation of any valid animal model ethanol intake (Cicero 1980). However, use of an oral self-administration paradigm by necessity resulted in the introduction of a number of confounding variables, including factors associated with taste, thirst, absorption and delayed onset of central effects (Gill & Amit 1989; Sherman et al., 1990). All of these factors contributed to an increase in the difficulty of establishing ethanol intake and subsequently interpreting any changes in intake. Given the introduction of these confounds and the availability of the intravenous paradigm, it is difficult to accept that a superficial homology to human behavior would have been, in and of itself, sufficient to result in the preferential use of the oral paradigm.

Nonetheless, a second factor which added further to the distinctness of the animal models for ethanol intake was the perceived aversion of the rat to the oral intake of ethanol. While rats in general exhibit a preference for ethanol at concentrations below 6%, they nevertheless have been observed to exhibit a natural aversion to ethanol above this concentration (Erikson 1968; Myers 1968). The aversion to ethanol has been generally attributed to the aversive taste of the fluid or post ingestional effects of the drug (Deitrich & Melchior 1985; Woods, Ikomi, & Winger 1971). As a consequence, the consensus has been that rats will not generally consume sufficient amounts of ethanol to become overtly intoxicated, and that for greater quantities of ethanol to be consumed, specific training procedures must be instituted (e.g. Amit & Stern 1971; Samson 1986; Grant & Samson 1985). In the absence of obvious alternatives, researchers continued to work with rodents and have developed a plethora of different induction techniques designed to produce more meaningful levels of ethanol intake in rodents.

Finally, a factor which has shaped ethanol research and has been perhaps the greatest hindrance to the development of standardized animal models in alcohol research has been the emphasis placed on the satisfaction of a set of criteria deemed necessary for a "valid" animal model of alcoholism (e.g. Lester & Freed 1973; Cicero 1980). In general these criteria have been rigid to the point that not a single model has been able to satisfy all of the criteria (Cicero 1980). As such, a great deal of ambiguity has been generated with regard to the relative validity or merits of any given ethanol intake paradigm.

It would appear that the rationale behind the development of such rigid criteria was the attempt to compensate for the perception that the rat did not drink pharmacologically relevant amounts of ethanol in traditional preference paradigms. In practical terms, this implied that it was not apparent that animals drank to intoxication or that they became physically dependent on ethanol. Clearly, this perception became evident when critics (Cicero 1980; Lester 1966; Lester & Freed 1973) suggested that ethanol preference studies do not, in all probability, produce detectable blood levels of ethanol or intoxication. It was

further suggested that, in fact, ethanol consumption may be driven simply by the its caloric properties (Cicero 1980). Therefore, it was argued that ethanol preference in animals was unlikely to reflect the influence of a central pharmacological effect and as a consequence was potentially of little relevance to human alcoholism (Cicero 1980; Lester 1966; Lester & Freed 1973; Dole, Ho & Gentry 1985).

Despite evidence which suggested that pharmacologically relevant amounts of ethanol are consumed within the traditional preference paradigm (Gill, France & Amit 1986; Weiss, Mitchner, Bloom & Koob 1990; Ferraro, Carrazzo & Vogel 1991), the aforementioned criteria (e.g. Lester & Freed 1973; Cicero 1980) are currently still being used as a source for the validation of particular models (e.g. Mcbride, et al., 1990; Lankford, Roscoe, Pennington & Myers 1992), and by implication a repudiation of others.

TECHNIQUES FOR THE INDUCTION OF ETHANOL INTAKE

A practical consequence of these historical influences has been the development of methodologies which address the presumed inherent preference-avoidance behavior observed in rats when presented with ethanol as a drinking fluid. As stated earlier, laboratory rats tend to show a preference for low concentrations of ethanol (below 5% v/v) and avoid higher concentrations Thus, various acquisition or induction techniques have been employed to establish and maintain increased consumption of higher concentrations of ethanol (i.e. pharmacologically meaningful), typically 10% /v (Amit & Stern 1971; Samson 1986; Grant & Samson 1985).

Induction techniques which involve the least amount of overt manipulation or coercion have been those based on the periodic presentation of ethanol (Amit & Stern 1971; Halloway, Bird, & Davenport 1984) and sucrose-fading (Samson 1986; Grant & Samson 1985). Both of these induction techniques are notable for the fact that the animals exposed to them consumed ethanol in a purely voluntary manner. In the periodic presentation paradigm, increasing concentrations of ethanol are presented to animals on an intermittent schedule, for example on alternate days. It would appear that an important element of this

paradigm was the "alcohol deprivation effect (ADE) (e.g. Sinclair 1972; Sinclair & Senter 1968). Specifically, ADE has been characterized by a transient increase in ethanol intake which occurs following a period of ethanol deprivation (e.g. Sinclair 1972; Halloway, et al., 1984). In general, the intermittent schedule of presentation resulted in the consumption of larger quantities of ethanol at concentrations which would normally be avoided.

The sucrose substitution paradigm, on the other hand (Samson 1986; Grant & Samson 1985), attempted to diminish the magnitude of an animals avoidance of higher concentrations of ethanol through the gradual substitution of ethanol for sucrose. Initially, animals are presented with a highly desirable sucrose solution. Over time the concentration of the sucrose solution was gradually decreased, while the concentration of ethanol was gradually increased. This substitution procedure was sustained until the ethanol solution was devoid of any sucrose. Through the utilization of this technique, oral self-administration of ethanol solutions up to 40% have been achieved.

One paradigm which, more recently, has received attention as a means to induce ethanol intake in rats has been the restricted access paradigm (e.g. Marcucella & Munro, 1986). In this paradigm, animals were maintained on a schedule in which the access to ethanol was systematically reduced to a period of 10 or 20 minutes daily. The actual duration of the restricted ethanol exposure, however, has varied across studies. Typically, under restricted access conditions rats drank immediately following ethanol tube placement and continued to drink continuously for a 2-4 min. period (Gill et al., 1986; Spivak & Amit, 1987; Stewart & Grupp, 1984). This short bout of drinking has been demonstrated to result in levels of ethanol intake sufficient to produce detectable levels of blood and brain ethanol (Gill, France & Amit 1986; Stewart & Grupp, 1984). Furthermore, the behavioral and pharmacological effects of these amounts of ethanol appeared to be meaningful since following the intake, these animals exhibited locomotor excitation (Gill et al., 1986; Spivak & Amit, 1987) as well as deficits in a passive avoidance task (Gill et al., 1986).

In contrast to the induction procedures described above, increases in ethanol intake have been also been achieved through more restrictive procedures which involve food or fluid deprivation. One such technique was schedule induced polydipsia (Falk 1961). Essentially, this technique was found to induce excessive ethanol drinking as a function of food deprived rats intermittently receiving small pellets of food with concurrent access to water. In fact, rats undergoing this manipulation were reported to drink up to one half of their body weight in water in 3 hours (Tang, Kenny & Falk 1984). Excessive consumption of ethanol has been induced simply by substituting ethanol for water while using this procedure. It was evident that ethanol consumption often persists following the termination of the polydipsia procedure (e.g. Tang, Kenny & Falk 1984).

Finally, a fourth procedure which was often used in oral operant self-administration paradigms involves prandial ethanol drinking. Ethanol consumption was induced with this technique by supplying a portion of a food deprived animals food ration in chambers equipped with a fluid delivery system. Under these conditions, post prandial drinking occurs readily (Henningfield, Meisch 1975). Following a period of time the food administration could be withdrawn and the animal would continue to consume the ethanol so long as the food deprivation was maintained.

As can be observed from the preceding descriptions, concern over the inherent preference-avoidance behavior observed in rats has resulted in a substantial degree of diversity in the development of voluntary ethanol induction techniques. A consequence of this situation has been that investigations of the mechanism mediating ethanol reinforcement were often evaluated from within the context of radically different methodological environments. Interestingly, while induction methodology effectively became an independent variable, their influence on reinforcement processes, or ethanol intake data, in general, remained obscure.

Available research would suggest, however, that the use of the different induction techniques described above in fact yielded results which appear to be strikingly similar in

their descriptive characteristics (e.g. Samson, Pfeffer & Tolliver 1988; Meisch & Carroll 1987; Boyle, Spivak, Smith, & Amit 1994). Illustrating the similarity in the pattern of intake between induction techniques, it has been reported that the amount of ethanol consumed during restricted access has been found to be comparable to reports of the typical size of an ethanol drinking bout in both restricted and nonrestricted access paradigms in free-feeding rats (Gill et al., 1986; Marcucella & Munro, 1986; Samson, Hodge, Tolliver, &. Haraguchi, 1993). Similarly, the outcome of animal research utilizing divergent ethanol acquisition techniques has correlated well with regard to the influence of pharmacological manipulations on the self-administration of ethanol in humans (Amit, Sutherland, Gill, & Ogren 1984; Amit, Brown, Sutherland, Rockman, Gill, & Selvaggi 1985).

Thus, as a function of the assessment of the amount and pattern of intake across induction techniques, it may be parsimonious to assume that the use of these different induction techniques may represent different but equally valid approaches to the study of ethanol intake. Given this premise, it can be argued that historical influences within the field of alcohol research have unnecessarily emphasized the significance of the preference-avoidance behavior observed in rats (Lester & Freed 1973; Cicero 1980)

Furthermore it is argued here that a consequence of the undue attention directed towards the induction of ethanol intake has been the failure to act upon more critical limitations which are inherent in the use of the traditional behavioral measures of voluntary intake. The limitations of the traditional measures have become particularly evident as animal models have increasingly been used to evaluate the involvement of specific neurobiological mechanisms in the mediation of ethanol intake. Specifically, these limitations have been expressed through the production of confusing and inconsistent interpretations of the role of neural substrates in the mediation of ethanol induced reinforcement (e.g. Weiss, Mitchener, Bloom & Koob 1990; Pfeffer & Samson 1988).

In the following section, the inherent limitations of the predominant measures of ethanol intake currently in use were discussed. Specific emphasis was placed on their

failure to exhibit the degree of behavioral resolution necessary for the differentiation of the effects of pharmacological manipulations on ethanol intake.

TRADITIONAL BEHAVIORAL MEASURES OF ALCOHOL INTAKE

The voluntary intake of ethanol has been evaluated primarily through the use of behavioral measures inherent in two basic oral self-administration paradigms. These consisted of the two bottle preference technique (e.g. Gill, France & Amit 1986; Sinclair & Senter 1968) and that of the operantly maintained oral self-administration paradigm (e.g. Meisch & Carroll 1987; Weiss, Mitchener, Bloom & Koob 1990). However, numerous variations of each paradigm have evolved. Essentially, the differences have centered on the type of induction used to initiate drinking, the concentration of ethanol used and the dependent measures obtained (e.g. Amit & Stern 1971; Samson 1986; Grant & Samson 1985). In the case of the operant paradigms, differences also appeared in the type of reinforcement schedules used (Meisch & Lemaire 1993).

TWO BOTTLE PREFERENCE PARADIGMS

A paradigm which continues to sustain wide popularity, despite substantial criticism (Cicero 1980), has been the two bottle preference paradigm. Through the use of this paradigm, ethanol reinforcement has been evaluated and established on the basis of an observed preference for ethanol over other solutions, principally water. The two bottle preference paradigm has generally been characterized by the use of behavioral measures limited to total and/or absolute ethanol intake, total fluid intake and preference ratios, collected over periods of varying lengths of time.

Historically, the two bottle preference paradigm has made a substantial contribution to the process of establishing the reinforcement efficacy of ethanol within an animal model. For example, it has been demonstrated that rats will consume pharmacologically relevant amounts of ethanol when given a free choice between ethanol and water (e.g. Gill 1989). In general the findings of ethanol preference studies have been consistent with the outcome of operant studies which have demonstrated that rats will work to obtain and consume

ethanol (Meisch & Carroll 1987). Further, as was mentioned earlier, the outcome of preference studies have correlated well with the influence of pharmacological manipulations on the self-administration of ethanol in humans (Amit, Sutherland, Gill, & Ogren 1984; Amit, Brown, Sutherland, Rockman, Gill, & Selvaggi 1985).

However, as was also suggested earlier, criticisms have been made regarding the interpretation of the observed preference for ethanol within the two bottle paradigm (Lester & Freed 1973; Cicero 1980). In essence it has been suggested that ethanol preference in the two bottle paradigm may be driven by the properties of ethanol other than its central reinforcing effects. These would include ethanol's caloric value, taste or olfactory properties (Cicero 1980). However, the contention that ethanol intake in rats may be driven by factors other than its central reinforcing effects, has been discussed and effectively questioned elsewhere (e.g. Gill 1989). Nonetheless, the assumptions underlying these criticisms appear to have some validity. Specifically, there would appear to be support for the supposition which suggests that the crude behavioral measures, inherent in preference paradigms, are deceptive and do not readily permit the identification of those mechanisms which regulate ethanol intake (Cicero 1980).

The poor behavioral resolution of the measures inherent in the two bottle preference paradigm has become particularly evident when changes in the reinforcing efficacy of ethanol have been inferred from the simple changes in the level of ethanol intake. In particular, decreases in ethanol intake or preference induced as a function of the manipulations of the GABA (June, Lummis, Colker, Moore & Lewis 1991), serotonin (Svensson, Fahlke, Hard & Engel 1993), dopamine (Pfeffer & Samson 1988), and opiate receptor systems (Froehlich, Harts, Lumeng, Li 1990) have all been reported. Furthermore, these effects have been attributed to a decrease in the reinforcing efficacy of ethanol. These findings, considered together, would lead one to assume that these divergent receptor systems all interact at some level with the substrate(s) mediating ethanol's central reinforcing effects.

However, this position was called into question when the influence of pharmacological manipulations on measures beyond those encompassed in the two bottle paradigm were examined. Specifically, accumulated data have indicated that serotonerigic manipulations decrease the intake of food (Gill, Filion, & Amit 1988), the consumption of preferred fluids (Gill & Amit 1989; Montgomery, & Burton 1986) and have been suggested to decrease motivated behavior in general (Amit, Smith, & Gill, 1991). Thus, at least in regard to influence of the serotonergic system on ethanol intake, the two bottle intake paradigm initially failed to provide sufficient resolution to demonstrate the nonspecific nature of the pharmacological manipulations.

Implicit in the interpretations of the changes in ethanol preference has been the assumption that changes in ethanol intake are indicative or synonymous with changes in the reinforcing efficacy of the ethanol (e.g. Weiss, Mitchener, Bloom & Koob 1990). However, this assumption, as the critics have implied (e.g. Cicero 1980), is suspect in that drug intake in general, and voluntary ethanol intake in particular, are subject to multiple influences of which the drugs reinforcing properties are but one (e.g. Yokel 1987; Wise 1987; Gill & Amit 1989; Sherman, Rusniak, & Garcia, 1990).

The extensive experience derived previously from the IV self-administration literature has indicated that pharmacological manipulations may have multiple effects which impinge upon the behavioral response being measured (E.g. Yokel 1987; Wise 1987; Porter, Villanueva 1989; Trujillo, Belluzzi & Stein 1989).

Clearly, however, due to the self-administration of ethanol through an oral route, use of the ethanol preference paradigm implied that an even greater number of potentially confounding influences must be considered (Gill & Amit 1989; Sherman et al 1990). In particular, changes in fluid intake may be subject to the influence of changes in sensory processes (including taste and smell), the rate of ethanol absorption and metabolism, as well as the general well being of the organism (Gill & Amit 1989; Sherman et al., 1990).

The aforementioned suggested, therefore, that induced changes in the voluntary intake of ethanol may be a function of numerous factors other than the changes in the reinforcing value of ethanol. A critical limitation of the two bottle preference paradigm appeared to be the failure of the paradigm to provide the behavioral resolution necessary to differentiate the relative influence of these diverse factors.

ORAL OPERANT PARADIGMS

A second technique used increasingly to examine the mechanisms regulating ethanol intake has been the operantly maintained oral self-administration paradigm (e.g. George 1988; Samson, Tolliver & Shwarz-Stevens 1990; Weiss et al., 1990). Conceptually, this paradigm is derived from research involving the intravenous self-administration of psychoactive substances (e.g. Deneau et al., 1969). Interestingly, however, this approach did not appear to improve upon the traditional two bottle preference paradigm described earlier. In general, it will be argued that operant paradigms have been characterized by poor behavioral resolution and specificity, when employed to identify the processes mediating ethanol intake.

Use of the operant paradigm has been appealing, since it meets some of those criteria proposed by Cicero (1980), at least with regard to self-administration. Specifically, ethanol was consumed through an oral route, significant blood ethanol levels could be achieved with this process (e.g. Weiss et al., 1990) and animals worked for the ethanol under various schedules of reinforcement (e.g. Elmer, Meisch, Goldenberg, & George 1988).

The basic operant paradigms in common use have mostly involved the initiation and maintenance of a fixed ratio schedule of ethanol reinforcement. Other schedules, however, have also been used. Typically, the initiation of operant responding has required, as in the case of preference paradigms, the use of acquisition techniques in order to induce and maintain stable rates of oral ethanol consumption. Test chambers have been equipped with a single lever for ethanol reinforced responding (e.g. Samson, Tolliver, Shwarz-Stevens,

1990; George 1990) or two levers, for combinations of reinforcements including food and fluid, ethanol and water or ethanol and an inactive lever) (e.g. Weiss et al., 1990).

Essentially, in a single lever design, changes in ethanol reinforcement were evaluated on the basis of changes in the rate of bar pressing. However, in a single lever design, in order to demonstrate that a drug has in fact supported the observed rate of responding one must demonstrate appropriate changes in responding (a decrease) when a vehicle was substituted (Caroll, Stitzer, Strain & Meisch 1990). Under a two lever condition, a higher rate of responding on the lever associated with a given drug, (relative to the vehicle or inactive lever) was generally interpreted as an indication of the drug possessing reinforcing properties. In addition, changes in reinforcement were often inferred as a function of specific changes in the rate of responding for the lever which administered drug.

However, interpretations of changes in the reinforcing efficacy of ethanol derived from the rate of operant responding were subject to difficulties of interpretation comparable to those described for the two bottle oral preference paradigm. Previous research has indicated that changes in the rate of operant responding do not necessarily reflect a comparable change in motivational properties (Wise 1987; Porter, Villanueva 1989; Trujillo, Belluzzi & Stein 1989). Typically, in operant self-administration studies an inverted U shaped curve was obtained as a function of the rate of responding and increasing concentrations of administered drug (Macenski & Meisch 1994). This relationship implied that as one increased the concentration of drug the rate of responding increased and subsequently decreased (Macenski & Meisch 1994; Dworkin & Smith 1988). High doses of drug generally exhibited lower rates of responding than moderate doses. However, in contrast, evidence suggested that a drug's reinforcing effects increased directly with dose (Dworkin & Smith 1988; Macenski & Meisch 1994). Thus, the operant self-administration paradigm appeared to provide a potentially confounded measure of reinforcing efficacy.

A number of hypotheses have been proposed to account for the discrepancy between reinforcement efficacy and the measures of rate. These have included the behavioral

titration of blood levels and motor impairment (Katz 1989). However, with drugs such as the psychomotor stimulants, it was suggested that the rate of responding may be a reflection of the unconditioned rate-modulating effect of a drug (Woods, Winger, France 1987). Further, the interoceptive stimulus properties of a self-administered drug, which permit the animal to detect the delivery of a drug, have also been suggested to modulate self-administration (Dworkin & Smith 1988). Thus, measures of rate, which have been obtained from operant self-administration studies, appear to be subject to the influence of several factors including the reinforcing, rate modulating and stimulus properties of a drug.

In practice, the failure to differentiate between the influences described above has resulted in questionable interpretations concerning changes in operant rates following pharmacological manipulations of ethanol intake (e.g. Weiss, Mitchener, Bloom & Koob 1990). For instance, decreases in ethanol intake have been induced by dopamine agonists. These reductions in intake have been interpreted as compensatory responses, on the part of the animal, to the potentiation of the reinforcing efficacy of ethanol produced by enhanced dopamine activity (Weiss, Mitchener, Bloom & Koob 1990). However, comparable decreases in ethanol rate measures have been induced following the administration of the dopamine antagonists pimozide (Pfeffer & Samson 1985) and haloperadol (Pfeffer & Samson 1988). The apparent independence of the measures of rate from that of the neuropharmacological effects of dopamine would make it difficult to establish any relationship between dopamine and ethanol reinforcement. Simply stated, these findings have suggested that simple response rates lack the resolution necessary to differentiate the effect of drugs which presumably possess inverse pharmacological properties.

In recognition of the limitations inherent in the use of operant rates as behavioral measures, attempts have been made to use operant paradigms less reliant on measures of rate. (Dworkin & Smith 1988). In particular, the progressive ratio operant paradigm has been used with greater frequency in studies examining the neurobiological basis of reward (Hubner & Moreton 1991; Richardson & Roberts 1991; Yanagita 1987). Under a

progressive schedule, the ratio of responses necessary for the delivery of a drug reinforcement is progressively increased, either across sessions or within sessions (Johanson 1988). Generally, once a stable rate of self-administration is established on a fixed ratio schedule, the ratio of responses are progressively increased until the subject fails to produce the number of responses, within a criterion period, necessary in order to receive a reinforcement. The largest ratio completed, referred to as the breaking point, is used as a measure of the maximal effort the animal will invest in order to receive an injection (Markou, Weiss, Gold, Caine, Schulteis & Koob 1993).

Limited use of the progressive ratio paradigm has, in fact, been employed in examining the reinforcing efficacy of ethanol (Elmer, Meisch, Goldenberg, & George 1988). It was demonstrated that C57BL/6J mice, which display a high preference for ethanol in a two bottle paradigm (Mclearn & Rodgers 1961), self-administer ethanol under a fixed ratio operant schedule. Further, it was demonstrated that as one increased the fixed ratio schedule, the mice increased their rate of responding, indicating a motivation to work harder to obtain the ethanol. On the other hand, Balb/cj mice, which display low preference for ethanol failed to demonstrate any change in responding as the response requirement was similarly increased (Elmer, Meisch, Goldenberg, & George 1988).

Use of the oral operant paradigm, however, has presented a unique challenge to the interpretation of changes in behavioral measures of ethanol intake, which are not readily resolved through the use of a progressive ratio paradigm. Specifically, research has suggested that the oral ethanol operant paradigm may be confounded by behavioral measures which are sensitive to manipulations influencing the performance of the operant in addition to changes in reinforcement efficacy of ethanol (Cousins, Sokolowski, Salamone 1993; Spear, Muntaner, Goldberg & Katz 1991; Salamone, Cousins, McCullough, Carriero, Berkowitz 1994; LeMagnen 1992).

Central to this argument is the assumption that oral operant self-administration paradigms in general, and ethanol self-administration in particular, represent a case of

second order conditioning. For example, when an operant is performed, the animal is presented with a bolus of ethanol. However, as a function of the latency between ingestion and the general experience of the post ingestive effects, the animal will not experience the pharmacologically reinforcing effects of ethanol for some time following the initiation of drinking (Carroll, et al., 1990; Nurmi, Kiianmaa, & Sinclair 1994). The immediate consequence of any operant response is the presentation of a fluid with specific stimuli, such as taste and smell. It is these stimuli which have been intermittently associated with the pharmacologically reinforcing effects of ethanol over the course of any 24 hour period (Gill 1989; Gill, France, & Amit 1986). Thus, the taste and smell of the ethanol acquire the status of secondary reinforcers.

It has been argued that, at best, the outcome of operant studies utilizing second order conditioning may result in behavioral measures which are functionally independent of the type of reinforcer utilized. In this regard, it has been suggested that the changes observed in the rate of oral operant responding under a complex schedule of secondary reinforcement may provide us with information regarding the secondary reinforcer (palatability etc.) but not necessarily anything about a substances (ex food) specific postingestive reinforcing effects (LeMagnen 1992). In support of this argument, operant responding following a drug challenge (cocaine, chlordiazepoxide or chloropromazine) has been reported to be independent of whether responding was maintained by cocaine (IM) or by food when using a complex schedule of secondary reinforcement (Valentine, Katz, Kandel & Barret 1983). Similarly, when the behavior maintained by either cocaine or methohexital was compared under two different schedules of drug delivery, the rates and patterns of responding depended more on the schedule of drug delivery than on the particular drug maintaining responding. (Spear, et al., 1991).

Further emphasizing this potential dissociation between rates of responding and reinforcement processes, it has been suggested th... oral operant responding may be acutely sensitive to manipulations which influence nonreinforcement related mechanisms (Cousins,

Sokolowski, Salamone 1993; Salamone, Cousins, McCullough, Carriero, Berkowitz 1994). For example, it has been demonstrated that the systemic administration of dopamine antagonists (Salamone, Steinpreis, McCullough, Smith, Grebel & Mahan 1991) or the depletion of dopamine in the nucleus accumbens (Cousins, Sokolowski, Salamone 1993) decreased the rate of operant responding for food. However, these same manipulations failed to produce a reduction in the motivation for food, as indicated by an increase in the nonoperant intake of concurrently available free food. On the basis of these findings, it has been suggested (Cousins, Sokolowski, Salamone 1993) that the effects of dopamine depletion on operant rates may reflect influences on the behavioral mechanisms regulating the execution of the operant response, as opposed to the simplistic notion that these manipulations impair food motivation or reward. Interestingly, this specific relationship between the performance of the operant response and dopamine activity was further reflected by findings which indicated that an increase in dopamine release from the NA was more closely associated with highly active operant responding (for food) than with the consumption of free food (Salamone, Cousins, McCullough, Carriero, Berkowitz 1994).

The above therefore suggested that both the traditional two bottle preference and oral operant self-administration paradigms have potential limitations resulting in inadequate descriptions of ethanol intake. It would appear to be the case that both preference and operant paradigms have lacked the behavioral specificity or resolution necessary to differentiate between the effects of pharmacological manipulations on ethanol intake. Additionally, research has suggested that the oral operant paradigm may be confounded by behavioral measures which are more sensitive to manipulations influencing the performance of an operant than to changes in reinforcement efficacy (Cousins, Sokolowski, Salamone 1993; Salamone, Cousins, McCullough, Carriero, Berkowitz 1994).

It is suggested, therefore, that neither of these paradigms are sufficient, in and of themselves, to adequately address questions relating to the neurobiological mechanisms of ethanol reinforcement. Consequently, for the evaluation of the mechanisms regulating

ethanol intake the microstructural approach will be presented as a high behavioral resolution alternative.

MICROSTRUCTURAL ANALYSIS

Despite the ongoing use of traditional paradigms, it was recognized rather early that an understanding of the mechanisms which act to regulate ethanol intake could not be achieved solely on the basis of simple ethanol intake measures. It had been suggested, for example, that the factors contributing to the limitation of ethanol intake could not be known without knowledge of the temporal distribution of food, water and ethanol consumption (Lester 1966). Fortunately, techniques which would be required in order to adopt such a microanalytic approach to the study of behavior have been developed and utilized in other fields of behavioral neurobiology (e.g. Blundell & Latham 1980; Blundell & Latham 1978; Fletcher & Burton 1986; LeMagnen & Devos 1980).

In particular studies utilizing microstructural analyses in order to elucidate the mechanism regulating feeding behavior, have provided the basis upon which techniques for the analysis of voluntary intake of ethanol have been derived.

Microstructural Analyses of Consumatory Behavior

Microstructural techniques used to examine short and long term feeding responses, have clearly contributed to advances in the understanding of both the behavioral mechanisms and processes mediating feeding (LeMagnen 1992). Typically a microstructural analysis has examined the organization of the behavioral components which comprise the initiation, maintenance and termination of feeding responses. These temporal and structural measures have included the onset of the first eating bout (meals), the number, size and duration of food bouts, eating rate and the occurrence of the cessation of feeding (Blundell & Latham 1978).

In normal free feeding rats, feeding behavior has been characterized by a prandial periodicity, in which food meals or bouts are separated by intervals in which there is an absence of eating (LeMagnen 1992). The definition of a meal in the literature has varied but

has been generally based on the time interval following the termination of eating. The end of a meal or bout was said to occur if the rat fails to initiate another bout of eating within 5 to 40 minutes (LeMagnen 1992).

Superimposed upon the prandial periodicity, is a diurnal or circadian periodicity. The diurnal periodicity has been characterized by the expression of large meals separated by short intervals during the night, and small meals separated by large intervals during the day (LeMagnen & Devos 1980). Generally, 70 to 90 % of the 24 hour food intake occurs during night portion of the dark/light cycle. While day to day intake is fairly constant there is a significant correlation in the intake of food between the nocturnal and subsequent diurnal intake as well as between the diurnal and subsequent nocturnal intake (LeMagnen & Devos 1980).

An analysis of drinking patterns in free feeding rats suggested that normal drinking was closely associated with food intake (e.g. Fitzsimons & LeMagnen 1969). Meal-associated drinking was suggested to reflect, in the absence of internal stimuli, anticipation of future water requirements based upon the consumption of food. Thus, food stimuli may act as conditioned stimuli to drink (Fitzsimons & LeMagnen 1969).

An examination of the pattern of food intake indicates that the overall pattern of food intake, in free feeding animals, would appear to be a function of two distinct mechanisms. The first mechanism periodically initiates eating while a second independent process governs the amount consumed until satiation is achieved (LeMagnen 1992).

The Initiation of Eating and Satiety

The signal to eat or initiate a bout or meal, in free feeding animals, has been described as a result of the combination of systemic and sensory stimuli (Louis-Sylvestre & LeMagnen 1980). This combination has been referred to as the appetite. The systemic signal to eat seems to consist of a blood borne or humoral signal which reflects a nutritional deficit. Data suggested that this humoral signal may result from a transient decrease in blood

glucose. In free feeding rats, this signal will act intermittently on brain targets and may give rise to hunger and arousal and the initiation of meals (Louis-Sylvestre & LeMagnen 1980).

The sensory afferent pathways originating from the oral cavity and which are activated by food in the mouth provide the sensory signal to eat and may contribute to the recognition of the palatability of the food (LeMagnen 1992). Elements which contribute to and influence palatability include visual, olfactory and taste signals. In addition, palatability is influenced by the signals derived from oral processing of the food (i.e. chewing). Recent studies have suggested that the palatability of food is independent of systemic processes, and governs the amount of food eaten until satiation was achieved and feeding ended (meal size). Furthermore, systemic stimuli and palatability are substitutive and additive on the initial strength of stimulation to eat (LeMagnen 1992).

It is interesting to note that it has been suggested that palatability (including taste) can be conditioned to the postingestive nutritive properties of food (Jacobs 1974; Sclafani & Mistenbaum 1988). In this situation the postingestive nutritive effects of food served to function as a reinforcer, further driving food intake. In fact, research has suggested that both learned and unlearned palatability (sweet taste and greasy texture) will be maintained, under specific situations, only if it is reinforced by the post ingestive nutritive activity of the food. For example, it has been demonstrated that food deprived rats will initially consume large quantities of saccharine or sucrose. Over time, however, sucrose intake increased, while the intake of the non-nutritive saccharine extinguished (LeMagnen 1954).

Satiety, and by implication meal size, has been described as a balance struck between the initial strength of stimulation (both sensory and systemic) and the negative feedback provided by oral and gastric stimuli (Davis & Cambell 1973; Davis & Smith 1988; LeMagnen 1992). In this manner, manipulation of the taste and postabsorptive effects of food may potentially increase or decrease the size of a given meal and therefore the onset of satiation.

The Characterization of Feeding Behavior following Pharmacological Manipulation

The use of the microstructural analysis, described above, to characterize normal feeding behavior has also been used effectively to further the understanding of the mechanisms mediating the effects of pharmacological manipulations on consumatory behavior in animals (Blundell & Latham 1980; Blundell & Latham 1978; Fletcher & Burton 1986; Blundell, Latham & Lesham 1976) and humans (Rogers & Blundell 1979). Within the feeding literature it has been clearly acknowledged that the exclusive use of, and reliance upon simple intake measures may mask information necessary for identifying and discriminating the mechanisms mediating the effects of various classes of drugs on food intake (e.g. Blundell & Latham 1980). Simple decreases in food intake, by far the most common result of pharmacological manipulations (a claim which can be equally made for ethanol intake as well), may be a function of influences on natural processes of hunger or satiation, or alternatively may simply reflect nonspecific obstructions to feeding (Blundell, Rogers & Hill 1985). An example of nonspecific effects would include the disruption of behavioral organization caused by certain stimulants. Thus drugs with a seemingly common functional outcome, at a crude level, have been examined and were shown to have differential effects upon the microstructural components underlying feeding (e.g. Blundell & Latham 1980; Burton, Cooper & Popplewell 1981).

In an attempt to behaviorally differentiate between the effects of different classes of equipotent anorexic drugs, specifically amphetamine and Fenfluramine, alterations in the internal structure of individual bouts have been examined (Burton, Cooper & Popplewell 1981; Blundell & Latham 1980; Blundell & Burridge 1979). Results of these microstructural analyses have suggested that these drugs intervene in different sets of processes responsible for the organization of feeding behavior (Blundell & Latham 1980; Burton, Cooper & Popplewell 1981). Typically, animals treated with amphetamine exhibited a longer initial latency to begin eating, followed by infrequent short bouts of rapid

eating. On the other hand, the effects of Fenfluramine were characterized by much more restricted effects which were limited to a reduction in the rate of eating and bout size. These results were interpreted to suggest that amphetamine acted in a nonspecific manner to impede the organization of behaviors necessary for feeding, whereas fenfluramine acted to influence satiety processes (Blundell & Latham 1980).

Thus, the use of the microstructural analyses in feeding studies revealed a technique which has been useful in both characterizing the normal pattern of consumption and providing a means for discriminating between the subtle behavioral effects of pharmacological manipulations.

MICROSTRUCTURAL ANALYSES OF ETHANOL INTAKE

While the microstructural approach has not heavily permeated the field of alcohol research, it has been tentatively applied to characterize the pattern and structure of ethanol intake (Gill, France, & Amit 1986; Gill 1989; Samson, Tolliver, Schwarz-Stevens 1990; Dole & Gentry 1984).

Consistent with the pattern observed with food and water intake (Fitzsimons & LeMagnen 1969), ethanol intake has been shown to exhibit both prandial and circadian periodicity with the majority of ethanol intake bouts occurring during the night portion of the day/night cycle (Dole & Gentry 1984; Gill, France, & Amit 1986). In the context of a microstructural analysis of food, water and ethanol intake in male Long Evans rats, Gill (1989) further defined the structure of and interaction between consumatory behaviors, in a two bottle preference paradigm. In this paradigm, a microcomputer controlled data acquisition system was utilized to dynamically monitor food and fluid intake (Gill, Mundl, Cabilio & Amit 1988). Feeding and drinking activity were monitored continuously over a 23 hour period. Data from this analysis indicated that on average, rats consumed 2.5 gm/kg/day (preference ratio = .58) derived from 10% ethanol within approximately 9 discrete ethanol bouts. Each bout consisted of approximately 2.25 mls of ethanol. Gill, France, & Amit (1986) have demonstrated that the amount of ethanol consumed within the

range of a normal ethanol bout such as that described above, was sufficient to produce pharmacologically meaningful or intoxicating effects. Thus, given the number of discrete bouts which occur during the dark portion of the day/night cycle, a rat will potentially experience numerous "pharmacologically reinforcing" events throughout this period. In addition, the sporadic nature of the ethanol bouts indicated that an animal's blood ethanol levels would rise and fall throughout that cycle. The absence of sustained blood ethanol levels would represent an important factor explaining the failure to observe physical dependence in this animal model (Amit, Sutherland, & White, 1976).

Interestingly, it would appear that the microstructure of ethanol intake by Long Evans rats in the free choice preference paradigm, described above, are not entirely consistent with the structure of intake reported for the same strain of rats self-administering ethanol in an operant paradigm (Samson, Tolliver, Schwarz-Stevens 1991). While the cumulative levels of daily ethanol intake were comparable, the size of individual ethanol bouts within the operant paradigm were reported to be smaller and more numerous than those obtained with the two-bottle preference procedure (Samson, Tolliver, Schwarz-Stevens 1991). In point of fact, the size of the ethanol bouts obtained within the continuous access operant paradigm (Files, Andrews, Samson, Lumeng, & Li 1992; Samson, Tolliver, Schwarz-Stevens 1991) are routinely smaller than those reported in free drinking preference paradigms (Murphy, Gatto, Waller, Mcbride, Lumeng & Li 1986; Gill, Filion, & Amit 1988; Gill, France, & Amit 1986). Further, it has been demonstrated that ethanol consumed within the simulated bout of a restricted access paradigm, is most consistent with the level of ethanol consumed in a bout in the preference paradigm (Gill, France, & Amit 1986; Boyle, Spivak, Smith, & Amit 1994)

It would appear therefore that the use of operant paradigms may result in the underestimation of the amounts of ethanol consumed during individual bouts of drinking within a free choice situation. The difference in the size of ethanol bouts is significant in that the determination of bout size may be a crucial element necessary for the understanding

of those behavioral mechanisms regulating consumatory behavior, such as satiety (Blundell & Latham 1980, LeMagnen 1992). The smaller bouts obtained as a function of operant ethanol self-administration may indicate that there are differences in the behavioral mechanisms which regulate the intake of ethanol in free choice preference and operant paradigms.

A number of other factors may also account, however, for the paradigmatic differences in the description of the bout microstructure. In this regard the discrepancy between the size of ethanol bouts in free drinking rats and those same rats maintained on an operant schedule may perhaps be explained as a function of the inherent response requirements of the paradigm or the actual definitions of what constitutes a bout.

Nonetheless, most microstructural analyses of ethanol intake to date have clearly demonstrated that rats drink sufficient amounts of ethanol to become repeatedly intoxicated over the course of the dark portion of the day/night cycle. This fact would argue in favor of the notion that rats consume ethanol for its pharmacological properties.

However, an analysis of the temporal pattern of ethanol intake has also indicated that the prandial relationship of ethanol intake may be similar to that observed for water (Gill, 1989; Samson, Tolliver, Schwarz-Stevens 1991). More specifically, 57% of fluid bouts have been associated with food intake regardless of type of fluid, with 55% of ethanol bouts following food bouts (Gill, 1989). The similarity in the prandial pattern of water and ethanol intake, would appear to legitimize criticisms which essentially suggested that the animals in these studies are not consuming ethanol for its pharmacological properties (Dole & Gentry 1984; Dole, Ho & Gentry 1985). In particular, it has been suggested that if animals are drinking ethanol for pharmacological effects, then one would expect ethanol intake to be dissociated in time from the intake of food and water (Dole, Ho & Gentry 1985).

It can be argued, however, that a simple analysis of prandial relationships, typical of the few microanalytic studies performed to date, may have masked differences which

differentiate water and ethanol intake. In this context, it would be of interest to examine the temporal and structural pattern of both ethanol and water intake with specific attention to those parameters which may dissociate the intake of the two fluids on the basis of their motivational valence. Such parameters may include among others the relative strength of approach towards the goal object. They could also include the relative size of each fluid bout following the introduction of ethanol and water and the total consumption within the first hour of fluid presentation.

A Microstructural Analysis of the Effects of Pharmacological Manipulations on Ethanol Intake

An area in which the microstructural approach holds perhaps most promise involves the delineation of the pattern and structure of effects, both direct and indirect, induced by pharmacological manipulations on ethanol self-administration. To date, this procedure has been used to examine the nature of the effects of manipulations of the dopaminergic on operant responding (e.g. Samson, Tolliver, Haraguchi, & Hodge 1992; Samson. Tolliver, Haraguchi, Kalivas 1991; Samson, Hodge, Tolliver, & Haraguchi 1993; Hodge, Samson, Haraguchi 1992) and serotonergic neurotransmitter systems on ethanol preference (e.g. Gill, Filion, Amit 1988; Gill & Amit 1989; Higgins, Tomkins, Fletcher, Sellers 1992; Svensson, Fahlke, Hard, Engel 1993).

While the microstructural approach has been presented in terms of an evolution of the two bottle preference paradigm, the basic concept behind the microstructural approach has been used to differentiate between the effects of divergent manipulations of the dopamine receptor system on ethanol-reinforced operant responding (Samson, Hodge, Tolliver, Haraguchi 1993; Pfeffer, Samson 1985; Hodge, Samson, Haraguchi 1992; Samson, Tolliver, Haraguchi, & Hodge). The nature of the behavioral measures derived from operant microstructural studies have been less detailed than those which have similarly examined the influence of pharmacological manipulations using ethanol preference paradigms. Generally, within the context of the operant paradigm, the microstructural

analysis provided an examination of the temporal pattern of ethanol reinforced responding and the temporaly localized patterns of response rates evaluated by the comparison of interresponse times (e.g. Samson, Hodge, Tolliver, Haraguchi 1993).

Collectively, these studies have suggested that the effects of divergent manipulations of the dopamine receptor system on ethanol intake can be differentiated on the basis of the pattern of operant responding. Specifically, the administration of dopamine agonists, peripherally or through microinjections into the nucleus accumbens, have produced bi-directional dose dependent effects on ethanol induced behavioral responding. Dopamine antagonists, in contrast produced decreases in responding (Samson, Hodge, Tolliver, Haraguchi 1993; Pfeffer, Samson 1985; Hodge, Samson, Haraguchi 1992). The microstructural analysis of operant responding for ethanol has indicated that the administration of DA agonists, such as amphetamine, resulted in a slowing of the momentary response rate followed by a continued prolongation of responding throughout the limited access session, a phenomena seen with both systemic injections and those administered to the nucleus accumbens (NA). The D2 agonist quinpirole produced similar findings. On the other hand, microinjections of raclopride, a D2 antagonist, into the NA resulted in both an increased latency to initiate responding and an early termination of responding (e.g. Samson, Hodge, Tolliver, Haraguchi 1993).

However, the use of the microstructural approach in conjunction with an operant paradigm is potentially subject to some of the same limitations, described in an earlier discussion, attributed to the use of traditional operant paradigms in the assessment of voluntary intake of ethanol. It would appear that the observed global effects of dopaminergic manipulations may not be specific to ethanol reinforced operant responding, but in fact may be generalized to operant behavior reinforced by a variety of reinforcers, such as water (Cohen 1991; Barrett, Katz, & Glowa 1981). Specifically, for drug, food and nonpharmacological reinforcers, amphetamine, a dopamine reuptake inhibitor and agonist, has been demonstrated to increase operant responding at low doses and suppress

responding at higher doses. Similarly, the dopamine antagonist, chlorpromazine has been demonstrated to decrease operant responding for second order schedules of reinforcement, again, independent of the type of reinforcer employed (Valentine, Katz, Kandel, Barrett 1983).

Thus, it would appear that the observed effects of dopaminergic manipulations on gross measures of operant behavior may potentially be independent of the type of reinforcer utilized. This finding is consistent with the apparent sensitivity of oral operant measures to dopamine manipulations, as was discussed earlier. Therefore, if these microbehavioral effects (e.g. temporal pattern of responding) of dopaminergic manipulations on ethanol intake are to have any meaning with regard to ethanol reinforcement specifically, it is incumbent upon the authors to demonstrate that these microbehavioral effects are also not characteristic of reinforced operant behavior in general.

In what is probably the most comprehensive and methodologically sound microstructural investigations conducted to date, serotonergic reuptake inhibitors have been examined with regard to their simultaneous effects on the pattern and structure of food, water and ethanol intake (Gill & Amit 1989; Gill, Filion, Amit 1988).

In general, increased serotonergic system functioning which follow the administration of selective serotonergic reuptake inhibitors (SSRI), has been demonstrated to produce a decrease in the level of voluntary ethanol intake (Gill & Amit 1987; Haraguchi, Samson, & Toliver 1990). Microstructural analyses of voluntary ethanol intake following the application of SSRI's have indicated that the decreases in ethanol intake have been associated with both decreases in the size (e.g. Gill et al., 1988) and frequency (e.g. Gill & Amit 1987) of individual ethanol bouts.

However, microstructural analyses further suggested that the effects of the SSRI's are not specific to ethanol intake but rather are accompanied by decreases in food intake in addition to a generalized decrease in preferred fluids (Gill & Amit 1989; Montgomery, & Burton 1986). The effects of SSRI's on food intake have been consistently characterized

by a reduction in both the size and duration of the meals (Gill, et al., 1988; Gill & Amit 1987). The lack of specificity of the effects of the SSRI's have been essentially supported in a more recent microstructural study (Higgins, Tomkins, Fletcher, Sellers 1992). In this study the authors have suggested that the suppressant effects of many 5-HT agonists on ethanol intake may be a function of a more generalized effect on consumatory behavior.

Interestingly, use of traditional preference paradigms has persisted despite their observed inadequacy in detecting nonspecific effects. One still encounters research, for example, in which the specificity of novel pharmacological manipulations on ethanol intake are inferred without careful consideration of the potential for indirect effects upon other consumatory behaviors (e.g. Svensson, Fahlke, Hard, & Engel 1993).

THE PRESENT INVESTIGATION

It has been suggested that traditional research paradigms lack the behavioral resolution necessary to differentiate the processes acting to regulate voluntary ethanol intake. In order to address this limitation, the microstructural approach has been proposed as a high behavioral resolution preference paradigm which provides the means for identifying the behavioral mechanisms which act to regulate ethanol consumption. In the two phases which comprise the present thesis, the differences and similarities in the processes which act to shape ethanol intake during both the normal acquisition of voluntary ethanol intake and following specific pharmacological manipulations are characterized.

In Phase 1 of the present thesis the microstructure and temporal pattern of consumatory behavior in unmanipulated animals was examined. Experiment 1 provided an analysis of the changes in the microstructure and temporal pattern of consumatory behavior which characterize the normal acquisition of voluntary ethanol intake under an alternate day, free choice paradigm. A perceived shortcoming of the microstructural paradigm is the display of low levels of voluntary ethanol intake in the test animals. In order to address this potential limitation in the microstructural paradigm, experiment 2 demonstrated the potential of an acute period of forced exposure to ethanol to enhance the voluntary intake of ethanol.

Experiment 3, further demonstrated the capacity of an acute period of forced ethanol exposure to increase the level of intake obtained specifically within a microstructural paradigm. In addition, experiment 3 provided insight into the mechanisms mediating the enhanced levels of ethanol intake following the forced ethanol exposure.

In Phase 2 of the present thesis the utility of the microstructural approach in differentiating the processes which mediate the subtle behavioral effects of pharmacological manipulations on voluntary ethanol intake was examined. Specifically, the ambiguity in the literature regarding the role of the gamma-aminobutyric acid (GABA) receptor system in mediating voluntary ethanol intake was examined. The introduction to Phase 2 discussed the GABAergic literature relevant to this area of ethanol research.

EXPERIMENT 1

A DESCRIPTIVE ANALYSIS OF THE STRUCTURE AND TEMPORAL PATTERN OF VOLUNTARY ETHANOL INTAKE WITHIN AN ACQUISITION PARADIGM.

Use of the microstructural approach has provided a means for understanding the behavioral mechanisms which act to mediate the voluntary intake of ethanol (Gill, France, & Amit 1986; Gill 1989; Samson, Tolliver, Schwarz-Stevens 1990; Dole & Gentry 1984). Illustrating this point, several studies have demonstrated that rats will consume ethanol within a number of discrete bouts, primarily during the dark portion of the daily light cycle (Gill, France, & Amit 1986; Gill 1989). However, the research, conducted to date, has been limited and centered upon an examination of the structural changes in consumatory behavior within a maintenance paradigm. In the context of a maintenance paradigm animals are examined which have already acquired the ethanol drinking behavior and are in fact maintaining stable levels of intake. As a consequence of this, the processes which act to shape the acquisition of voluntary ethanol intake have not been identified to date.

Therefore, in the present experiment, the changes in the microstructure and temporal pattern of food, water and ethanol intake which accompany the acquisition of voluntary ethanol intake were examined using an automated drinkometer system.

METHOD

Subjects

Twenty five male Long Evans rats (Charles Rivers Canada Inc.) weighing 200-250g 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 hr light/dark cycle. Food and fluids were available ad libitum throughout the test period.

Drugs

Ethanol solutions with concentrations which ranged from 2 to 10% (v/v) were prepared from 95% stock ethanol mixed with tap water.

Apparatus

A microcomputer controlled data acquisition system (drinkometer) was utilized in the present experiment to dynamically monitor food and fluid intake. The system consisted of operant chambers (Grason-Stadler chamber) equipped with feeders which dispensed 45-mg standard Bioserve food 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 hour period. During a daily 60 min. computer shutdown period, the volume of each fluid tube was recorded and incorporated into the subsequent data analysis. All accumulated raw data were processed in order 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 when there was a transition to another input device. Subsequent data analysis yielded measures 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.

In order to avoid the confounding influence of inadvertent contact by the animals with the food cups and drinking spouts, only those bouts consisting of more than 5 consecutive events were included in the analysis. Complete design specifications for the computerized acquisition system have been previously detailed (Gill, Mundl, Cabilio & Amit 1988).

Procedure

Following a five 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. In order to avoid a position bias the position of the ethanol filled tube was altered on successive ethanol presentation days. The presentation of ethanol was initiated with a 2% (v/v) ethanol solution. Subsequently, the concentrations of ethanol were increased by 2%, following every second ethanol presentation, until a final concentration of 10% ethanol was achieved. The 10% concentration of ethanol was available for a total of 4 presentation sessions. On all intervening days, the fluid tubes were filled with water .

RESULTS

In the present experiment, changes in the structure and pattern of ethanol intake across increasing concentrations of ethanol were examined. Towards this end, multiple repeated measures one-way (consisting of bout variables) and factorial ANOVAs (consisting of repeated measures across ethanol concentrations, hours and days) were performed. The source of interactions within factorial ANOVAs were established using tests of simple main effects and simple interactions. Tukey post hoc analyses, where denoted, were used to determine the significance of differences in means.

A Structural Analysis of Ethanol, Water and Food intake.

The results of the present experiment indicated a biphasic pattern of ethanol intake produced as a function of the presentation of increasing concentrations of ethanol in a free choice with water. Figure 1 illustrates the significant change in total ethanol intake (mls) across ethanol concentrations presented [$F(11,264)=743$, $p<0.00001$]. Post hoc analysis revealed that total ethanol intake decreased as the concentration of available ethanol wasincreased from 2% to 6%. Inversely, total ethanol intake was significantly increased as the concentration was further increased from 6 to 10%. Similarly, as observed in Figure 1, the presentation of increasing concentrations of ethanol also resulted in significant changes

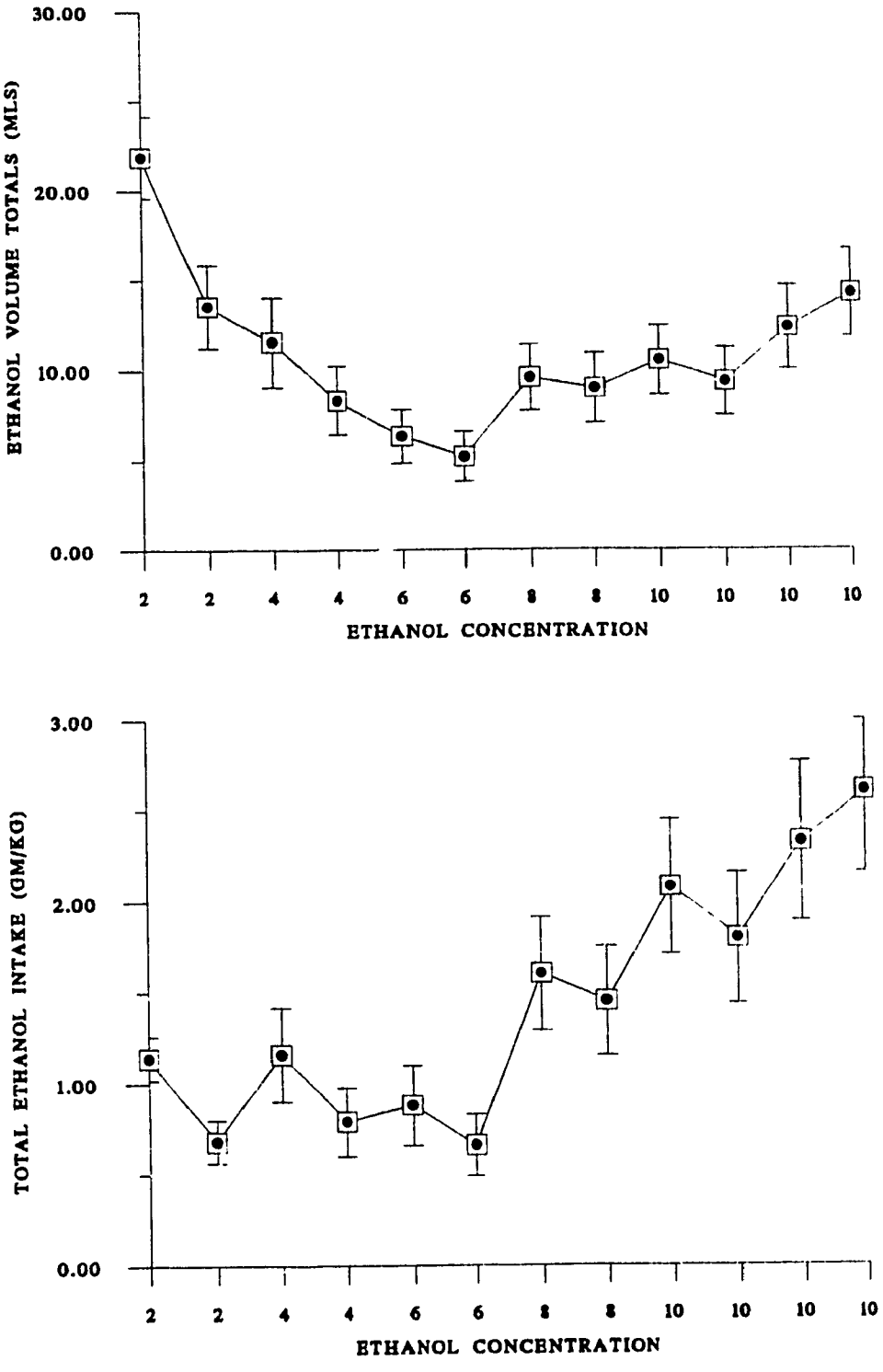

Figure 1. Total ethanol intake in milliliters and grams per kilogram across the presentation of increasing concentration of ethanol. Vertical lines represent S.E.M.

[F(11,264)=8.11, p<0.00001] in the amount of absolute ethanol (gm/kg) consumed. Absolute ethanol intake was observed to increase primarily across concentration of 6 to 10% ethanol.

Consistent with the ethanol intake values described above, the ethanol preference ratio's presented in Figure 2 displayed significant changes across ethanol concentrations. These changes were biphasic in nature [F(11,264)=7.67, p<0.00001]. Post hoc analysis further demonstrated that preference ratios decreased from 2 to 6 % and were significantly increased from 6 to 10%. Across the ethanol concentrations tested, maximal ethanol preference values were obtained at 2%, whereas the lowest ethanol preference was evident at 6% ethanol.

Total water intake values, which are presented in Figure 3, demonstrated a significant change across ethanol presentations [F(11,264)=6.34 p<0.00002]. However, in contrast to the biphasic pattern of ethanol intake described above, post hoc analyses suggested that water intake values were characterized solely by an increase in water intake across those sessions in which 2 to 6% ethanol was presented.

In an attempt to further define the nature of the differences between the intake of water and ethanol, a microstructural analysis of consumatory behavior was performed. An analysis of the size of ethanol and water bouts across ethanol presentations, presented in Figure 4, demonstrated that there was a significant interaction between the type of fluid consumed and the concentration of ethanol [F(11,264)=3.05 p<0.0008]. Mirroring the pattern of intake observed for total fluid intake, a test of simple effects, holding fluid type constant, revealed that the size of both ethanol [F(11,264)=3.43 p<0.0003)] and water [F(11,264)=2.71 p<0.004] bouts changed over ethanol concentrations. Specifically, Post Hoc analysis revealed that the changes in size of water bouts were primarily characterized by an increase in bout size over the presentation lower concentrations of ethanol. In contrast, the size of ethanol bouts were observed to be biphasic such that the size of ethanol

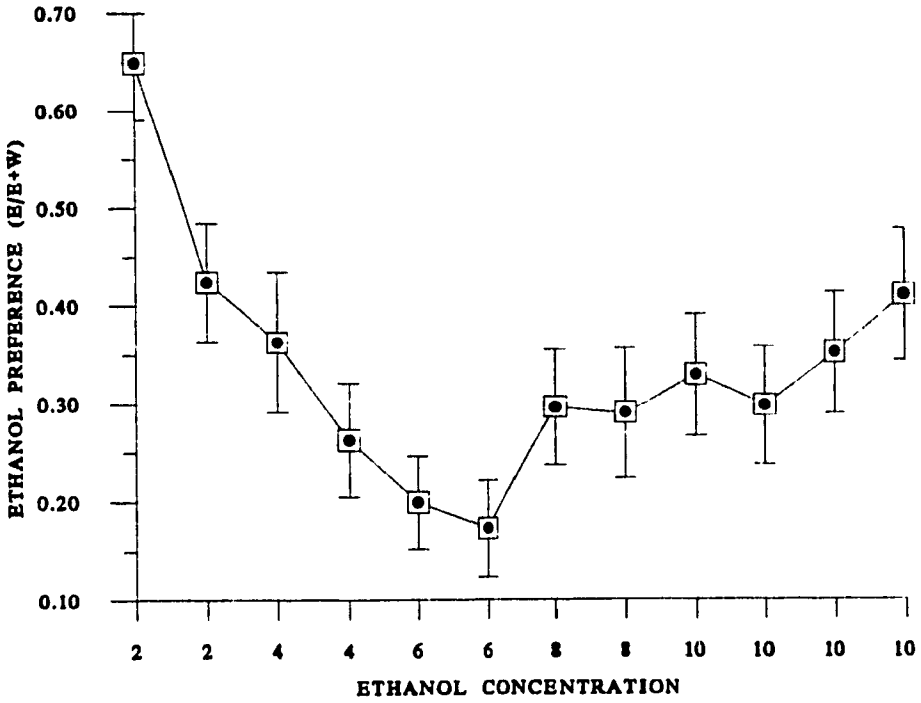

Figure 2. Ethanol preference ratio's across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

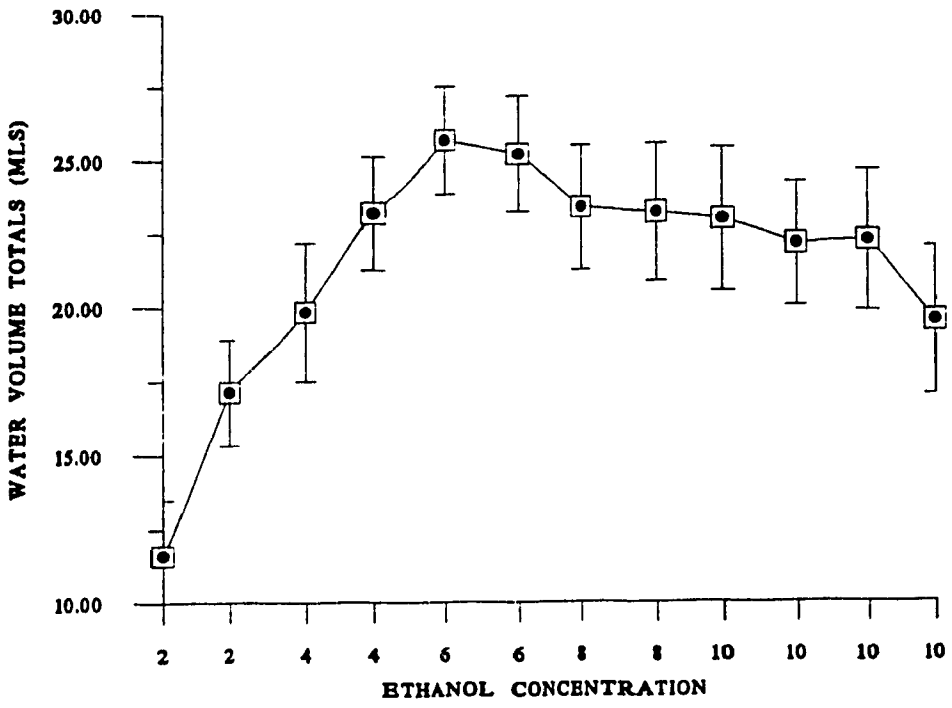

Figure 3. Intake of water (mls) across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

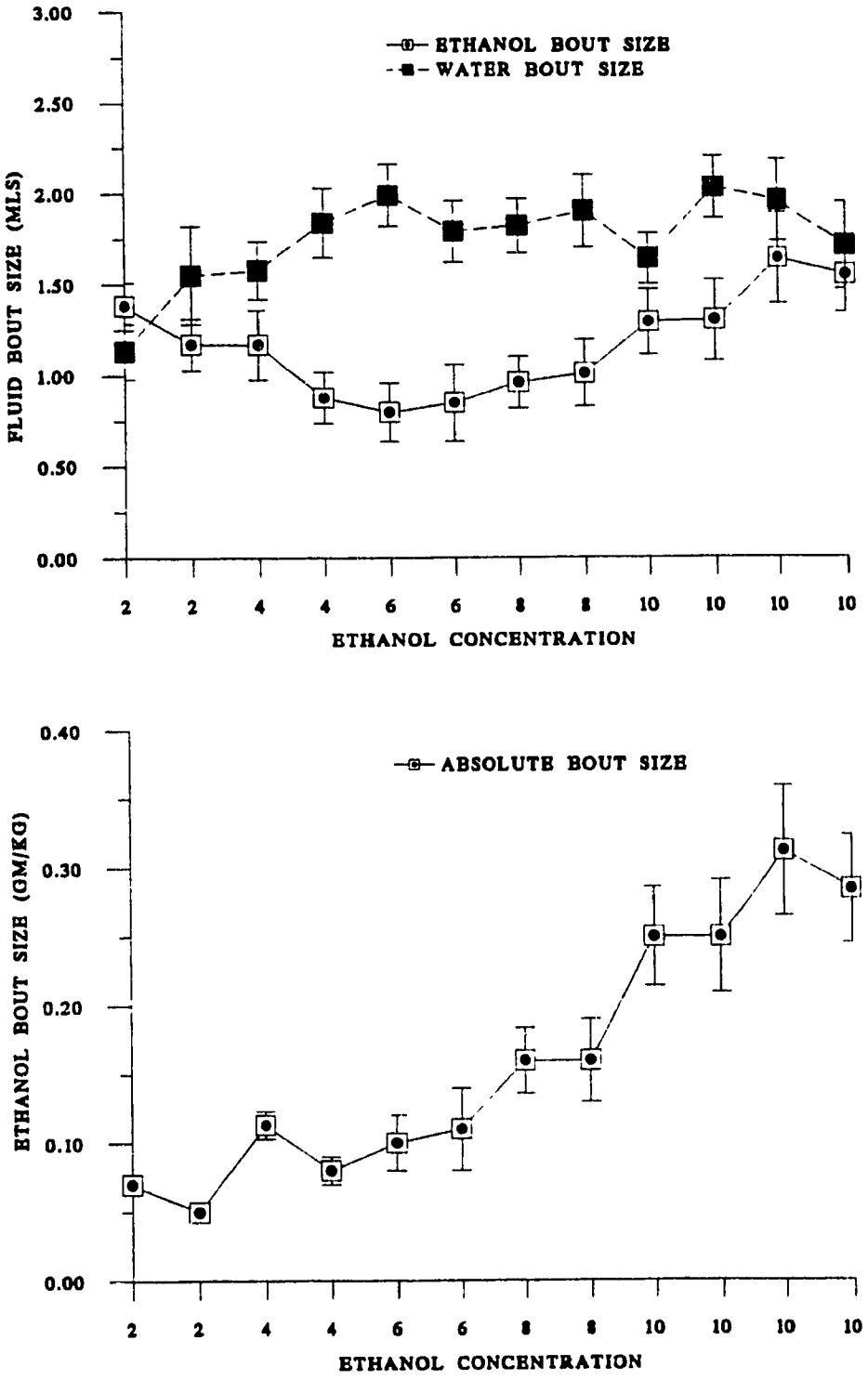

Figure 4. Mean bout size for the intake of water, ethanol (mls) and absolute ethanol across the acquisition curve. Vertical lines represent S.E.M.

bouts decreased over the lower concentrations (2 to 6%) and increased as a function of the presentation of higher ethanol concentrations (6 to 8% ethanol).

An analysis of the bouts of absolute ethanol intake, also presented in Figure 4, revealed a significant linear increase [$F(11,264)=13.86$, $p<0.00001$] across ethanol presentations. The biphasic pattern in the intake of raw ethanol was not evident.

An examination of the mean frequency and duration of water and ethanol bouts across ethanol concentrations are presented in Figure 5. An analysis of the frequency data demonstrated a significant interaction [$F(11,264)=6.35$ $p<0.0005$] between type of fluid consumed and ethanol concentration. The analysis of simple effects, holding fluid type constant, further demonstrated that the frequency of water bouts did not differ across ethanol concentrations [$F(11,264)=1.75$ $p<0.07$]. In contrast, the frequency of ethanol bouts changed significantly across ethanol presentations [$F(11,264)=6.55$ $p<0.00001$]. Specifically, number of ethanol bouts were observed to decrease as the concentration of the presented ethanol was increased from 2% to 6%. The frequency of ethanol bouts on the other hand remained constant between those trials when 6 and 10% ethanol were presented.

In the case of both the intake of ethanol [$F(11,264)=1.45$ $p<0.15$] and water [$F(11,264)=1.14$ $p<0.33$], the duration of bouts remain constant across ethanol presentations (Figure 5).

The changes, described above, in the size of ethanol and water bouts and the simultaneous absence of any changes in the duration of bouts suggested that there may be differences in the volume of fluid consumed per unit of time, i.e. the rate of intake, across ethanol presentations. Presented in Figure 6 are the patterns of both the rate of ethanol intake (mls/sec) and the size of ethanol bouts (mls). An analysis of these data suggested that the rate of ethanol intake changed significantly over the presentation of increasing concentrations of ethanol [$F(11,264)=1.98$, $p<0.031$]. Furthermore, these changes in the rate of ethanol intake appeared to be concentration dependent. While post hoc analysis suggested that the rate of ethanol intake at 6% was not significantly different from that at

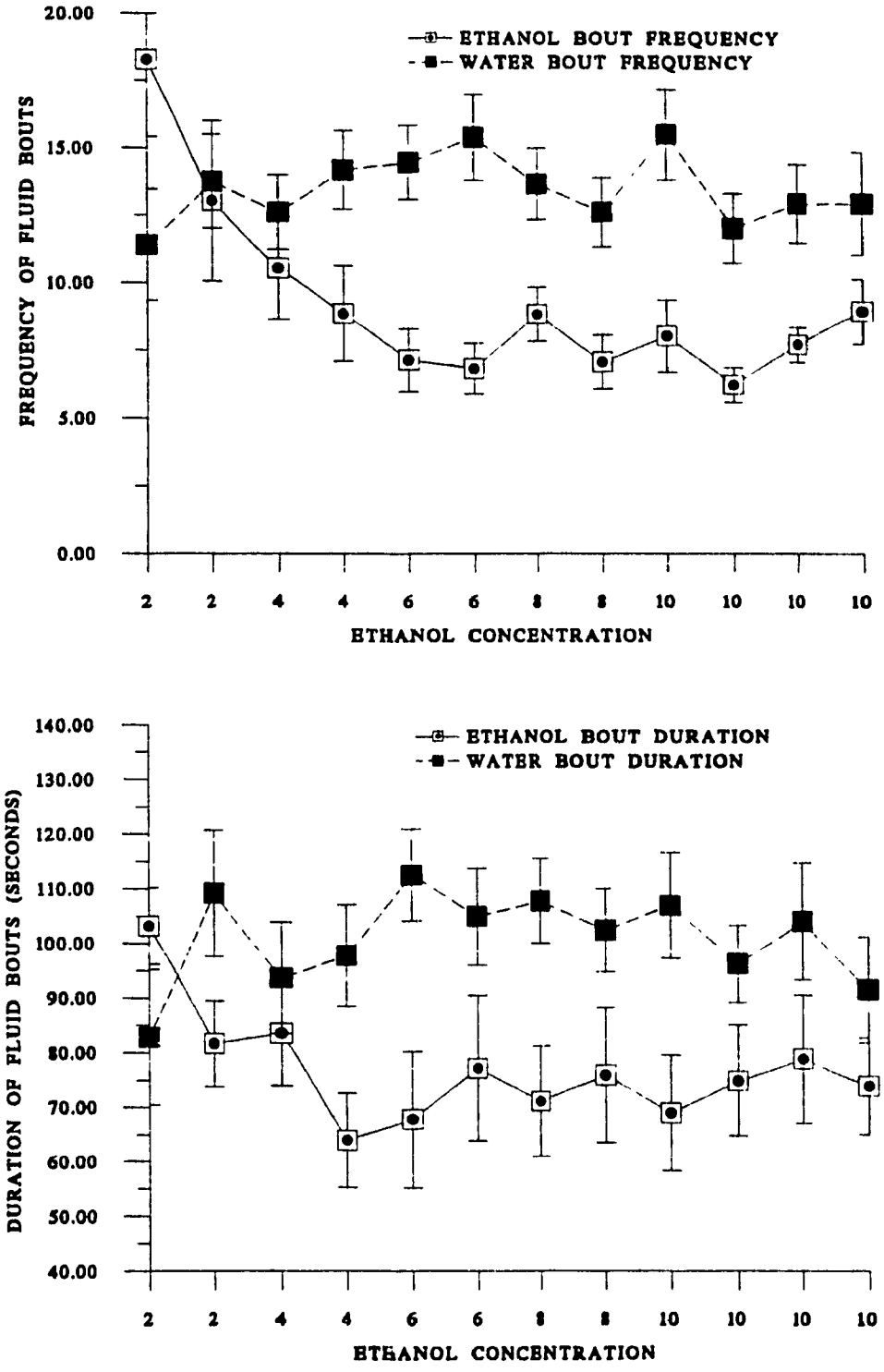

Figure 5. Mean bout frequency and duration values for ethanol and water across the acquisition curve. Vertical lines represent S.E.M.

2%, the rate of ethanol intake increased at concentrations from 6 to 10%. In addition, Figure 6 would suggest that unique to the presentation of higher ethanol concentrations the increase in the rate of ethanol intake paralleled the increase in the size of ethanol bouts.

While the rate of ethanol intake changed as a function of the concentration of ethanol presented, there were no significant changes [$F(11,264)=1.18$, $p<0.4$] in the rate of water intake, also presented in Figure 6.

The pattern of total food intake, as measured by total food pellets consumed, across ethanol concentrations is presented together with body weight values in Figure 7. The results revealed significant changes in food intake observed over days [$F(11,264)=4.64$, $p<0.00001$]. Post hoc analysis indicated that the increase in food intake was observed solely during the presentation of 6% ethanol. Body weight increased [$F(11,264)=3314.11$, $p<0.00001$] uniformly across ethanol concentrations (Figure 7).

A further analysis of food bout parameters are presented in Figure 8. The data suggest significant changes in both the frequency [$F(11,264)=3.25$; $p<0.0005$] and size [$F(11,264)=2.37$, $p<0.009$] of food bouts across ethanol concentrations. These results suggested that the transient increase in total food intake, observed during the second presentation of 6% ethanol, was principally a function of an increase in the frequency food bouts. The mechanisms mediating this limited and concentration dependent increase in the number of food bouts was unclear. However, the very circumscribed nature of the observed effect on food intake would argue against it being a compensatory response to the decrease in the intake of ethanol, which occurred at approximately the same time. In contrast to the observed effect on food intake, the decrease in ethanol consumption occurred gradually but consistently across the presentation of lower ethanol concentrations.

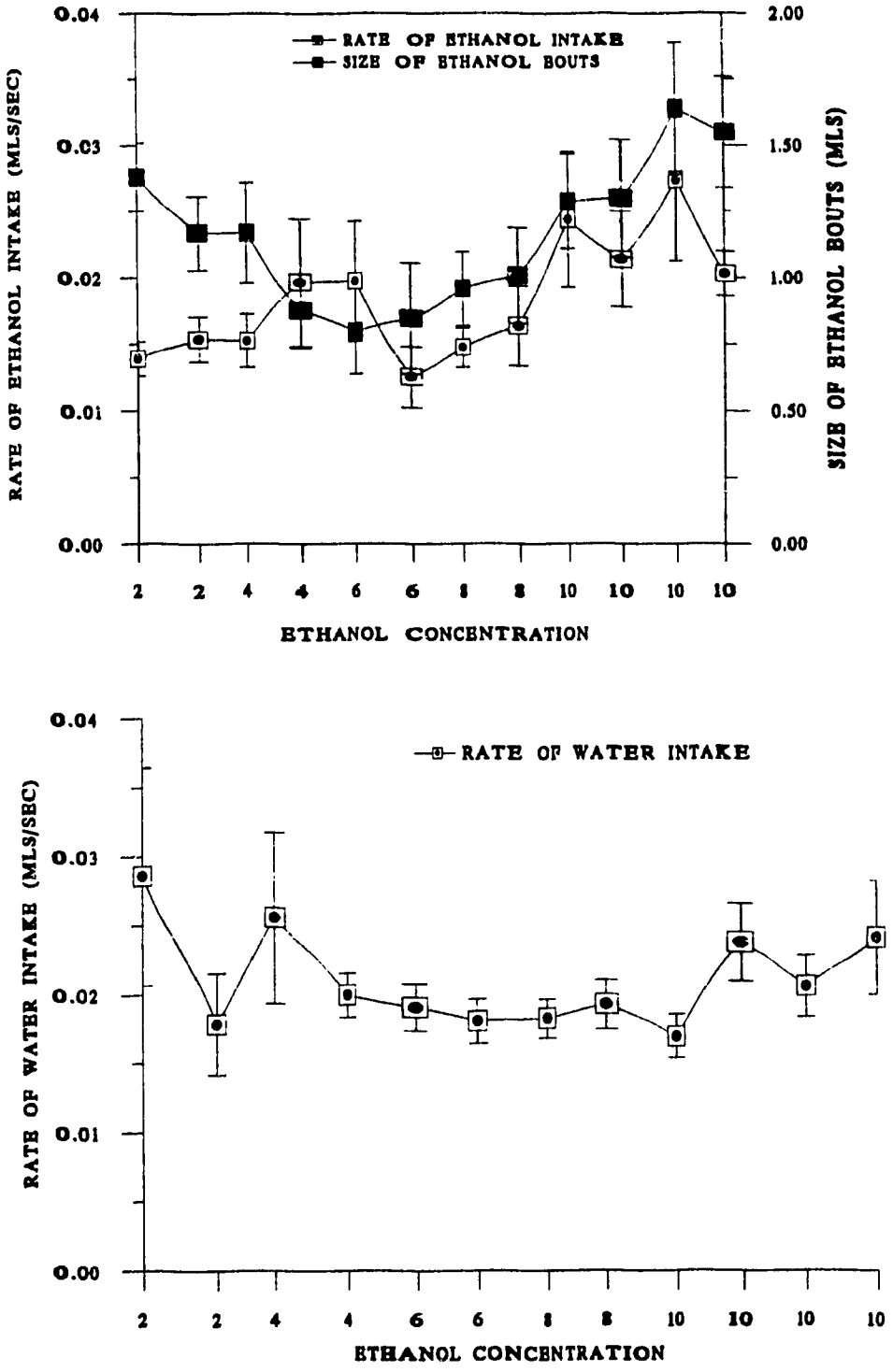

Figure 6. A comparison of the changes in the mean rate of ethanol intake with the changes in ethanol bout size across the acquisition curve (upper figure). The lower figure represents the rate of water intake. Vertical lines represent S.E.M.

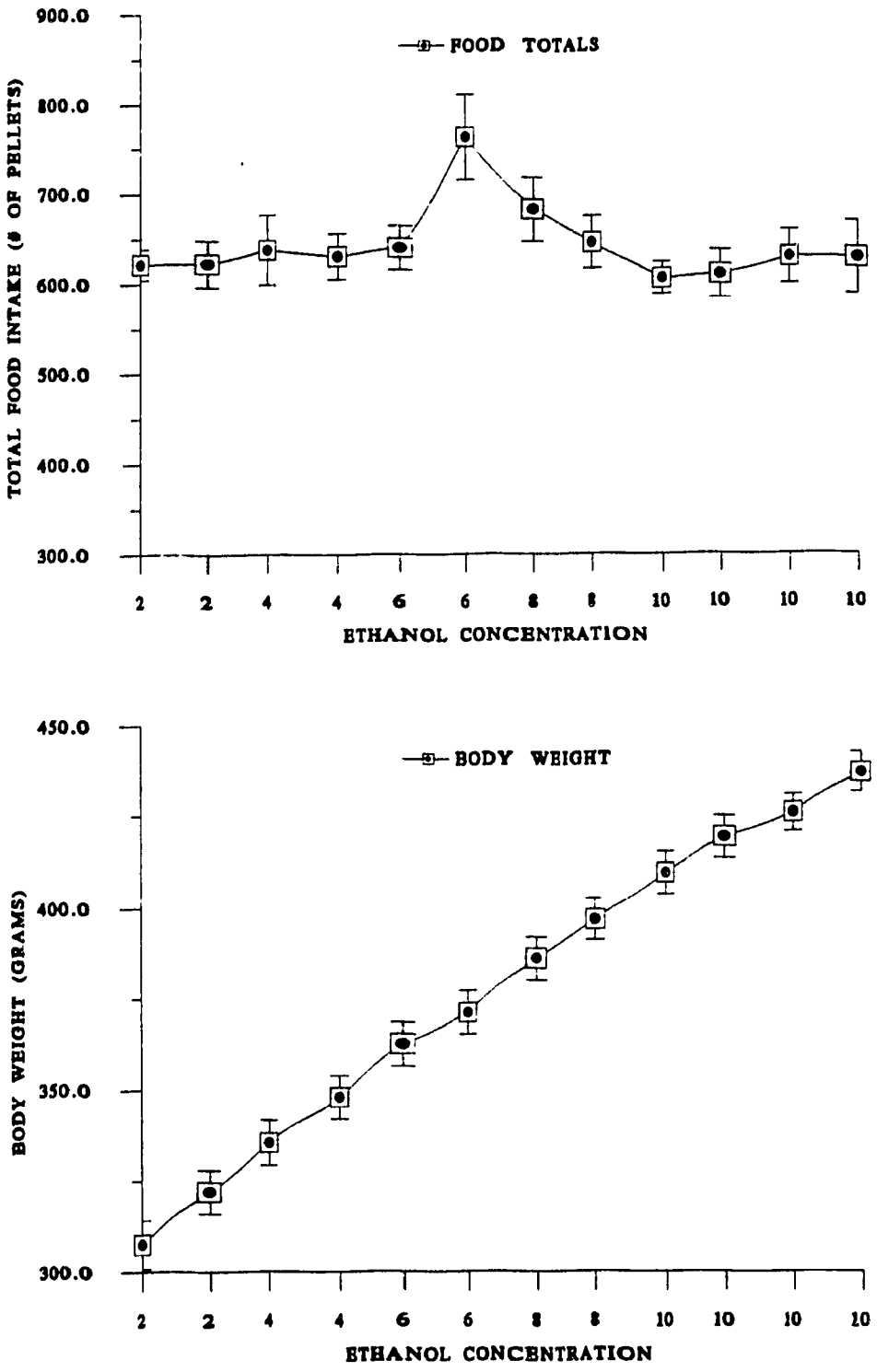

Figure 7. Total food intake and body weight values across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

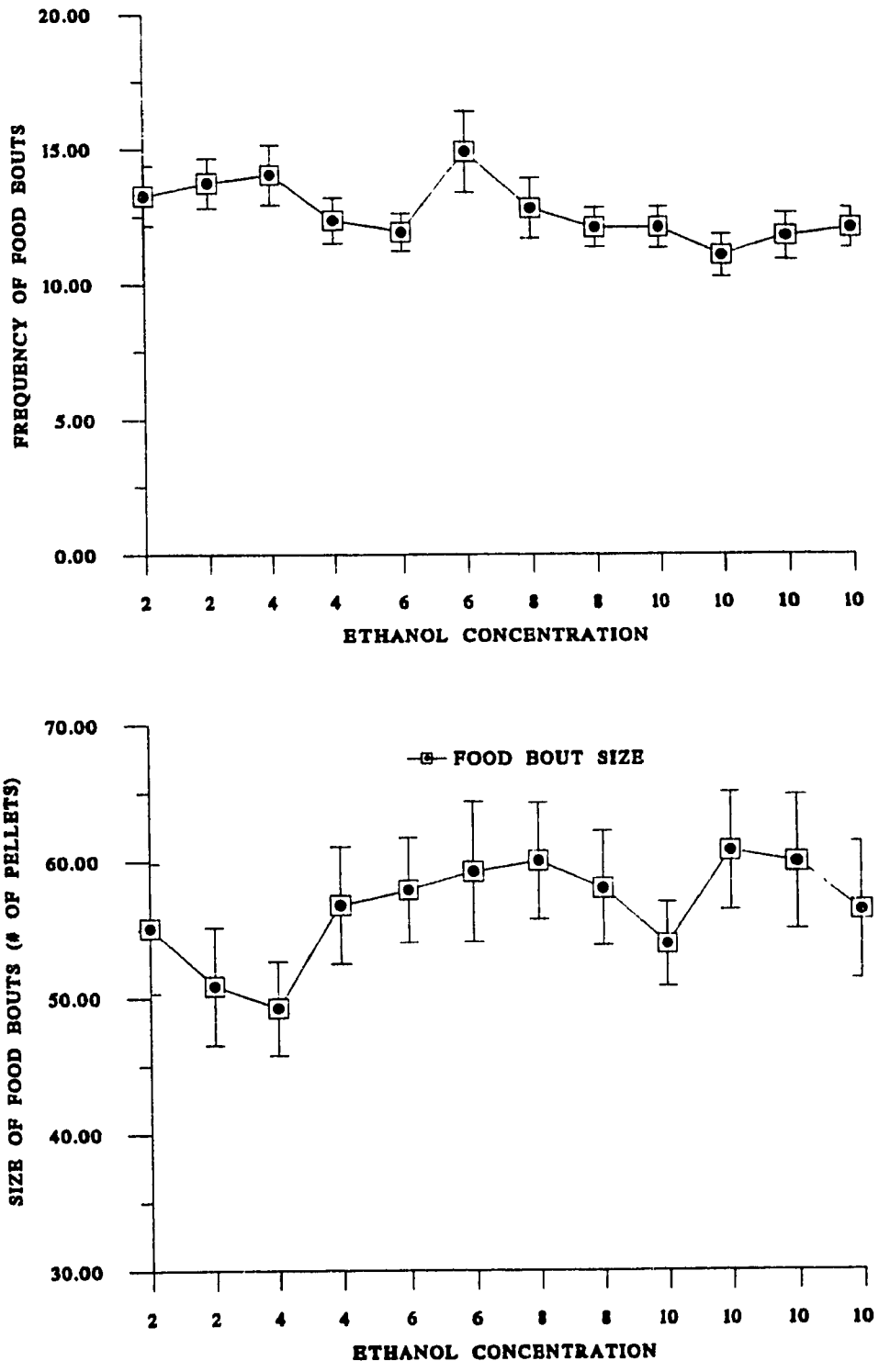

Figure 8. The frequency and size of food bouts across the range of increasing concentrations of ethanol. Vertical lines represent S.E.M.

Temporal Pattern of Ethanol, Water and Food Intake

In order to further characterize the pattern of consumatory behavior, a temporal analysis of the data was conducted using the data obtained during the second presentation of each ethanol concentration up to 8%, and all presentations at 10%.

The analysis of the temporal (hourly) pattern of ethanol intake revealed significant main effects for the intake of ethanol across both ethanol concentrations [$F(7,168)=3.53$, $p<0.002$] and hours [$F(22,528)=15.80$, $p<.00001$], with a significant concentration by hours interaction [$F(154,3696)=1.37$, $p<0.0021$]. A representative sample of the temporal pattern of ethanol intake utilizing the 2, 6 and 10 % ethanol concentrations is presented in Figure 9. The results of an analysis of these data indicated that a source of the interaction between ethanol concentration and hours was the singular pattern of ethanol intake observed during the presentation of 6% ethanol. In particular, further analysis indicated that the consumption of 6% ethanol was notable for the absence of the high levels of ethanol intake in hour one or the significant reduction in intake during hour two which characterized ethanol intake at other concentrations.

While the graphic representation of the temporal data presented above is desirable for its detailed hourly representation of intake, the inherent density of the information obtained prohibits the coherent display of more than a few levels of the ethanol concentration dimension. Therefore, in order to permit the presentation of all ethanol concentrations, in such a manner that the interaction between temporal parameters and ethanol concentrations are readily discerned, the 23 hour temporal data were transformed into intake values across 3 distinct temporal periods.

The justification for the selection of 3 distinct temporal periods of ethanol intake was based upon observations of the pattern of daily ethanol intake. As presented in Figure 9, the data would suggest that three distinct and internally consistent temporal periods subsume the diurnal/nocturnal pattern of ethanol consumption. Specifically, these included

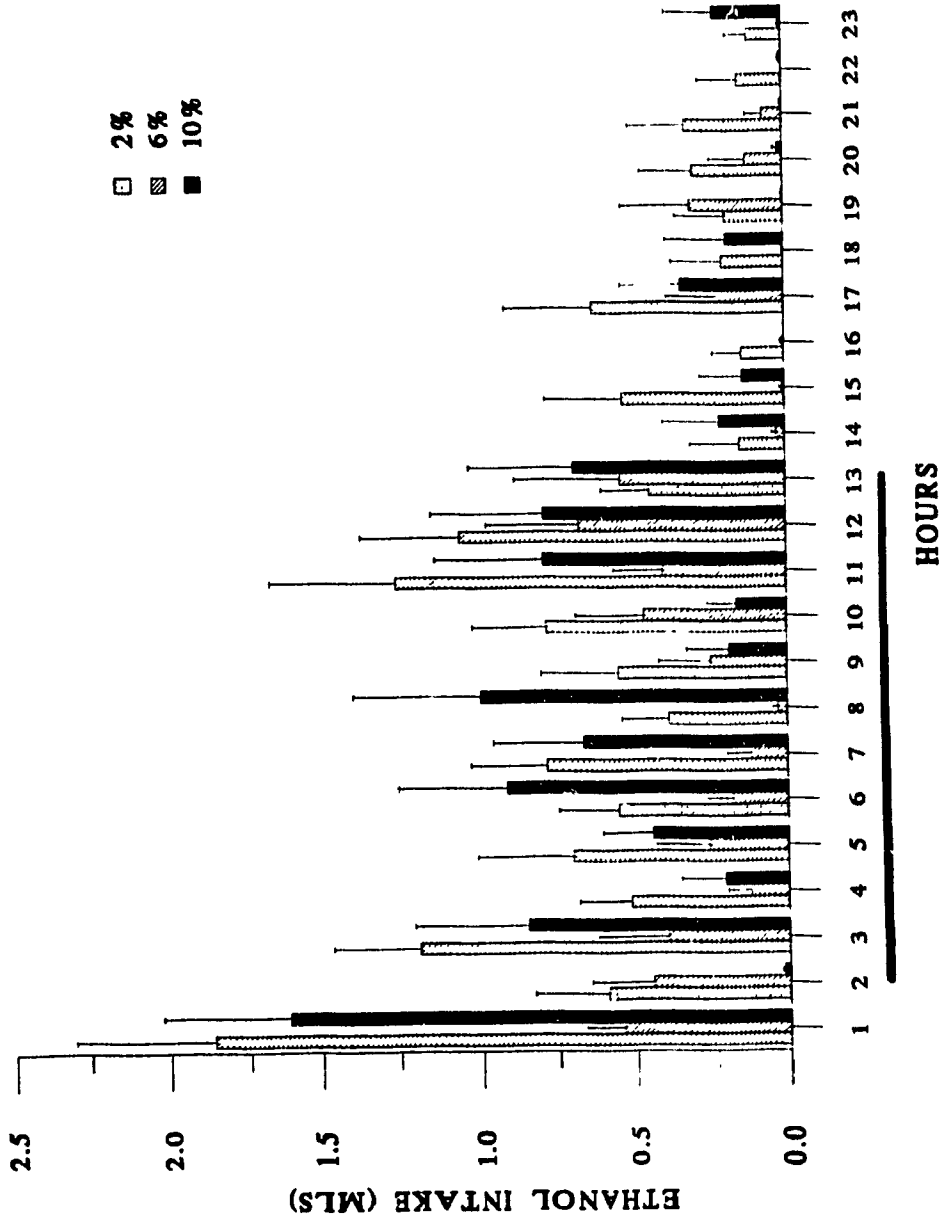

Figure 9. Temporal pattern of ethanol intake across the 23 hour daily sessions. Data is presented for the 2, 6 & 10% ethanol concentrations. The dark line indicates the lights out period. Vertical bars represent the S.E.M.

the period during which lights were off (nocturnal period), when the lights were on (diurnal period) and the hour immediately following the presentation of the fluid tubes.

The data suggest that of the total ethanol intake 69% occurred during the 12 hours comprising the lights off portion of the day/night cycle. This level of increased behavioral activity is consistent with the nocturnal nature of the rat. In contrast, only 15% of total ethanol intake was consumed during the 10 hour light portion (lights on) of the cycle (excluding the initial hour). The initial period (hour) following the presentation of fluid tubes (hour 1 in Figure 9), while technically occurring during the lights on period, was unique in so much as it represented the single largest hourly period of ethanol consumption. On average, 16% of the total ethanol intake occurred during the first hour.

Therefore, throughout subsequent sections of this thesis the transformed temporal representations, which consisted of the 3 distinct periods, and the associated analyses were utilized. In those cases where the transformation of the data obscured interactions which were evident in the analysis of the 23 hour temporal data both representations were included.

The mean ethanol intake values for the period following the initial presentation of the fluid tubes and for those during the dark and light portions of the day/night cycle are presented in Figure 10. An analysis of the transformed temporal data revealed that there were significant main effects for ethanol concentration [$F(7,168)=3.53$, $p<0.002$] and periods [$F(2,48)=40.47$, $p<0.00001$]. There did not appear to be any significant differences in the pattern of ethanol intake across temporal periods.

A representative sample of the temporal pattern of water intake across ethanol presentations is presented in Figure 11. The results of the analysis (all concentrations) described significant main effects for ethanol concentration [$F(7,168)=2.37$, $p<0.03$] and hours [$F(22,528)=16.69$, $p<0.00001$]. However there were no significant interactions [$F(154,3696)=0.9$, $p<0.79$].

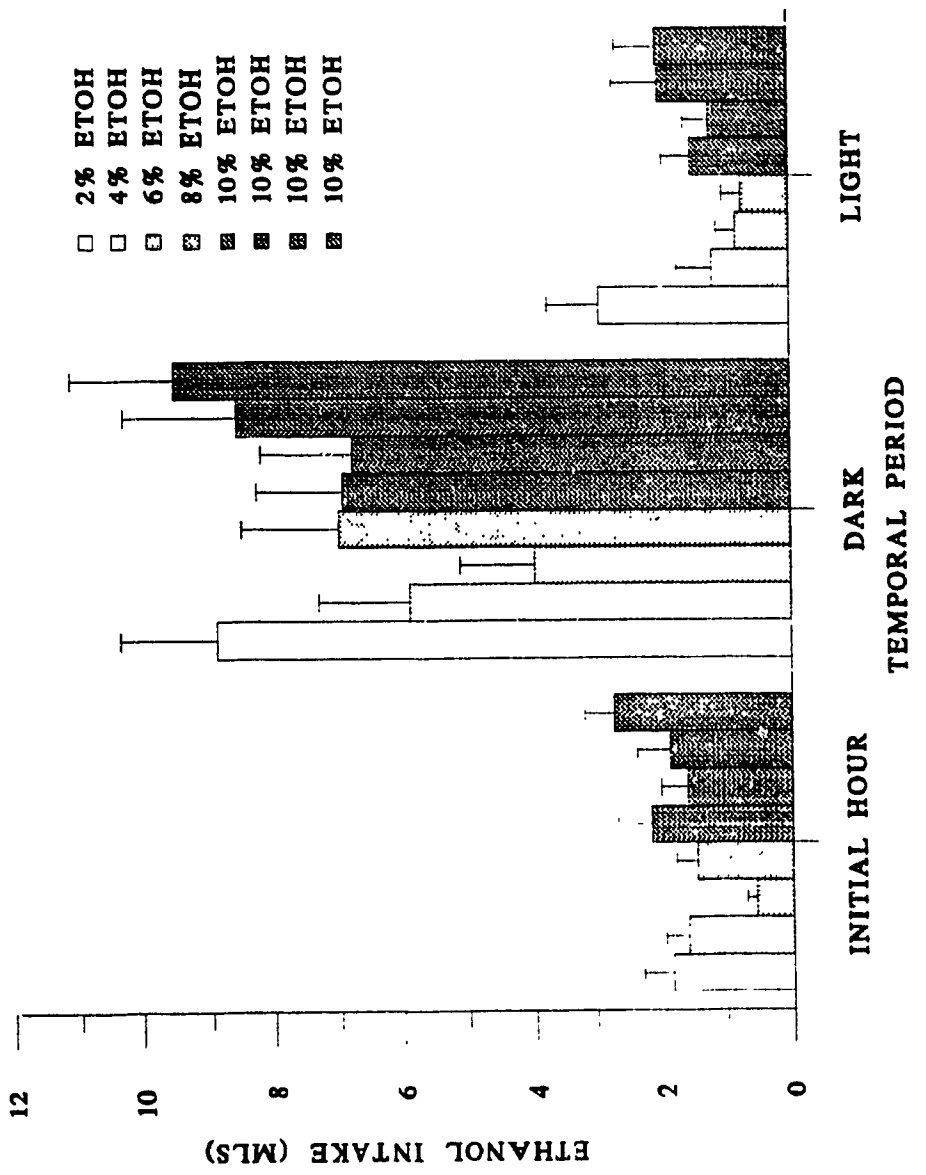

Figure 10. Mean ethanol intake values across descrete temporal periods, for each concentration of ethanol presented. Vertical bars represent the S.E.M.

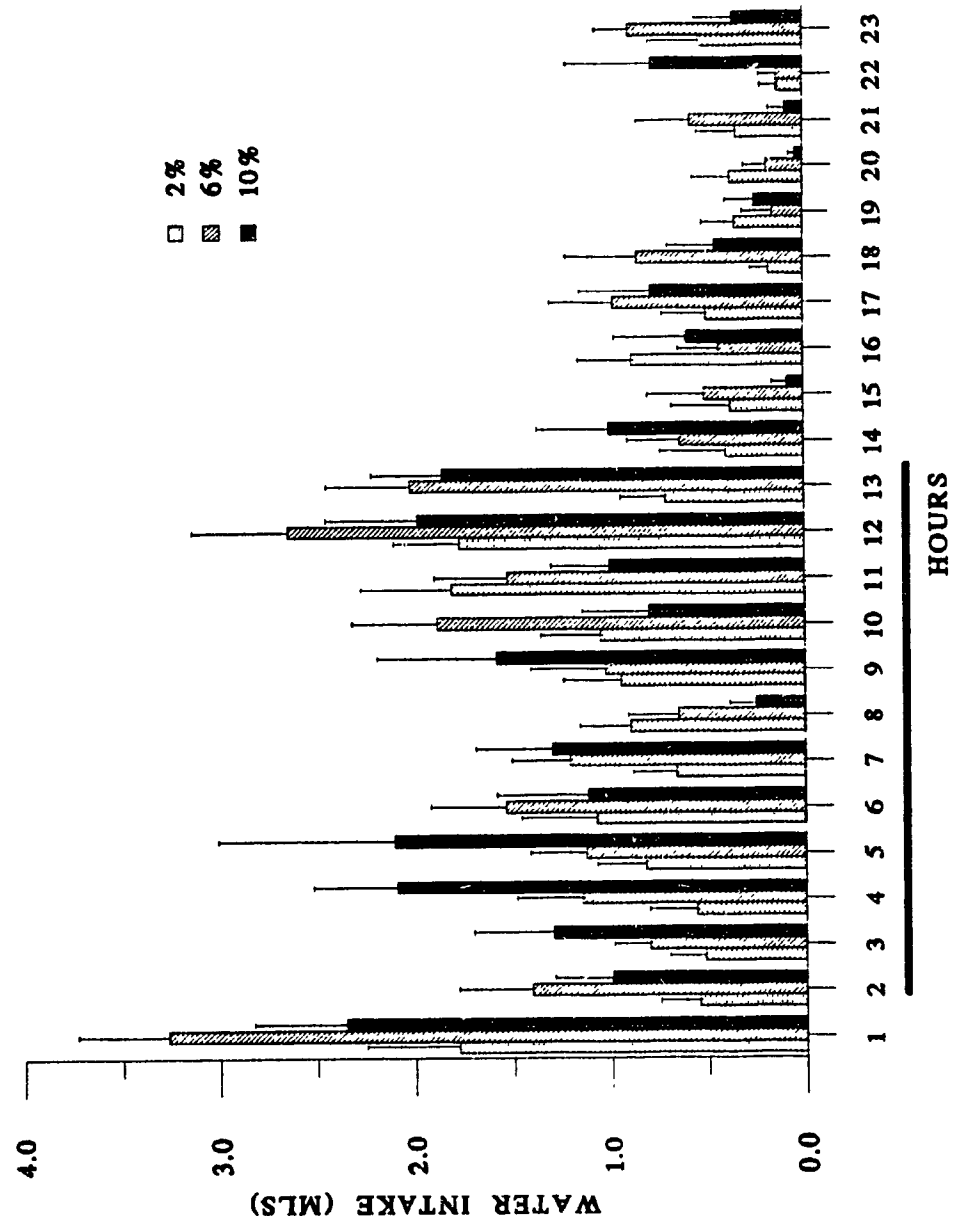

Figure 11. Temporal pattern of water intake across the 23 hour sessions. Data is presented for the sessions in which the 2, 6 and 10% ethanol concentrations were presented. Horizontal dark line represents the lights out period. Vertical bars represent the S.E.M.

Figure 12 presents the water intake data as a function of discrete temporal periods. Analysis of the temporal data also demonstrated significant main effects for concentration [$F(7,168)=2.37$, $p<0.025$] and temporal period [$F(2,48)=93.63$, $p<0.00001$]. Results further indicate that, in so much as there were no significant interactions [$F(14,336)=0.88$, $p<0.60$] water intake did not differ as a function of the temporal period. Generally, water intake was lowest, during the presentation of 2% ethanol, across all temporal periods.

A temporal analysis of food intake indicated a significant interaction between ethanol concentration and hours [$F(154,3696)=1.53$, $p<0.00001$] and ethanol concentration and temporal periods [$F(14,336)=2.53$, $p<.002$]. The pattern of food intake across temporal periods are presented in Figure 13. In light of the significant two-way interaction, a test of simple effects was performed holding periods constant. The results suggest that increases in food intake were limited to the first hour following fluid presentation [$F(7,168)=2.44$, $p<0.021$] and the dark portion [$F(7,168)=4.17$, $p<0.0004$] of the day/night cycle. There were no significant changes in food intake between concentrations during the light portion of the cycle [$F(7,168)=1.33$, $p<0.25$].

DISCUSSION

The results of the microstructural analysis reveal, for the first time, the involvement of differential, concentration dependent, behavioral mechanisms in the mediation of the acquisition of voluntary ethanol intake.

Increases in ethanol concentrations up to 6% were characterized by reductions in total ethanol intake, ethanol bout size and bout frequency. It appeared that the changes in ethanol intake were expressed uniformly across the 23 hour daily period. Furthermore, the presentation of ethanol concentrations below 6% produced changes in the parameters of ethanol intake, such as bout size, which were contrary to those changes observed in water intake.

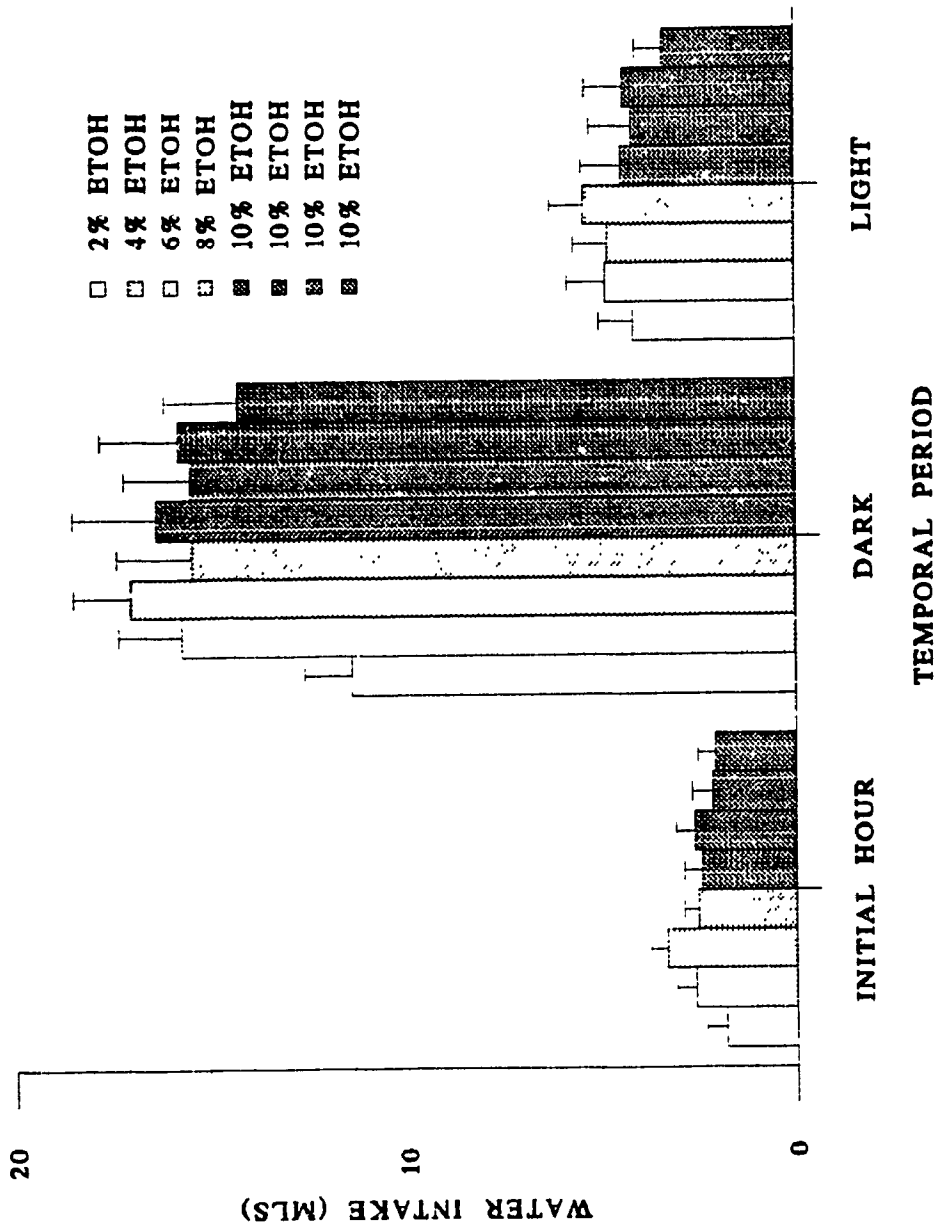

Figure 12. Mean water intake values observed during acquisition, as a function of descrete temporal periods. Vertical bars represent the S.E.M.

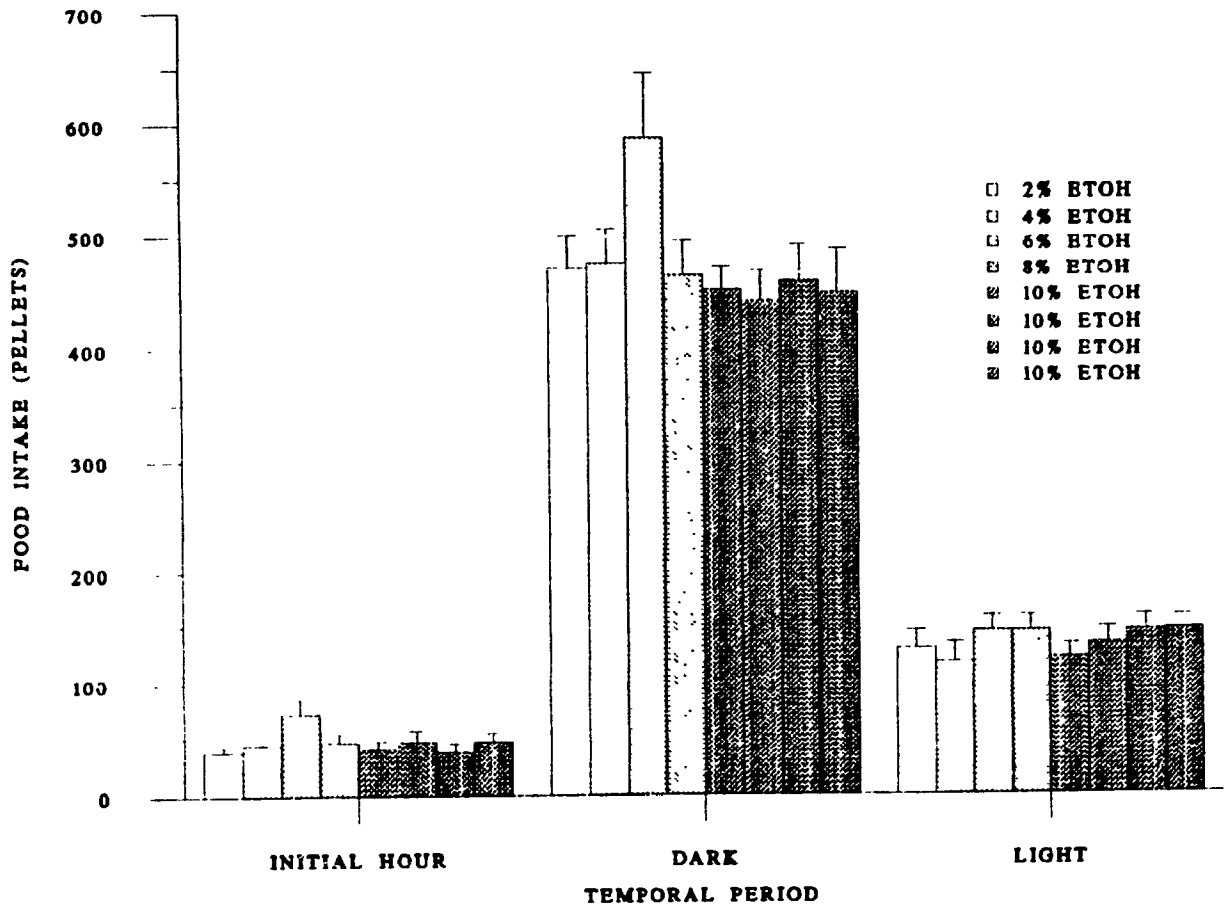

Figure 13. Mean food intake values observed during acquisition, as a function of descrete temporal periods . Vertical bars represent the S.E.M.

The reduction in multiple indices of ethanol intake would suggest that the ethanol solutions were increasingly aversive. Furthermore, the low levels of ethanol consumption (total ethanol intake <1.0 g/kg/23 hours; ethanol bout size <0.08 gm/kg) are not consistent with levels of ethanol intake which are pharmacologically meaningful (Gill et al., 1986). The results would suggest that for ethanol concentrations below 6% there may be some validity to criticisms (Dole, Ho & Gentry 1985; Dole and Gentry 1984) which argued that ethanol intake may be regulated by factors such as its taste, rather than its pharmacological properties. A factor which may play a role in this phenomenon is the increasingly aversive taste and smell of an ethanol solution which naturally accompanies increases in the concentration of ethanol solutions. In fact, some studies have suggested that as the concentration of ethanol is increased, the perceived taste of the solution changes from sweet to bitter-sweet (Bice, Kiefer, & Elder 1992; DiLorenzo, Kiefer, Rice & Garcia 1986).

In contrast, as the concentration of the ethanol solution was increased from 6 to 10% the behavioral pattern of ethanol intake changed in a manner such that the total intake of ethanol and the size of ethanol bouts were increased. Furthermore, the increase in the size of ethanol bouts was paralleled by an increase in the volume of ethanol consumed per unit time, or in other words the rate of ethanol intake. Interestingly, these changes in the parameters of ethanol intake were observed to increase despite an increase in the presumably aversive sensorial properties of the ethanol solution. Further, the changes in the size of ethanol bouts and rate of ethanol intake were independent of any effects upon food or water intake parameters. These results would suggest therefore that the reinforcing efficacy of the ethanol solution was enhanced as a function of the increase in ethanol concentrations from 6 to 10%. Presumably, this change in reinforcing efficacy was a function of ethanol's post-ingestive pharmacological effects.

The outcome of the present analysis would suggest that during acquisition there is a clear dissociation in the microstructure of ethanol intake above and below 6%. Furthermore, these data would suggest that a critical process which occurs during

acquisition is the reorganization of the structure of ethanol intake. Specifically, potential pharmacological effects were maximized through a process in which ethanol was consumed more rapidly, and ethanol intake was consolidated into a smaller number of larger ethanol bouts.

While the use of a microstructural analysis in the present study has contributed to a better understanding of the behavioral mechanisms regulating ethanol consumption, the paradigm does possess a potential limitation which must be addressed. In particular, animals used in the present study were characterized by substantially lower levels of voluntary ethanol intake than those obtained with similar strains used within traditional preference paradigms (e.g. Gill 1989; Gill, Amit, & Ogren 1985). The rats used in the present microstructural study achieved a maximum intake of only 2.5g/kg.

It is important to note that the traditional preference paradigm and the automated drinkometer system, used for the microstructural analysis, maintain differential environments and response requirements. In light of these differences it should not be surprising to find that the total level of ethanol intake in these two paradigms tend to be different. This point has previously been emphasized by Files, Andrew, Samson, Lumeng and Li (1992). Specifically, it has been demonstrated that the introduction of a lever press contingency into a preference paradigm results in a level of total ethanol intake lower than that obtained with the same animals in a two bottle home cage preference paradigm (Files, Andrew, Samson, Lumeng and Li 1992).

Interestingly, traditional induction techniques, such as sucrose-fading, have not generally been effective in increasing overall levels of ethanol intake or reducing the variability in the expression of ethanol self-administration within the microstructural paradigm (Samson, Tolliver, & Schwarz-Stevens 1991).

Nonetheless, the lower levels of ethanol intake observed in microstructural studies may act to diminish the future utility of the paradigm. Low baseline levels of voluntary

ethanol intake may result in the introduction of potentially confounding factors, such as a floor effect, when examining manipulations which decrease ethanol intake.

Therefore, in the following experiment a common preference-enhancing procedure was evaluated for its capacity to enhance the level of ethanol intake in a unselected strain of rats within a two bottle preference paradigm.

EXPERIMENT 2

AN EXAMINATION OF THE INFLUENCE OF FORCED ETHANOL EXPOSURE ON VOLUNTARY ETHANOL INTAKE.

A characteristic feature of the microstructural paradigm utilized in Experiment One, has been a resultant level of ethanol intake which is substantially lower than that observed within the traditional two bottle preference paradigm (e.g. Gill, 1989; Samson, Tolliver, Schwarz-Stevens 1991). While the behavioral mechanisms which account for the low level of ethanol intake are not clear, paradigmatic differences in response requirements and environments may potentially be a causative factor.

One approach which has been demonstrated to maintain high levels of ethanol intake within both traditional preference and microstructural paradigms involved the use of selectively bred lines of alcohol preferring rats. These animals, as a group, display high levels of ethanol intake and preference (Mcbride, Murphy, Lumeng & Li 1990; Froehlich, Harts, Lumeng, & Li, 1990; Froehlich, Zweiflel, Harts, Lumeng, & Li, 1991). It has been suggested that genetically selected strains, such as the line of ethanol-preferring (P) rats, are endowed with an innate preference for ethanol which makes them particularly suitable for studying the neurobiological correlates of ethanol intake (Mcbride, Murphy, Lumeng & Li, 1989; Penn, McBride, Lumeng, Gaff, and Li, 1978). The P line of rats have been demonstrated to consume more than 5 g of ethanol/kg/day. In addition, the P rats have been described as being capable of drinking amounts of ethanol sufficient to achieve pharmacologically meaningful blood ethanol levels. Furthermore, following chronic consumption these animals have been shown to develop tolerance and dependence (Li, Lumeng, McBride, & Waller, 1979).

In microstructural analyses of the pattern of intake in a free access situation, P rats have consumed over 6 gm/kg of ethanol per day (Murphy, Gatto, Waller, McBride, Lumeng, & Li 1986). The ethanol intake was further characterized by ethanol bouts consisting of 1.1 gm/kg of ethanol. Ethanol intake was described as occurring within 6 to

8 individual bouts over a 24 hour period. Most of the ethanol bouts were suggested to occur (66%) during the dark portion of the light cycle. These results would suggest that within a microstructural paradigm, P rats consumed substantially more ethanol overall, than has been reported for Long Evans rats (Gill, France & Amit 1986; Gill 1989).

However, there is some evidence in the literature to suggest that total ethanol intake and preference in P rats is not as robust or uniform if a period of forced ethanol consumption is not used as part of the pre-test screening process for ethanol preference (Bice & Kiefer 1990; Morzorati, Lamishaw, Lumeng, Li, Bemis, Clemens, 1988; Lankford, et al., 1992; Weiss et al., 1990; Gill et al., 1986). Such a period of forced ethanol exposure (included in the preference procedure) has been routinely used to verify the robust level of ethanol intake in P rats prior to their inclusion in voluntary intake studies (Li et al., 1979; Froehlich et al., 1990). It may be argued, therefore, that in those studies in which the P rats consume 5 gm/kg or greater (e.g. Penn et al., 1978), an important element contributing to the high level of observed ethanol intake may have been the inclusion of a period of forced ethanol exposure.

Potentially, findings from this line of research would suggest that an acute period of forced ethanol exposure, when administered as a pretest, may help induce enhanced levels of individual ethanol intake, rather than simply reflect high basal levels of intake in these animals. If verified, use of the forced choice technique may serve as a simple and inexpensive adjunct to the microstructural preference paradigm, designed to increase mean ethanol intake in unselected rats. It should be added, however, that this finding would also help to clarify the nature of the increased ethanol intake observed in genetically selected lines of animals, such as the P rats.

Therefore, the present experiment examined the capacity of an acute period of forced ethanol exposure to enhance voluntary ethanol intake in unselected rats. Furthermore, the extent to which prior ethanol experience interacted with the effects of forced ethanol exposure were also examined.

METHOD

Subjects

Twenty male Long-Evans new colony rats (Charles Rivers Canada Inc.) weighing 175 - 200 g at the start of the experiment were individually housed in stainless steel cages in a room controlled for constant temperature, humidity and 12L : 12D cycle. Food was freely available throughout the test period.

Drugs

Ethanol solutions, with concentrations which ranged from 2 to 10% (v/v) were prepared from 95% ethanol mixed with tap water.

Procedure

Following a period of acclimatization, the rats were randomly assigned to three groups. Group 1 was exposed to a schedule within which a sequence of increasing concentrations of ethanol solutions was presented in glass Richter type tubes mounted on the front of the home cages, in a free choice with water on an alternate day schedule. Beginning with a 2% ethanol solution, the concentrations were increased every second ethanol presentation until an 8% concentration was achieved. The position of the ethanol-filled tube, in relation to the water-filled tube, was altered on successive ethanol presentation days to avoid the potential of a position bias. During the intervening days both tubes were filled with water. Following the final presentation of the 8% ethanol solution, these subjects were then presented with a forced exposure to ethanol in which 10% ethanol solution served as their sole source of fluid for 4 consecutive days. The subjects were then presented with a free choice between 10% ethanol and water on an alternate day schedule over a period encompassing ten ethanol presentations. The subjects were then presented again with a forced exposure to ethanol over 4 consecutive days, but with a 15% ethanol solution. The subjects were subsequently presented with a free choice between 15% ethanol and water over 10 alternate day ethanol presentations. Again, water and ethanol intake was measured throughout this period. Throughout this period, when 10 and 15%

ethanol was available, water and ethanol consumption was measured. In addition, the body weight of animals was recorded.

The second group of rats followed the procedure described above with the sole exception that they did not receive the initial exposure to increasing concentrations of ethanol prior to the 4 day period of forced exposure to the 10% ethanol solution.

In contrast, the third group was presented with the initial exposure to increasing concentrations of ethanol in a free choice with ethanol, as described above, followed by ten alternate day free choice presentations with each of the 10 and 15% ethanol solutions. The third group did not receive any forced exposure to the ethanol solutions.

RESULTS

In the present experiment, the effects of variations in the schedule of ethanol exposure on the intake of ethanol, water and body weight were examined using multiple three-way factorial ANOVAs (the variables consisted of schedule type and repeated measures for ethanol concentration and days). The source of interactions within factorial ANOVAs were established using tests of simple main effects and simple interactions.

The results of the present study indicated that an increased level of ethanol intake was produced as a function of a schedule of forced ethanol exposure. An analysis of ethanol intake during exposure to the presentation of 10 and 15% ethanol (free choice and alternate day) revealed that there was a significant interaction between the type of schedule utilized and test days [$F(18,153)= 2.31$ $p< 0.0031$]. In light of the significant two-way interaction, a test of simple main effects and simple interactions was performed holding schedule groups constant. As can be seen in Figure 14, the rats under the forced exposure schedule, without gradual pre-exposure to ethanol, increased their level of absolute ethanol intake over the test period [$F(9,153) = 7.06$ $p<0.0001$]. The animals intake increased to a maximum of 5.2 gm/kg during alternate day presentation of 10% ethanol. Similarly, the group of rats which received a pre-exposure to increasing concentrations of ethanol in addition to the forced exposure also exhibited significantly increased levels of absolute

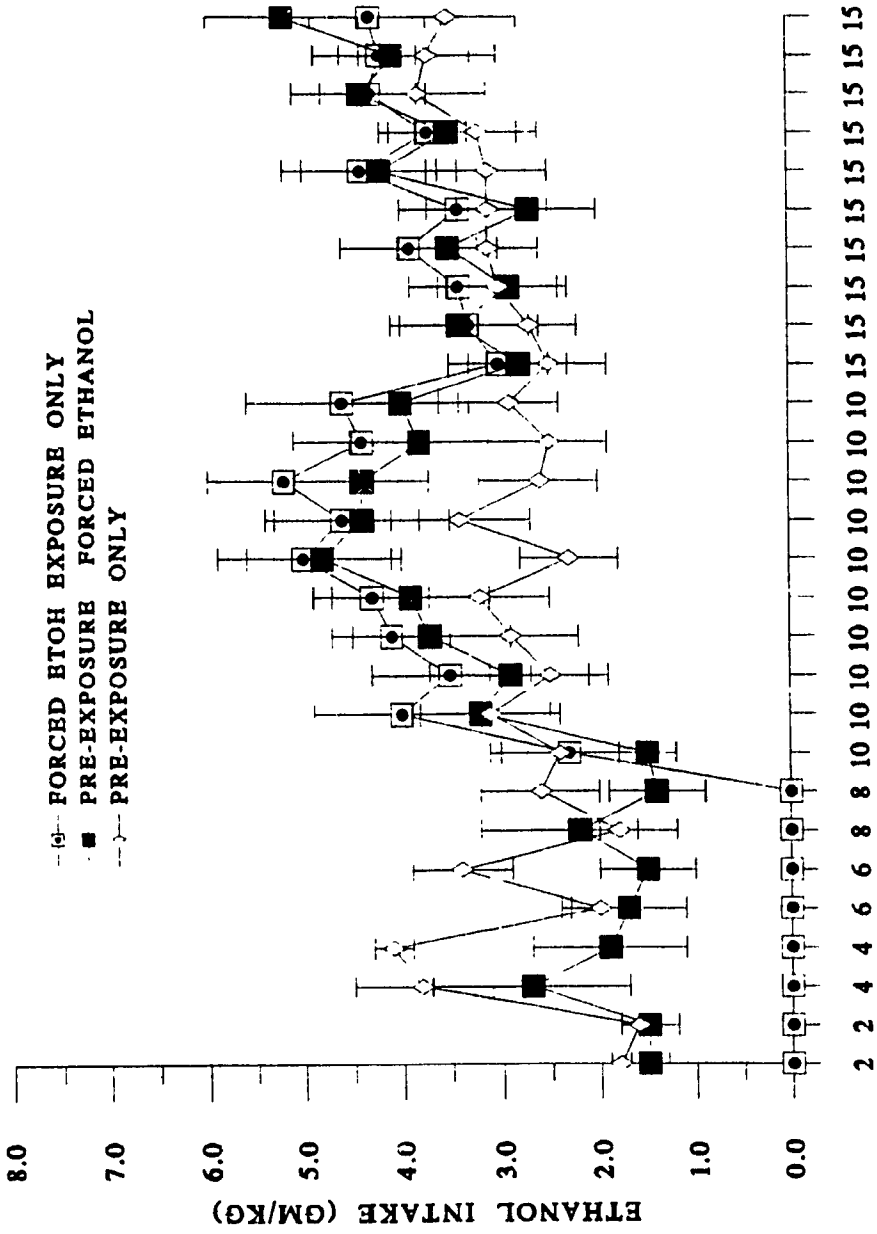

Figure 14. Mean level of absolute ethanol intake as a function of the schedule of forced ethanol exposure. Vertical bars represent the S.E.M.

ethanol intake over the test period [F(9,153) = 12.54 p<0.00001]. Those rats not receiving the schedule of forced ethanol exposure failed to exhibit any significant changes in their level of absolute ethanol intake [F(9,153)=1.57 p<0.1286]. The maximum level of ethanol intake (at 10%) observed in these animal was 3.4 gm/kg.

In a similar manner, an examination of the ethanol preference ratios presented in Figure 15, indicated a significant interaction between schedule type and test days [F(18,153) = 3.12 p< 0.0002]. Subsequent tests of simple main effects and interactions demonstrated that, consistent with what had been observed with absolute ethanol intake, ethanol preference levels significantly increased over days for those rats receiving only the forced exposure [F(9,153) = 9.87 p<0.00001] and those receiving both the pre-exposure to ethanol and the forced ethanol exposure [F(9,153) = 17.43 p< 0.00001]. However, in contrast to what was observed with absolute ethanol intake, rats which received the pre-exposure only schedule also exhibited significant increase in preference levels over the test period. [F(9,153)=4.18 p<0 0001]. While all three groups exhibited increases in preference over days, differences in maximal preference levels were evident. In subjects receiving the forced exposure to ethanol, a maximum preference of 0.6 was attained, while a maximum preference of 0.44 was observed in those rats not subjected to the forced ethanol exposure.

The analysis of total fluid intake values, as presented in Figure 16, revealed a significant three-way interaction between schedule type, ethanol concentration and days [F(18,153) =2.65 p< 0.0007]. In addition, a main effect for schedule type [F(2,17) = 4.79 p<.02], revealed that the groups differed in terms of their total overall fluid intake, with the pre-exposure only group exhibiting significantly lower levels of total fluid intake.

Finally, the analysis of body weight values, as presented in Figure 17, demonstrated a significant two-way interaction between schedule type, ethanol concentration [F(2,17) =2.23 p< 0.004]. However, the main effect for schedule type was not significant, [F(2,17)=2.23, p<.14].

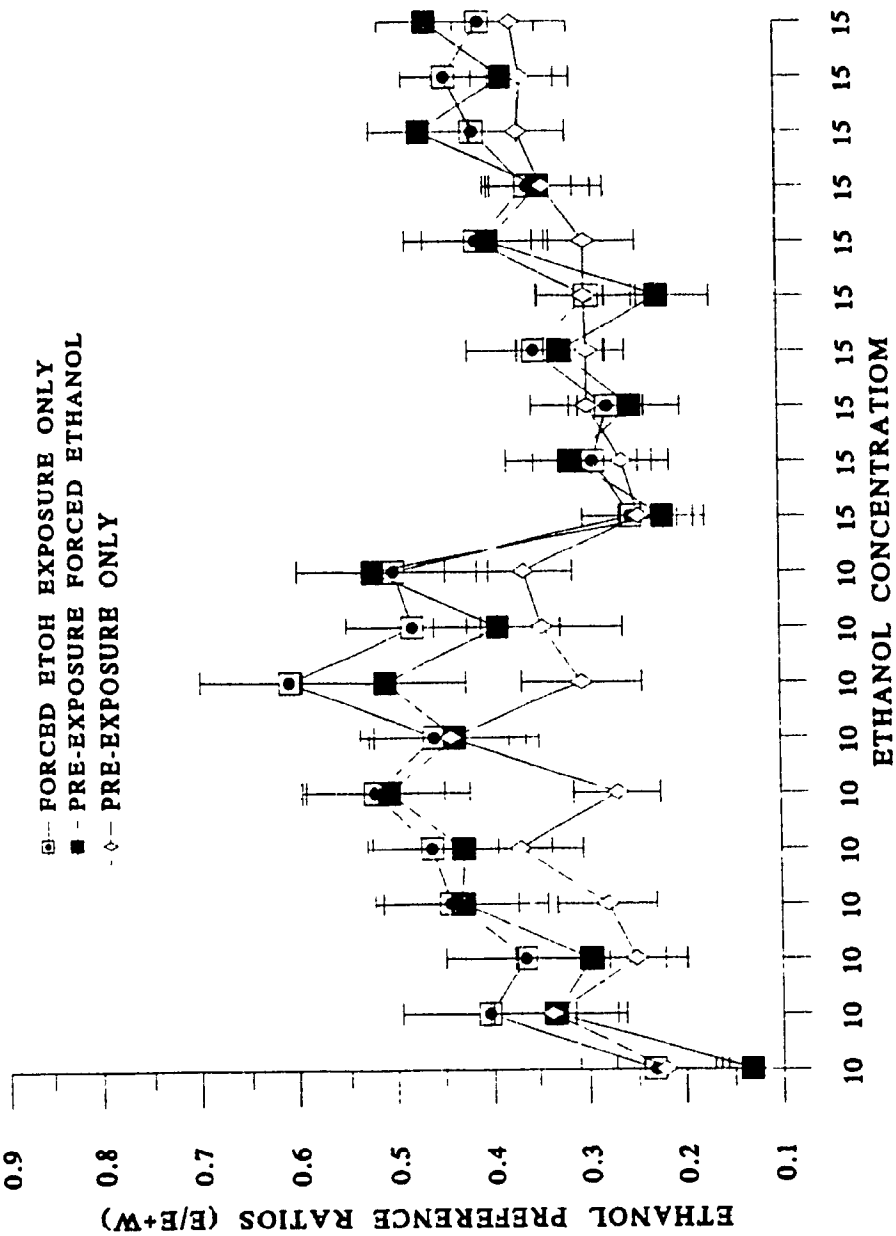

Figure 15. Mean ethanol preference ratios, as a function of the schedule of forced ethanol exposure. Vertical bars represent the S.E.M.

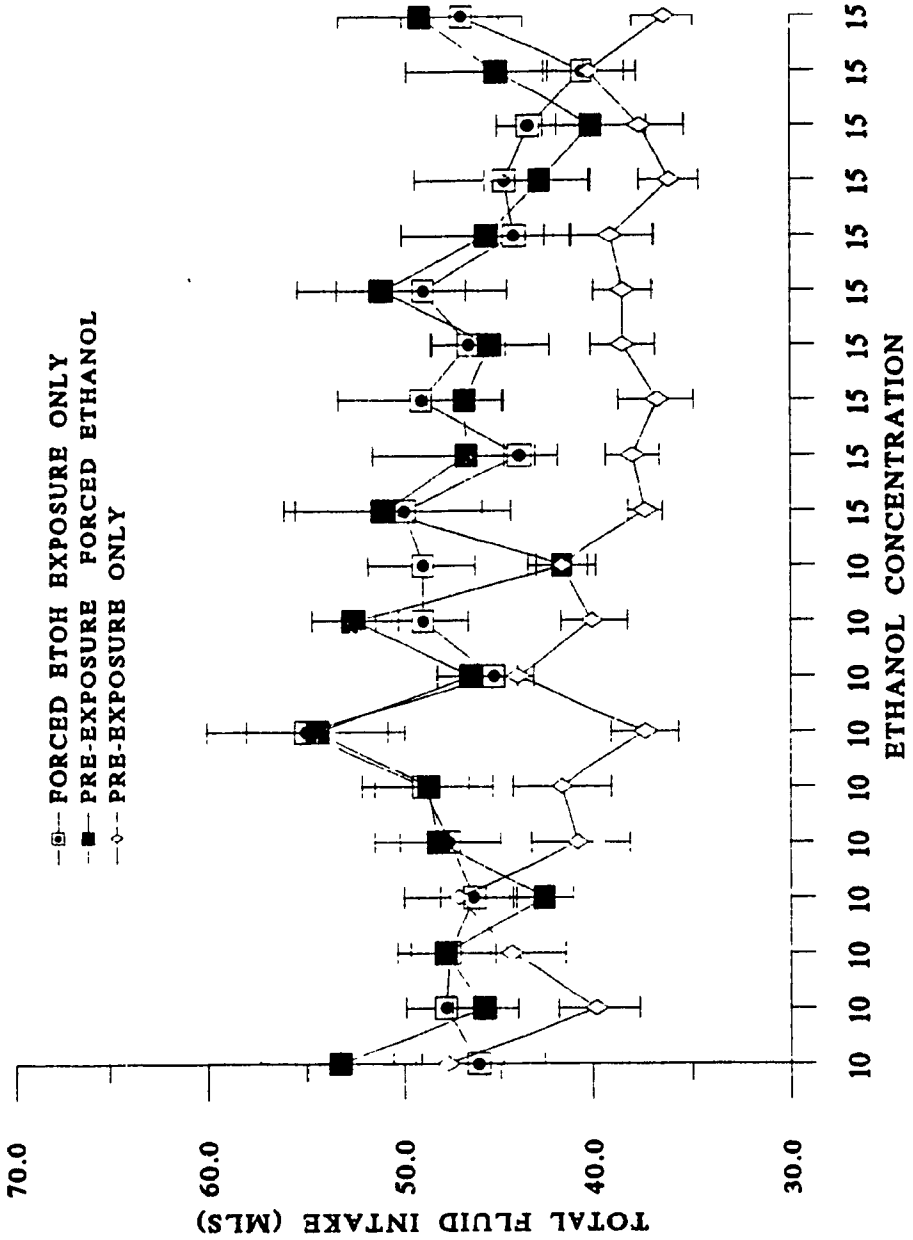

Figure 16. Mean total fluid intake values, as a function of the schedule of forced ethanol exposure. Vertical bars represent the S.E.M.

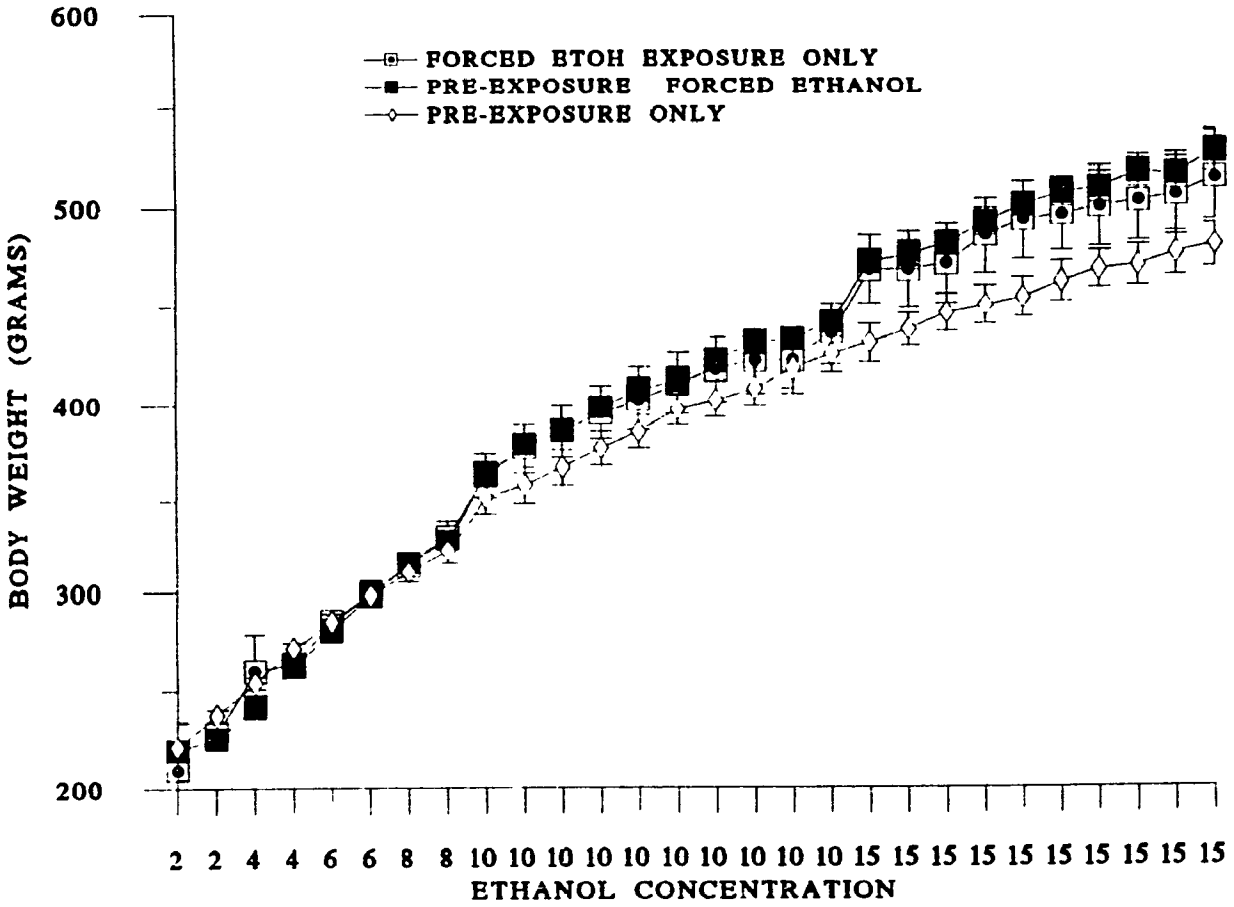

Figure 17. Mean body weight values, as a function of the schedule of forced ethanol exposure. Vertical bars represent the S.E.M.

DISCUSSION

These results suggested that increased voluntary ethanol intake can be achieved as a function of the manipulation of schedules of ethanol presentation. The results of Experiment 2 revealed that, within an alternate day maintenance paradigm, the prior administration of four days of forced ethanol exposure was sufficient to produce an increase in the amount of ethanol consumed in non-selected rats. Subjects receiving only the four days of forced exposure to 10% ethanol, consumed ethanol exceeding 5 g/kg with an ethanol preference ratio of .60. This level of intake is comparable to that observed in strains of rats which have been specifically bred for high ethanol intake (Li et al., 1979; Froehlich et al., 1990).

Furthermore, the effects of forced ethanol exposure were independent of any prior experience with ethanol drinking. Specifically, forced ethanol exposure enhanced the voluntary ethanol intake of ethanol in ethanol naive rats in addition to those which experienced the traditional ethanol acquisition procedure.

The results of the present examination are important in terms of their implications for the use of certain lines of rats selected for high ethanol intake. The use of an acute period of forced ethanol exposure has been used routinely to verify the robust level of ethanol intake, in selectively bred "P" and "HAD" lines of rats, prior to their inclusion in voluntary intake studies (Penn et al., 1978; Li et al., 1979). While the present experiment suggests that forced exposure to ethanol can produce high levels of ethanol intake in unselected rats, previous studies suggest that without the forced ethanol exposure selected P rats exhibit less than maximal levels of ethanol intake (Bice & Kiefer 1990; Morzorati, Lamishaw, Lumeng, Li, Bemis, Clemens, 1988; Lankford, et al., 1992; Weiss et al., 1990; Gill et al., 1986). These findings would suggest therefore, that the common forced ethanol exposure procedure (Li et al., 1979; Penn et al., 1978; Froehlich et al., 1990; 1991), is one factor which may substantially contribute to the high level of ethanol intake and preference historically ascribed to lines of selectively bred rats.

It must be noted that the findings of the present study would appear to be inconsistent with earlier research which suggested that forced ethanol exposure either decreased or had no effect upon ethanol intake (e.g. Eimer & Senter 1968; Rodgers, Ward, Thiessen, & Whitworth 1967). However, this inconsistency can likely be attributed to differences in the schedule of ethanol availability observed following the forced choice (exposure) procedure. The early forced exposure studies (e.g. Rodgers, Ward, Thiessen, & Whitworth 1967) utilized continuous access schedules of availability, where as, the present study used an alternate day free exposure paradigm. Research that examined factors that affect the acquisition of ethanol intake (e.g. Halloway et al. 1984), has indicated that rats given periodic access to 10% ethanol will gradually increase their relative selection of ethanol. In contrast, the same strain of rats with continuous access to 10% ethanol, either forced choice or free choice, will not exhibit comparable increases in the intake of ethanol until switched to a periodic schedule of ethanol presentation (e.g. Wise 1973; Halloway et al. 1984) .

Nonetheless the results of the present experiment would suggest that a relatively brief exposure to forced ethanol consumption may serve as an efficient method for inducing high levels of ethanol intake in unselected rats. Thus, forced ethanol exposure may potentially serve as a useful adjunct to the microstructural paradigm. The following study provided a descriptive analyis of the influence of forced ethanol exposure on voluntary ethanol intake within the context of a microstructural paradigm.

EXPERIMENT 3

A MICROANALYSIS OF THE EFFECTS OF FORCED ETHANOL EXPOSURE ON ETHANOL INTAKE

The results of Experiment 2 indicated that an acute period of forced ethanol exposure was sufficient to induce increased levels of ethanol intake in a traditional 24 hour preference paradigm. The present experiment further examined the extent to which forced ethanol exposure increased voluntary ethanol intake within the microstructural paradigm. Furthermore, the behavioral mechanisms which mediate the effects of the forced ethanol exposure on ethanol consumption were evaluated on the basis of an analysis of the microstructure and temporal pattern of intake.

METHOD

Subjects

Thirty nine male Long Evans new colony rats (Charles Rivers Canada Inc.) weighing 175 - 200 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 was available ad lib throughout the test period.

Drugs

Ethanol solutions with a concentration of 10% (v/v) were prepared from 95% stock ethanol mixed with tap water.

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 which dispensed 45-mg standard Bioserve pellets. The feeders were activated by the interruption of photo beams resulting from the placement of a rats head into the food cup. Each photo beam interruption resulted in a single pellet being dispensed. In addition, each chamber was equipped with two plastic drinking tubes fitted with steel ball bearing spouts.

All feeding and drinking behavior were monitored continuously over a 23-h period. During a daily 60-minute 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 to have been 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 minutes or when there was a transition to another input device. Subsequent data analysis yielded measures 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 or drinking spouts, only those bouts consisting of more than five consecutive events were included in the analysis. Complete design specifications for the computer acquisition system have been previously detailed (Gill, Mundl, Cabilio & Amit 1988).

Procedure

Following a 7-day period of acclimatization to the operant boxes, the rats were presented over 4 consecutive days with a forced exposure to ethanol in which a 10% (v/v) ethanol solution was the sole fluid available.

Immediately following the period of forced ethanol exposure the rats were placed on an schedule in which 10% ethanol was presented in a free choice with water on alternate days. The position of the ethanol-filled tube was altered on successive days to avoid a position bias. Both tubes were filled with water on intervening days.

The analysis of the temporal and structural properties of ethanol intake was carried out over five consecutive ethanol presentations. These occurred following a period of time in which ethanol intake was allowed to stabilize.

RESULTS

The effects of a forced exposure schedule of ethanol presentation on the structure and pattern of ethanol, water and food consumption were examined. Differences in bout parameters across days were assessed through multiple one-way repeated measure ANOVAs. The effects of forced ethanol exposure on the temporal pattern of food and fluid intake were assessed using repeated measure factorial ANOVAs (with the variables hours or periods and days). The source of interactions within the factorial ANOVAs were established using tests of simple main effects and simple interactions.

A Structural Analysis of Ethanol, Water and Food intake

The effects of forced ethanol exposure on absolute ethanol intake (gm/kg) are presented in Figure 18. The intake of absolute ethanol did not differ significantly across days [$F(4,152)=2.13$ $p<0.085$]. The results suggested that within the drinkometer paradigm forced ethanol exposure induced levels of ethanol intake reaching a maximum of 3.6 gm/kg.

In contrast, ethanol preference, also presented in Figure 18, changed significantly over days [$F(4,152)=3.21$ $p<0.015$]. Results suggested that ethanol preference increased from day 1 to day 2, and remained constant thereafter. The data indicated that during the 4 final trials (out of 5) ethanol intake exceeded 0.5.

Figure 19 illustrates the total volume intake of 10% ethanol and water over days. Overall, there were no significant differences in total intake between water and ethanol following forced ethanol exposure [$F(1,38)=0.02$ $p<0.89$]. This suggested that the two groups consumed similar amounts of each fluid overall.

However, an analysis of the total intake of ethanol (mls) and water intake revealed a significant interaction between fluid type and days [$F(4,152)=2.58$ $p<0.04$]. An analysis of simple effects, holding fluid type constant, demonstrated that in the case of ethanol intake (mls) there were significant changes over days [$F(4,152)=3.26$ $p<0.014$]. Post Hoc analysis revealed that the intake of ethanol increased from day 1 to day 2. Following day 2

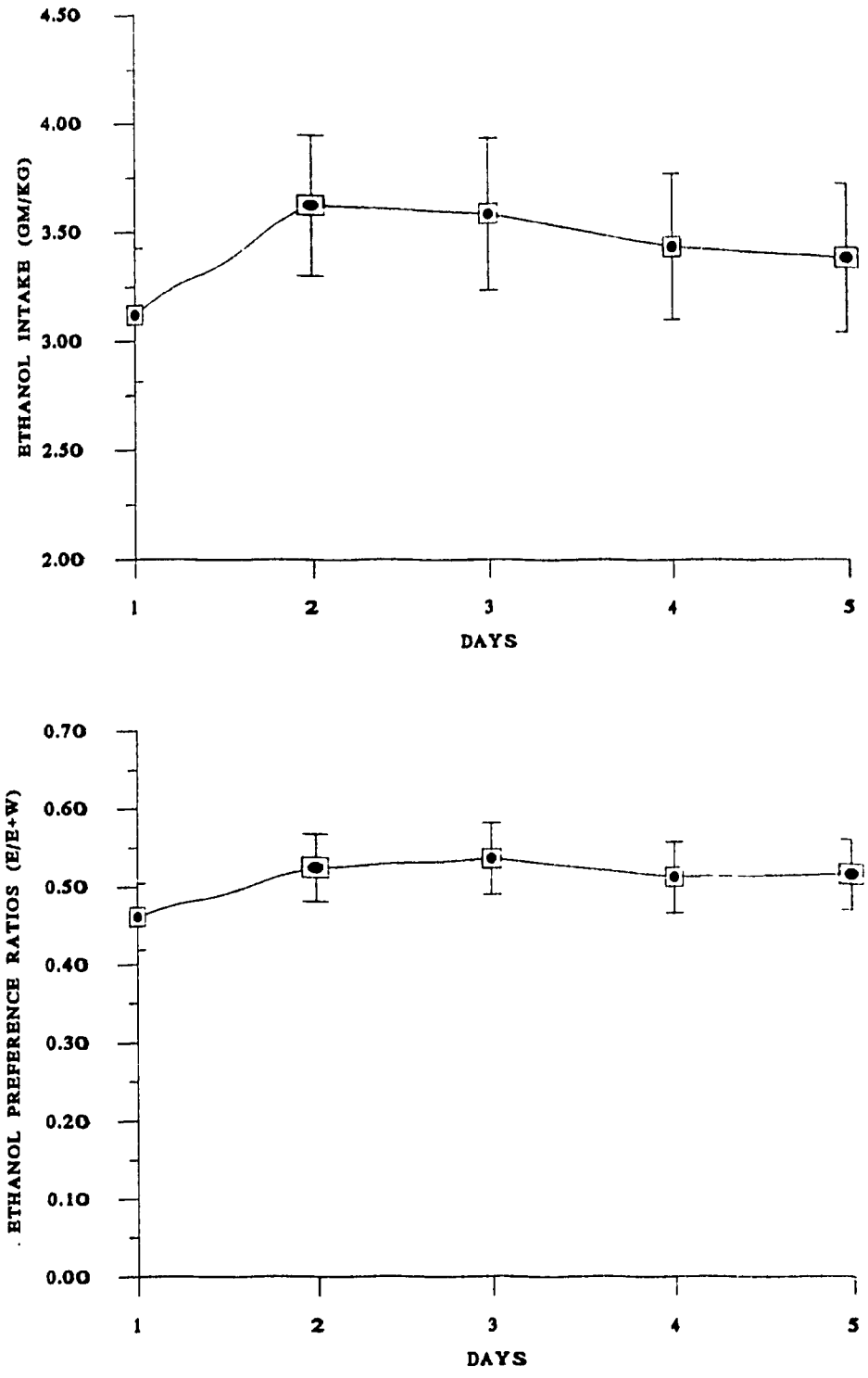

Figure 18. The effects of a period of forced ethanol exposure on absolute ethanol intake (gm/kg) and preference values. Data were recorded during a free choice ethanol maintenance period. Vertical lines represent S.E.M.

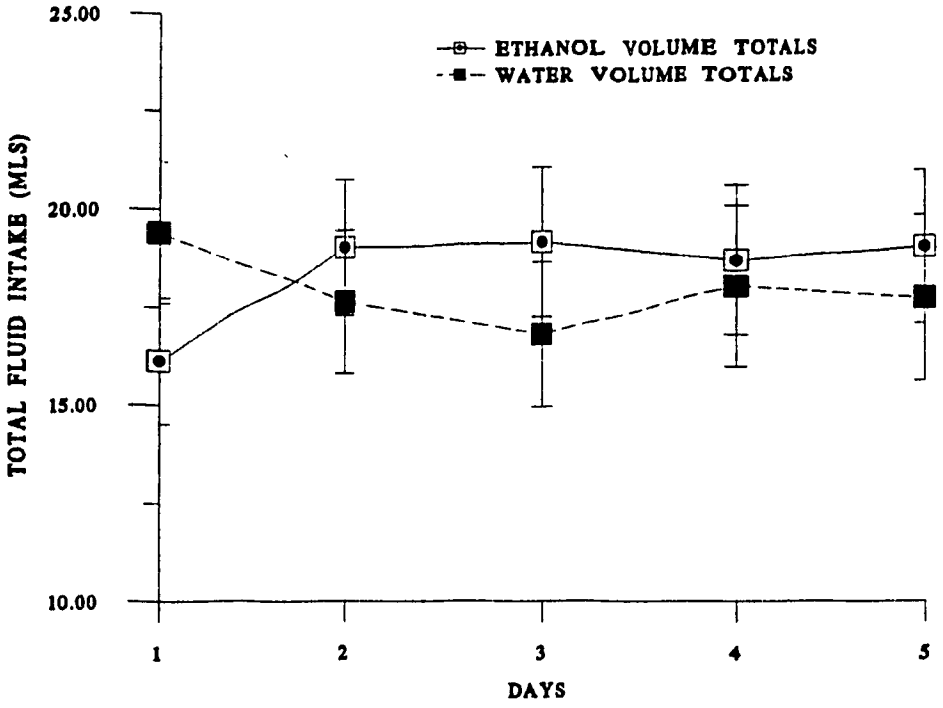

Figure 19. The effects of forced ethanol exposure on total ethanol (mls) and water (mls) intake values, recorded during a free choice ethanol maintenance period. Vertical lines represent S.E.M.

there were no significant changes in intake. In contrast, total water intake remained stable over days [$F(4,152)=0.99$ $p<0.45$].

An examination of the size and frequency of fluid bouts, presented in Figure 20, suggested a further differentiation in the effects of forced ethanol exposure on ethanol and water intake. A comparison of the size of fluid bouts indicated that following forced exposure to ethanol, ethanol bouts were significantly larger than the bouts of water intake [$F(1,38)=9.26$ $p<0.0042$]. On average, the largest of these daily bouts consisted of 4 ml or approximately 0.75 g/kg of 10% ethanol (Table 1). Inversely, there were significantly fewer ethanol bouts than water bouts [$F(1,38)=4.70$ $p<0.04$].

In Figure 21, the rate (volume consumed per unit of time) of water and ethanol intake are presented. An analysis of these values indicated that there were no significant differences in the rates of intake for ethanol and water [$F(1,38)=3.35$ $p<0.075$].

Food intake, presented in Figure 22 as the total food pellets consumed, significantly increased over days [$F(4,152)=2.91$ $p<0.025$]. Post hoc analysis suggested that by day 4, food intake was significantly higher than that of day 1. Similarly, body weight also increased as a function of days [$F(4,152)=123.8$ $p<0.000001$].

While total food intake increased, neither the size [$F(4,152)=2.05$ $p<0.09$] nor frequency [$F(4,152) = 1.03$ $p< 0.4$] of food bouts changed significantly over days (Figure 22).

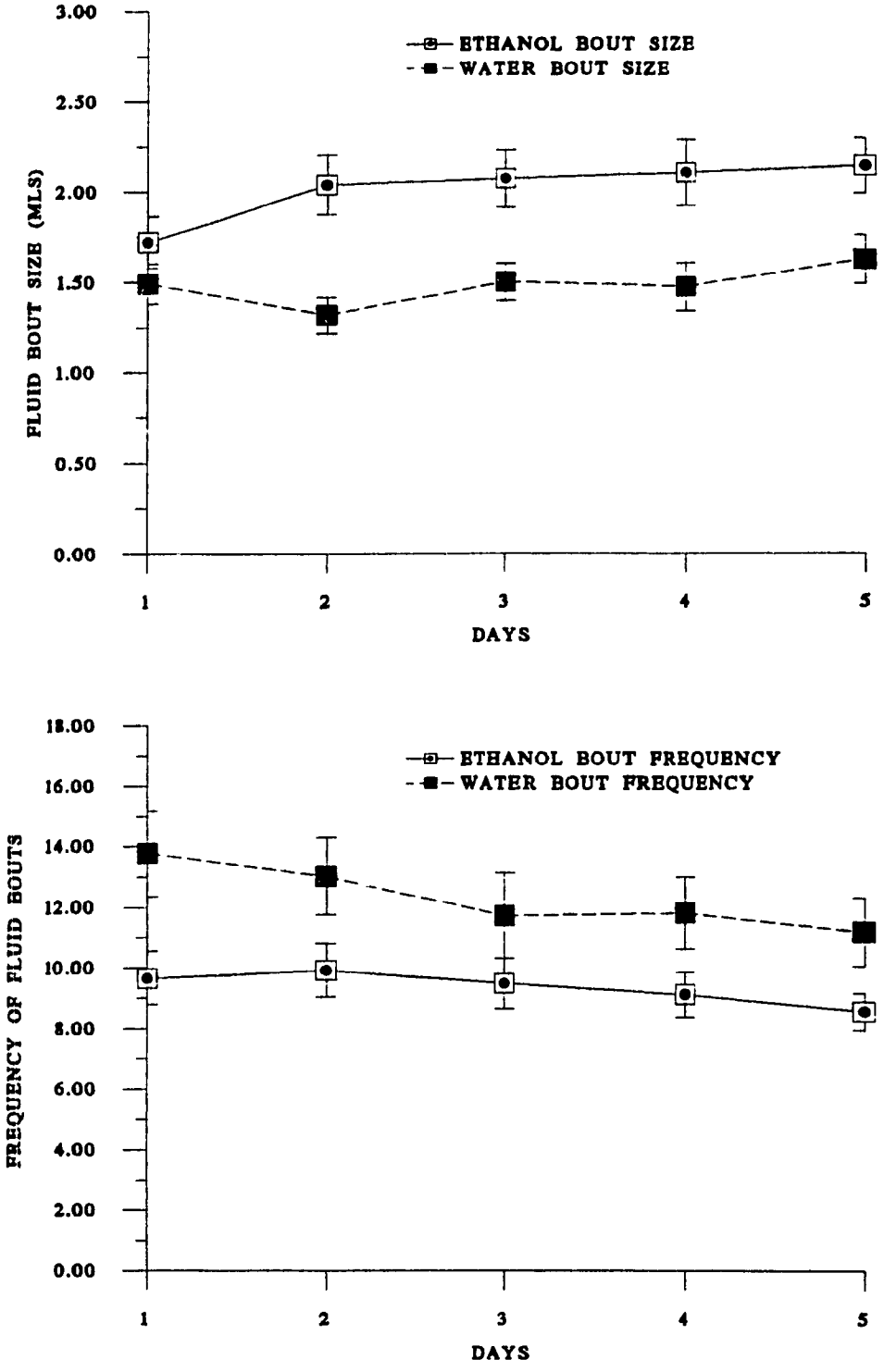

Figure 20. The effects of forced ethanol exposure on mean size (mls) and frequency of water and ethanol bouts. Data was recorded during a free choice maintenance period. Vertical lines represent S.E.M.

Table 1.

Microstructural characteristics of ethanol bouts following forced ethanol exposure

AVERAGE ETHANOL BOUTS SIZE - MLS (GM/KG)	Day 1	Day 2	Day 3	Day 4	Day 5
MEAN	1.72 (.334)	2.04(.334)	2.07(.391)	2.11(.391)	2.14(.392)
STD. ERROR	0.14	0.16	0.15	0.18	0.15
LARGEST ETHANOL BOUT MLS (GM/KG)					
MEAN	3.61(.709)	3.95(.759)	4.21(.796)	4.16(.775)	4.10(.743)
STD. ERROR	0.33	0.30	0.29	0.28	0.26

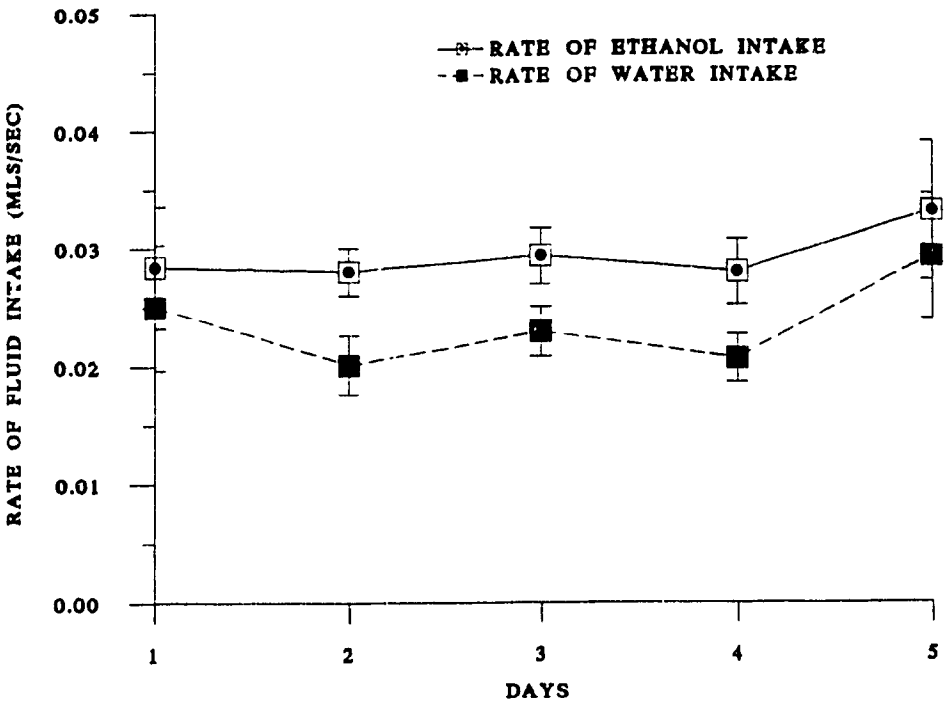

Figure 21. The effects of forced ethanol exposure on mean rate of ethanol and water intake (mls/sec) across an ethanol maintenance period . Vertical lines represent S.E.M.

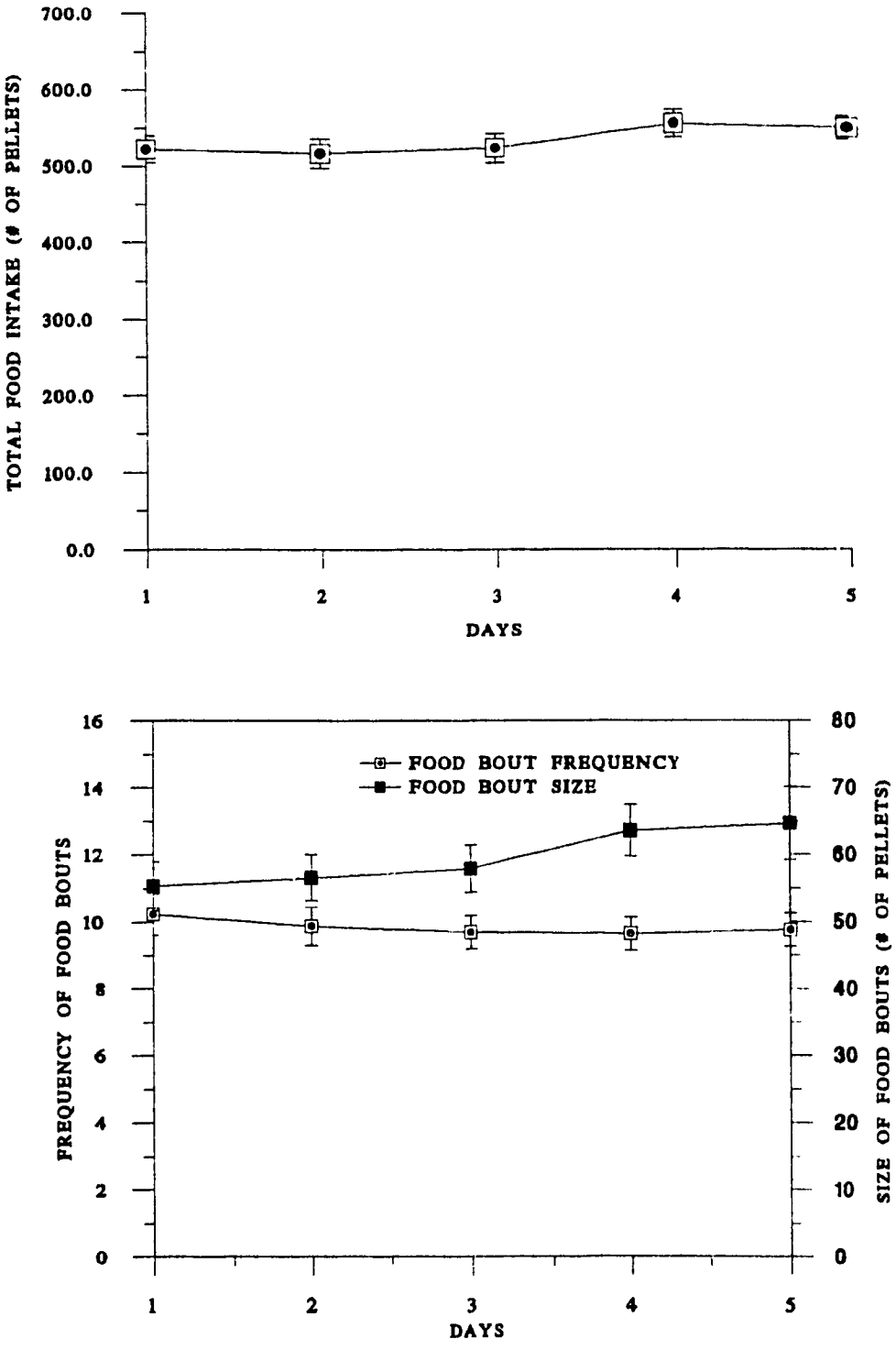

Figure 22. The effects of forced ethanol exposure on measures of total food intake (upper figure) and both size and frequency of food bouts (lower figure). Data were recorded during a free choice maintenance period. Vertical lines represent S.E.M.

A Temporal Analysis of Ethanol, Water and Food intake

Presented in Figure 23 are the ethanol intake values as a function of the test days and temporal periods. Theses results revealed a significant main effect for days [F(4,152)=3.13 p<.02). Post hoc analysis of the marginal means were interpreted to suggest an increase in ethanol intake from day 1 to 2, consistent with the findings reported earlier for total intake values. The results demonstrated the absence of any interaction [F(8,304)=1.39 p<0.198] between temporal periods and trial days.

Again as was reported with total intake findings, water intake, as presented in Figure 24, did not differ significantly over days [F(4,152)=1.0 p<0.42]. The interaction between temporal periods and trial days was not significant [F(8,304)=0.61 p<0.78]

The temporal pattern of food intake, presented in Figure 25, revealed a significant main effect for days [F(4,152)=2.84 p<0.027]. The results indicated the absence of any interaction between temporal periods and trial days [F(8,304)=1.06 p<0.4]

DISCUSSION

The present experiment indicated that the total intake of absolute ethanol (3.59 g/kg) was substantially greater than that observed in Experiment 1, in which mean levels of intake only approached 2 - 3 g/kg.

The largest bouts consumed per day were comparable to those reported in the literature to produce meaningful psychopharmacological effects (Gill et al., 1986). In addition, comparisons with the outcome of Experiment 1 and the reports of previous studies (Gill, Filion & Amit 1988) revealed that the increased level of total ethanol intake observed in the present experiment was a function of an increase in the size of ethanol bouts.

Perhaps indicative of the incentive value of the available alcohol, the subjects exhibited a relatively large preference (.63) for alcohol during the first hour following the daily presentation of the alcohol and water tubes. During this initial period, it was observed that subjects exhibited substantial and diverse activity which appeared to be characterized

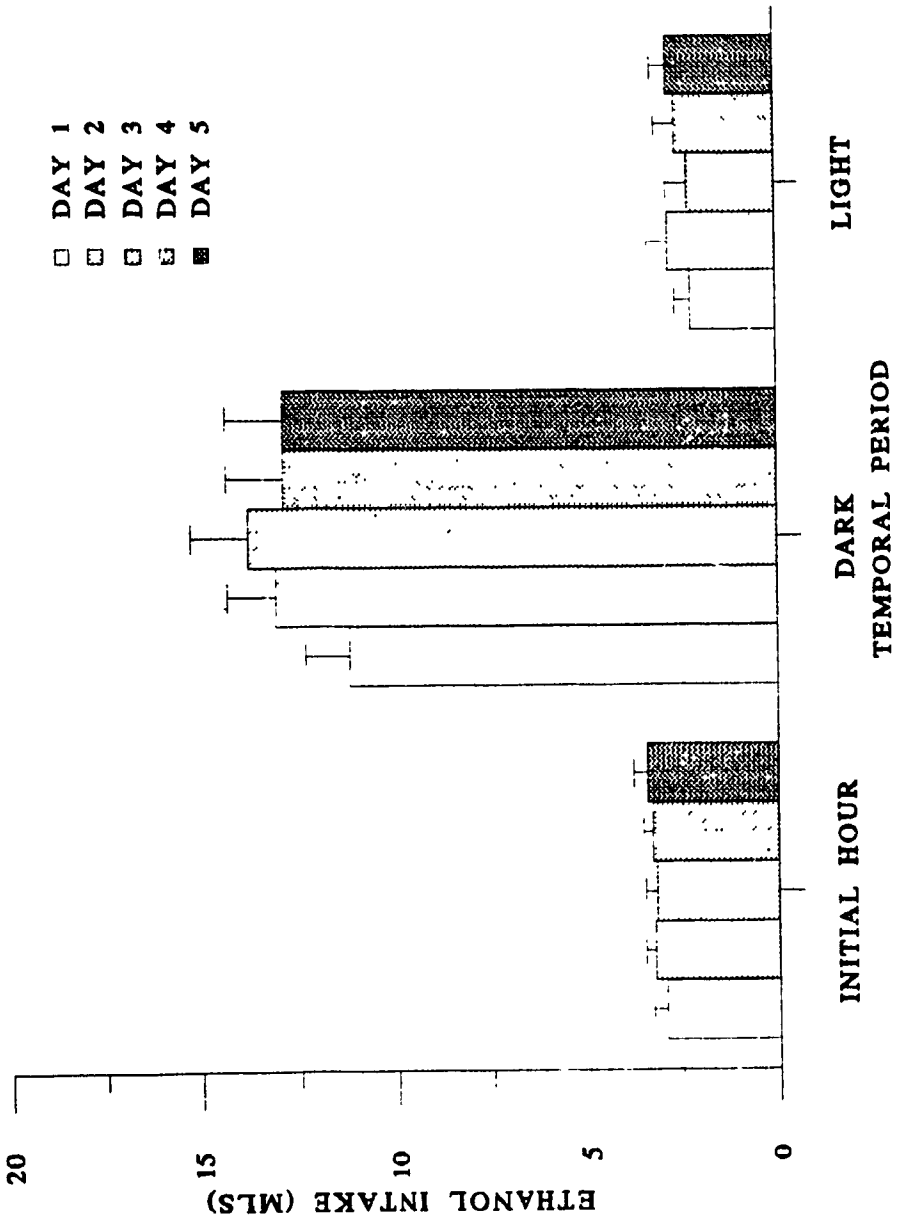

Figure 23. The effects of a period of forced ethanol exposure on the temporal pattern of ethanol intake observed throughout an ethanol maintenance period. Vertical bars represent the S.E.M.

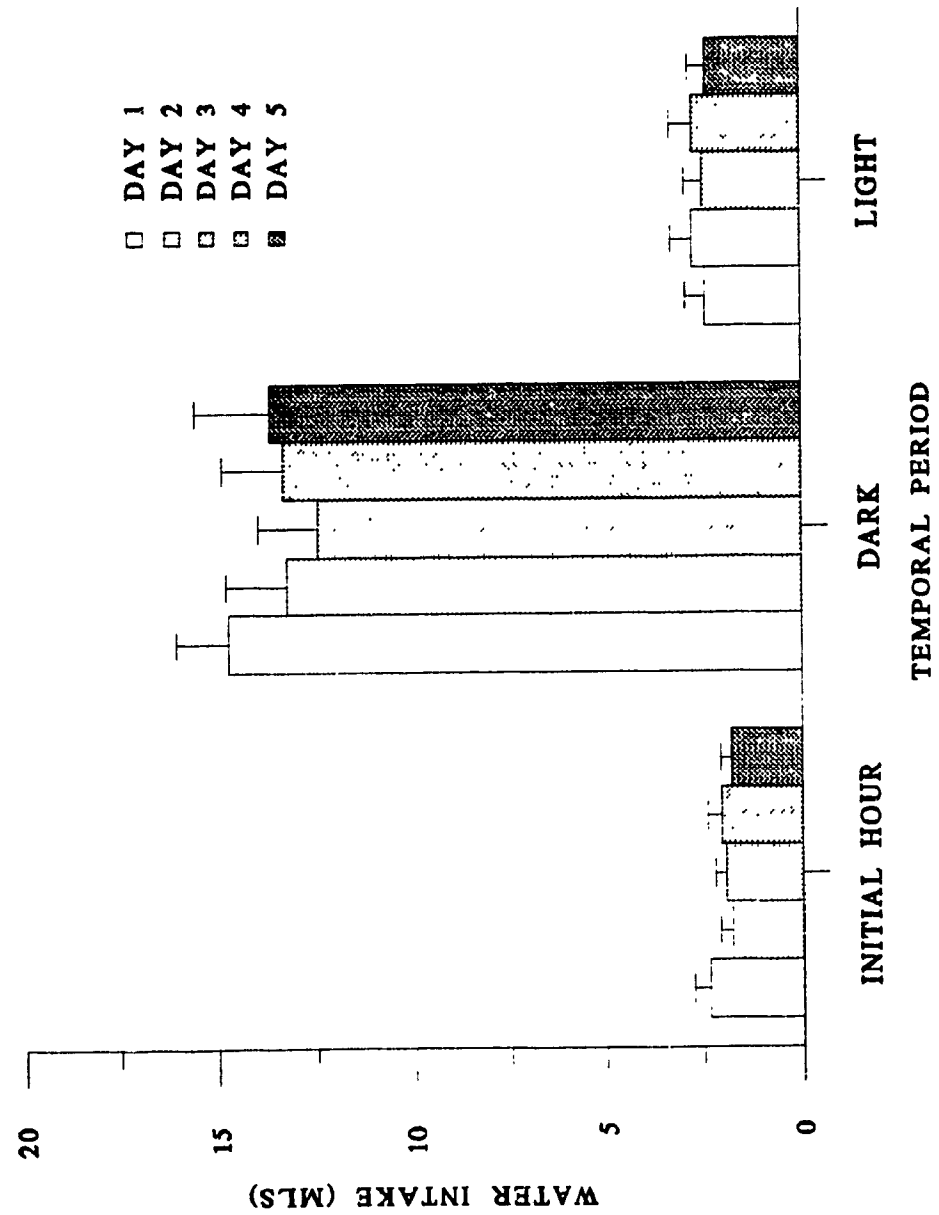

Figure 24. The effects of a period of forced ethanol exposure on the temporal pattern of water intake (mls) observed throughout an ethanol maintenance period. Vertical bars represent the S.E.M.

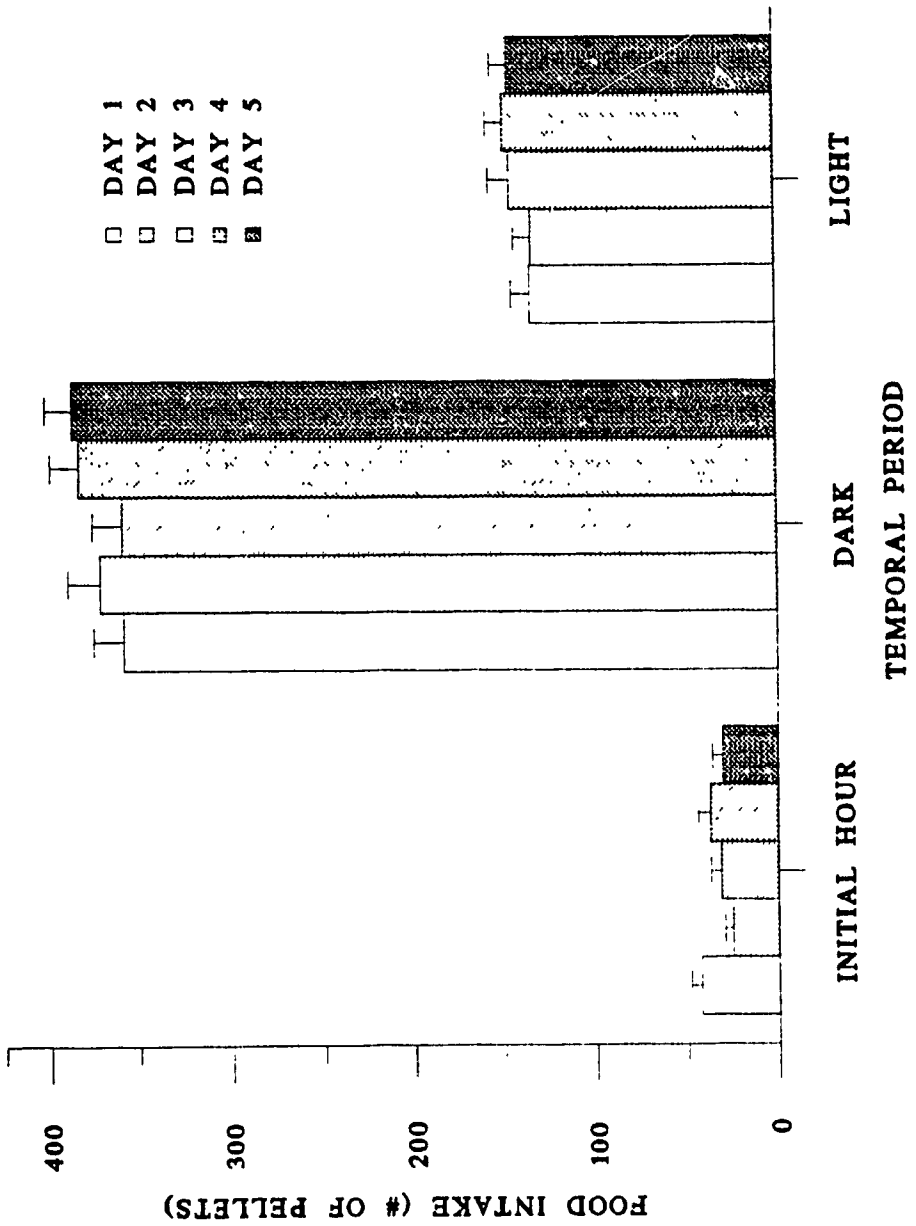

Figure 25. The effects of forced ethanol exposure on the temporal pattern of food intake (pellets) observed throughout an ethanol maintenance period. Vertical bars represent the S.E.M.

by a clustering of numerous smaller bouts of ethanol and water consumption. The larger bouts of ethanol intake were typically observed during middle to late portion of the dark cycle.

The present findings together with the outcome of Experiment 2 indicate that a relatively brief exposure to forced ethanol consumption may serve as an efficient method for inducing enhanced levels of ethanol intake in unselected rats.

GENERAL DISCUSSION PHASE 1

It was argued in the present thesis that the microstructural approach to the study of ethanol intake represented a fine grained behavioral approach which potentially provided a means for identifying the mechanisms which regulate ethanol consumption. Fulfilling this potential, use of this paradigm has provided a description of the reorganization of the microstructure of ethanol consumption, a critical process, which occurs during the acquisition of voluntary ethanol intake. Furthermore, the biphasic nature of the results observed in the present examination suggested that differential behavioral mechanisms mediate the organization of ethanol intake above and below the 6% ethanol concentrations.

The results of Experiment 1 were interpreted to suggest that, unique to the presentation of ethanol concentrations above 6%, ethanol intake was consolidated into a smaller number of increasingly larger ethanol bouts. Furthermore, the increases in the size of ethanol bouts were associated with increases in the rate of ethanol intake. This process is noteworthy in that the increases in bout size and rate of intake maximized an animals potential to achieve higher blood levels and to therefore experience the central pharmacological effects of ethanol. Potentially, it is the enhancement of ethanol's pharmacological effects which would explain the increase in ethanol intake which occurred despite the increasingly bitter nature of the ethanol solutions (Bice, Kiefer, & Elder 1992; DiLorenzo, Kiefer, Rice & Garcia 1986).

In contrast, the presentation of ethanol concentrations from 2% to 6% were characterized by a decrease in multiple ethanol bout parameters. The decrease in the size of ethanol bouts coupled with the low concentration of ethanol suggested that, for concentrations below 6%, ethanol intake was minimally influenced by the drugs central pharmacological effects. The intake of ethanol at these concentrations was more likely a function of the changes in the taste of the ethanol solutions, or other non-pharmacological factors.

Interestingly, the use of a radically different induction technique produced changes in the microstructure of ethanol drinking behavior that appeared to be an elaboration of those processes that mediated the increase in ethanol intake observed in Experiment 1. In particular, the increased level of ethanol intake, observed following the use of an acute period of forced ethanol exposure in Experiments 2 and 3 of the present thesis, appeared to be primarily a function of an increase in the size of ethanol bouts.

Illustrating this point, Figures 26 through 28, provide a qualitative comparison of ethanol intake, in addition to the size and frequency of ethanol bouts following the traditional acquisition paradigm (Experiment 1) and forced ethanol exposure (Experiment 3). Although a direct statistical comparison is not possible due to the interstudy comparison , the relative configuration of the ethanol bout parameters (including bout size and frequency) produced by these two distinct induction techniques are notable for their remarkable similarity. It would appear that the differences in the level of ethanol intake observed as a function of the traditional acquisition paradigm and an acute period of forced ethanol exposure were attributable primarily to differences in the size (Figure 28) and not the frequency (Figure 27) of ethanol bouts.

Furthermore, reports in the literature of microstructural studies have revealed that differences in ethanol intake are attributable to differences in the size and not the frequency of ethanol bouts utilizing, for both heterogeneous (e.g. Gill 1989) and genetically selected lines of rats (Murphy et al. 1986). Remarkably, the frequency of daily ethanol bouts have been consistently reported to be in the vicinity of 8 bouts, for preference paradigms which use a wide variety of induction techniques (Gill 1989; Murphy et al. 1986).

These findings would suggest that similar behavioral mechanisms may mediate the impact of radically different techniques used to induce ethanol intake. These findings would support the assertion, discussed earlier, suggesting that the use of different induction techniques may represent different but equally valid approaches to the study of ethanol intake.

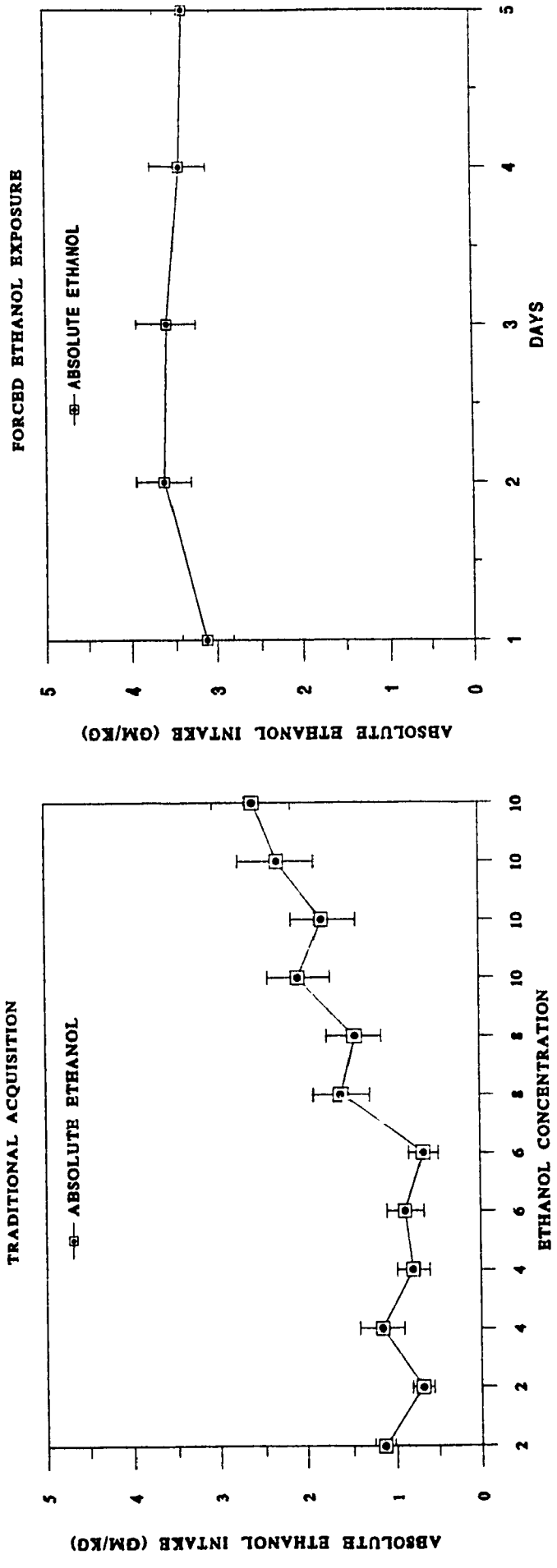

Figure 26. A comparison of absolute ethanol intake observed within both traditional acquisition and forced ethanol exposure paradigms. Vertical lines represent S.E.M.

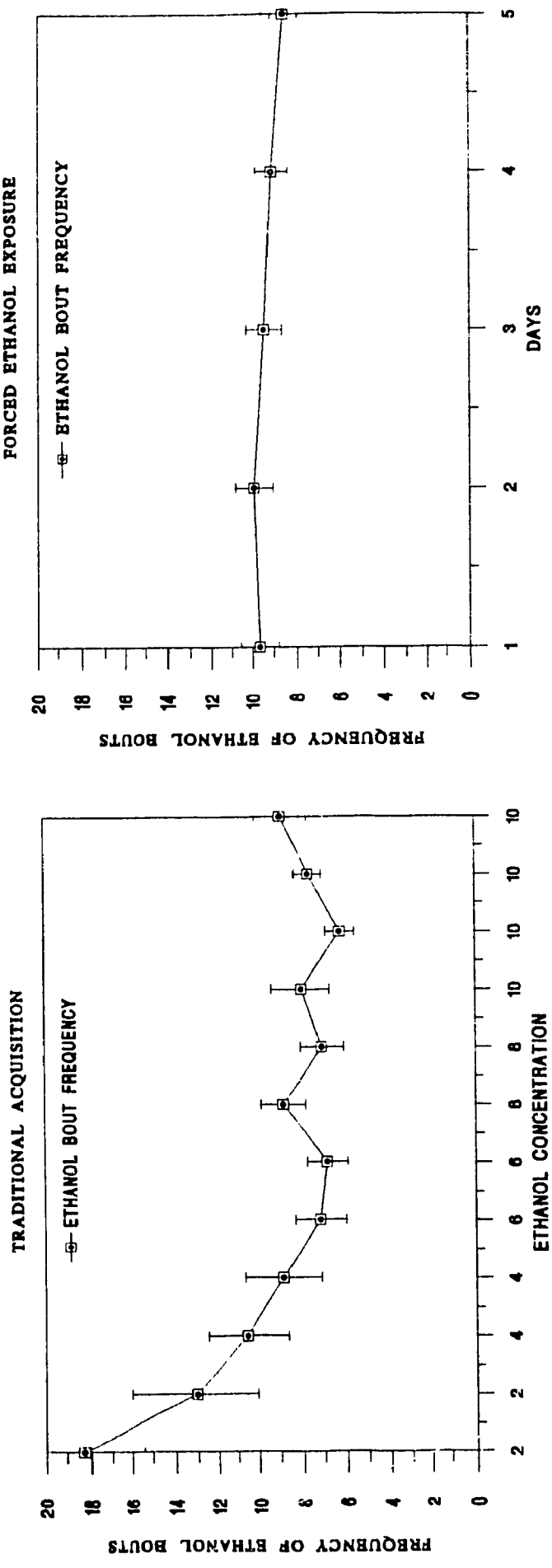

Figure 27. A comparison of the frequency of ethanol bouts observed during both traditional acquisition and forced ethanol exposure paradigms. Vertical lines represent S.E.M.

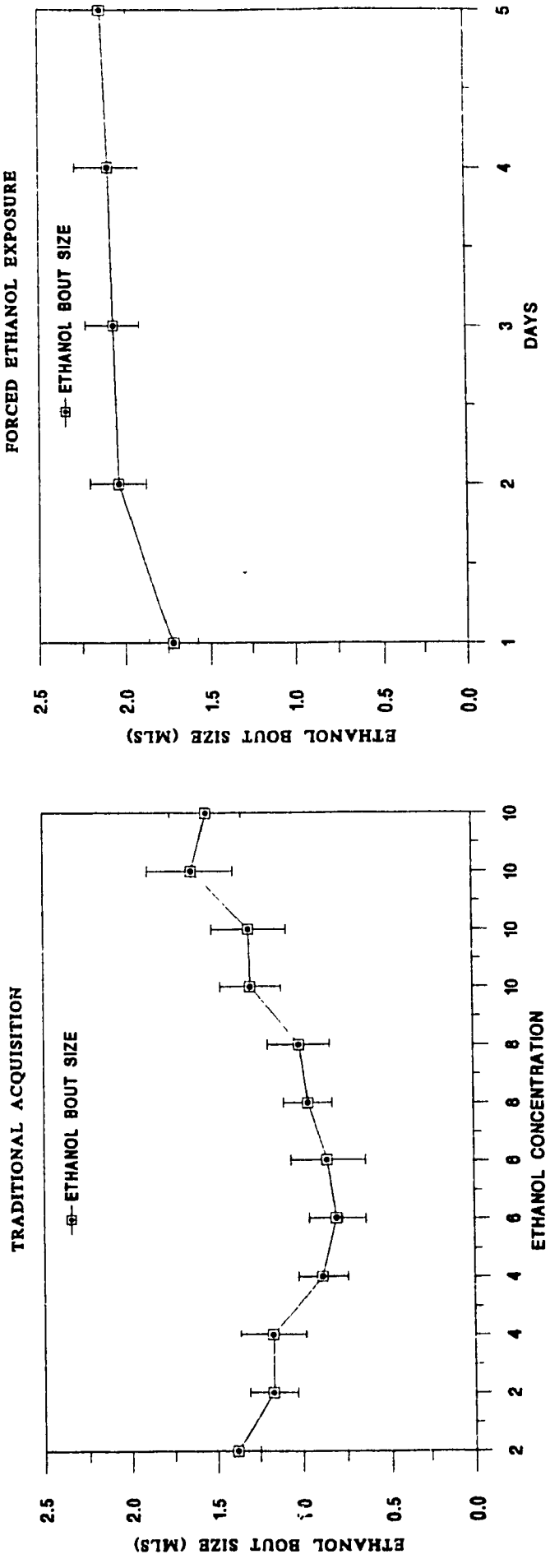

Figure 28. A comparison of the size of ethanol bouts observed within both traditional acquisition and forced ethanol exposure paradigms. Vertical lines represent S.E.M.

However, the findings of the present thesis are not consistent with several reports in the literature which have described the microstructure of oral operant ethanol self-administration (e.g. Files et al. 1992; Samson, et al. 1991). This literature suggested that, within the context of oral operant self-administration, rats will consume amounts of ethanol similar to those observed in the present thesis. However, the observed microstructural pattern of intake are quite different. Specifically, ethanol intake within operant studies has been characterized by the observation of a larger number of small ethanol bouts (Files et al. 1992; Samson, et al. 1991). As was stated earlier, in the present thesis, approximately 8 ethanol bouts of 10% ethanol per day were consumed, independent of the type of paradigm used (traditional acquisition or forced ethanol exposure). On average, each ethanol bout consisted of a minimum of .25g/kg. In contrast, however, Files et al. (1992) has reported that in a nonrestricted access operant paradigm, the structure of ethanol intake was characterized by approximately 25 daily bouts. Each bout was characterized by an intake of 0.16g/kg.

Curiously, while the mean ethanol bout size reported within oral operant studies (Files et al. 1992; Samson, et al. 1991) are considerably smaller than those observed in the present thesis, they are also smaller than those routinely reported in free drinking preference paradigms (Murphy, et al., 1986; Gill, et al., 1988; Gill, et al., 1986). Furthermore, it has also been demonstrated that ethanol consumed within the simulated bout of a restricted access paradigm, is also most consistent with the size of ethanol bouts observed in preference paradigms (Gill, et al., 1986). These findings would suggest, as was discussed earlier, that the pattern and structure of 24 hour ethanol intake may be a function of the operant schedules of ethanol reinforcement.

In summary, the microstructural analysis of the acquisition of voluntary ethanol intake suggested that biphasic, concentration dependent, behavioral mechanisms may mediate ethanol intake. Notably, increases in ethanol intake were a function of an increase in the size of individual ethanol bouts, but not the frequency. The induction of ethanol

intake through an acute period of forced ethanol exposure was observed to produce a configuration of ethanol bout parameters (e.g. frequency and size of bouts) which were similar to those observed in the traditional acquisition paradigm. Most significantly, the enhanced level of ethanol intake in the forced ethanol exposure paradigm appeared to be primarily a function of an increase in the size of ethanol bouts.

It was suggested, in an earlier discussion, that an area in which the microstructural approach holds perhaps most promise involves the delineation of the effects induced by pharmacological manipulations on ethanol self-administration. In the second phase of the present thesis, microstructural analyses of the effects of GABAergic manipulations on ethanol intake were examined. The analyses were used to address the ambiguity in the literature regarding the role of the GABAergic system in the mediation of voluntary ethanol intake. Knowledge of the processes which regulate ethanol intake in pharmacologically unmanipulated animals, obtained from Phase 1 of the present thesis, was used to interpret the significance of GABA induced changes in the structure of ethanol intake.

PHASE II - INTRODUCTION

The GABAergic System

It has been suggested that the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) may play a primary role in mediating some of the behavioral and pharmacological effects of ethanol (Liljequist & Engel 1982; Hakkinen & Kulonen 1976; Cott, Carlsson, Engel, & Lindqvist, 1976; Martz, Deitrich & Harris 1983).

Progress has been made in further defining the role of GABA in terms of two pharmacologically and functionally unique GABA receptor systems, specifically the GABA-A and GABA-B receptor systems (Hill & Bowery 1981). The GABA-A receptor, a major site of synaptic inhibition in the central nervous system, has been described as a complex unit in which a chloride (Cl-) ionophore is controlled by the GABA receptor and interacts with binding sites for benzodiazepines, picrotoxin and barbiturates (Ticku & Delgado 1989). The benzodiazepine and barbiturate sites are known to exhibit positive allosteric interaction with the GABA site, in other words they enhance GABA receptor binding and ionophore function, which results in membrane hyperpolarization (Peris 1992).

Molecular cloning techniques have further revealed a number of different GABA-A receptor subunits. These were divided into 5 classes of polypeptides each with its own subunits: alpha 1-6, beta 1-4, delta, gamma 1-3, and rho (Moss, Doherty, & Huganir 1992; Persohn, Malherbe, Richards 1992). The different receptor subtypes are known to vary in terms of their sensitivity to GABA (Levitan, Schofield, Burt, Rhea, Wisden, Kohler, Fujita, Rodriguez, Stephenson, Darlingson, Barnard & Seeburg 1988; Khrestchatisky, MacLennan, Chiang, Xu, Jackson, Brecha, Sternini, Olsen, & Tobin 1989).

Binding studies have revealed the presence of regional brain differences in terms of the expression of GABA-A receptor subtypes. It was suggested that these regional differences may account for the selective effects of certain GABA and benzodiazepine

agonists on sedation, anxiety and seizure activity (Peris 1993). Thus far, attempts to identify those subunits which may mediate GABA-A receptors sensitivity to ethanol have been equivocal. It has been suggested that the gamma 2l subunit and / or alternatively the alpha 6 subunit may be required for ethanol induced enhancement of the GABA-A chloride flux. (Weiss 1992; Wafford, Burnett, Leidenheimer, Burt, Wang, Kofuji, Dunwiddie, Harris & Sikela 1991). However, more recent data suggested that the presence of the Alpha 6 or gamma 2l subunit in and of itself does not result in the potentiation of GABA induced Cl- flux by ethanol (Marszalec, Kurata, Hamilton, Carter, & Narahashi 1994). Consistent with this finding, it has also been reported that the differential sensitivity to ethanol's hypnotic effects, observed in selectively bred mice, does not appear to reflect differences in the levels of alpha or gamma subunits (Zahniser, Buck, Curella, McQuilkin, Wilson-Shaw, Miller, Klein, Heidenreich, Keir, Sikela, 1992).

In contrast, to the complex Cl- ionophore associated with the GABA-A receptor, the Bicuculline insensitive GABA-B receptor is associated with a pertusis toxin sensitive G protein (Ticku & Delgado 1989). While the GABA-A system produces inhibition (pre or post synaptic) by modulating CL^- conductance, the GABA-B receptors are coupled to Ca^{++} or K^+ channels (presynaptic or postsynaptic respectively) (e.g. Kamatchi & Ticku 1990). Generally, GABA-B receptors are present in lower numbers within the CNS compared to the GABA-A receptors. It has been reported that the GABA-B receptor may play a role in inhibiting the release of amines, excitatory amino acids, peptides, hormones and GABA (e.g. Bowery 1989; Hausler, Monnet, & Peter 1993)

The Interaction Between Ethanol and the GABAergic System

A substantial body of data is available suggesting that ethanol may interact with the GABA-A receptor system. Specifically, it has been demonstrated, both in cultured spinal cord neurons and in vivo, that ethanol interacts and enhances GABA-A chloride channel flux both directly and indirectly, depending on the concentration of ethanol present (Mehta & Ticku 1988; Sanna, Concas, Serra, Biggio 1990). Low concentrations of ethanol have

been shown to potentiate the effects of GABA mediated CL^- influx possibly through a facilitation of coupling of receptor binding and chloride channel activation (Renolds, Prasad, & MacDonald 1992). Also, acute administration of ethanol may produce an increase in the number of benzodiazepine binding sites (Mosaddeghi, Burke, & Moerschbaecher 1992). Higher concentrations, on the other hand, may directly influence chloride channel functioning. However, direct or indirect, the effects of ethanol on chloride conductance were found to have been attenuated through the administration of GABA antagonists (Mehta & Ticku 1988).

Furthermore, while acute administration of ethanol (in vivo) potentiates the effects of GABA mediated Cl^- influx , prolonged treatment with ethanol has been suggested to induce a subsensitivity of the GABA but not benzodiazepine receptors sites on the GABA-A receptor complex (Szmigielinski, Szmigielska, & Wejman 1992; Sanna, Serra, Cossu, Colombo, Follesa, Cuccheddu, Concas, & Biggio 1993; Korpi, Uusi-Oukari, Wegelius, Casanova, Zito, & Kleinman 1992).

Related to the earlier discussion of the region-dependent differences in GABA-A subunits, there appear to be region-dependent differences in the way in which GABA-A receptor function is altered by ethanol (Proctor, Soldo, Allan, & Dunwiddie 1992; Freud, Lin, VanHorne, Harlan & Palmer 1990). Specifically, it has been demonstrated that ethanol enhanced potentiation of chloride flux occurs in microsacs prepared from cerebral cortical and cerebellar tissue, but not hippocampul tissue (Proctor, Soldo, Allan, & Dunwiddie 1992). This finding is paralleled by electrophysiological data suggesting that ethanol enhances the action of GABA on cortical but not hippocampul neurons (Proctor & Dunwiddle 1991).

While the GABA-B receptor is not generally considered a significant factor in the mediation of ethanol's effects, it has been suggested that the ethanol-induced enhancement of GABA-A receptor-chloride channel function may require activation of GABA-B receptors (Allan, Burnett, & Harris 1991). The GABA-B agonist baclofen potentiated the

effects of ethanol on Cl- uptake in membrane vesicle prepared from mouse cortex (Allan, Burnett, & Harris 1991). Likewise it was demonstrated that the GABA-B antagonists Phaclofen and 2-hydroxy-saclofen completely blocked the increase in Cl- flux produced by ethanol in the presence of either baclofen or GABA (Allan, Burnett, & Harris 1991).

The Relationship between the Sensitivity of the GABAergic Receptor System to Ethanol and the Expression of Ethanol Induced Behaviors

Support for the argument that the behavioral effects of ethanol may be mediated through GABA has been obtained through research with strains of animals selectively bred for differences in their response to ethanol's action (Martz, Deitrich & Harris 1983; Marley, Stinchcomb, & Wehner 1988; Allan, Spuhler & Harris 1988). These studies can be characterized as attempts to determine the extent to which sensitivity to the actions of ethanol is correlated with differences in responding to GABAergic manipulations at the behavioral, receptor and chloride flux level.

This body of research has also indicated that animals selected for their neurosensitivity to ethanol displayed a corresponding sensitivity to the incoordinating effects of baclofen and THIP (Martz, Deitrich & Harris 1983), and to behavioral effects of benzodiazepines, such as loss of righting reflex and hypothermia (Marley, Stinchcomb, & Wehner 1988).

Furthermore, it has also been demonstrated (Korpi, & Uusi-Oukari 1989) that in rats selectively bred for differential sensitivity to the hypnotic effects of ethanol, an acute administration of ethanol resulted in a down regulation of the GABA-A receptor function in the alcohol-sensitive rats. In a comparison of P and NP rats, P rats were found to have a greater GABAergic terminal density in the Nucleus Accumbens (McBride, Murphy, Lumeng and Li 1990). Similarly, there were more GABAergic terminals in the NA of High alcohol drinking (HAD) compared to low alcohol drinking (LAD) rats (Hwang, Lumeng, Wu, & Li 1990).

Similarly, genetic differences in the sensitivity to ethanol's hypnotic effects, in selectively bred animals, have been associated with the sensitivity of the GABA-A chloride

channels to the effects of ethanol (Wafford, Burnett, Dunwiddie & Harris 1990; Allan, Mayes, & Draski 1991; Allan, Spuhler & Harris 1988). Wafford, Burnett, Dunwiddie & Harris (1990) have demonstrated that the reactivity of specific cells (Xenopus oocytes) to the effects of ethanol may be altered by injecting these cells with brain mRNA from mice known to be differentially sensitive to the hypnotic effects of ethanol (LS vs SS). In particular, ethanol administration facilitated the responding of the GABA-A chloride channel in cells within which LS mRNA was injected and subsequently expressed. In contrast, ethanol induced chloride channel responding was antagonized in those cells with SS mRNA. Similarly, in rats selected for high (HAS) and low sensitivity (LAS) to an acute hypnotic dose of alcohol, the rats were also found to be differentially sensitive to the effects of ethanol on GABA-mediated chloride flux (Allan, Mayes, & Draski 1991).

The use of subjects selectively bred for differences in ethanol's action has provided some correlational evidence, described above, to suggest that the behavioral effects of ethanol administration may be a function of the relative sensitivity of the GABA-A receptor system to ethanol. However, the conclusions drawn from this line of research must be tempered by the apparent absence of consensus regarding the identification of the specific GABAergic mechanisms which are correlated with the sensitivity to ethanol's action. Emphasizing this point, a review of the literature by Crabbe & Harris (1991) noted that while various lines of animals selectively bred for differences in ethanol effects all differ in many aspects of the GABA-A receptor system, there is no single change in GABAergic functioning which is common to all selected lines.

Manipulations of GABAergic Function and their Effects on Ethanol Induced Behavioral Responding

Manipulations of the GABAergic system have been demonstrated to influence the effects of ethanol on various behavioral measures including intoxication (Martz, Deitrich & Harris 1983; Liljequist & Engel 1982; Hakkinen & Kulonen 1976; Cott, Carlsson, Engel, & Lindqvist 1976; Martz, Deitrich & Harris 1983; Allan & Harris 1989; Martz, Deitrich &

Harris 1983). In mice, both AOAA, a GABA-transaminase (GABA-T) inhibitor, and the GABA-A agonist THIP have been demonstrated to potentiate ethanol narcosis. In contrast, ethanol narcosis is also attenuated by the functional antagonist picrotoxin, a blocker of the chloride ionophore (Martz, Deitrich & Harris 1983). Picrotoxin is considered a "functional" GABA antagonist due to its indirect antagonism of the GABAergic system.

In a similar manner, AOAA has, in rats, been demonstrated to potentiate the degree of intoxication induced following ethanol administration, as assessed through performance on a tilted plane (Hakkinen & Kulonen 1976). On the same measure of intoxication the GABA-A antagonist Bicuculline attenuated the intoxicating effects of ethanol in rats (Hakkinen & Kulonen 1976). In still another measure of intoxication, the effects of manipulations of the GABA-A receptor system have been shown to influence ethanol-induced sleep time in mice (Liljequist & Engel 1982). Specifically, Liljequist & Engel (1982) demonstrated that the administration of the GABA-A agonist muscimol resulted in an increase in ethanol induced sleep time. At the same time, administration of the GABA-A antagonists bicuculline and picrotoxin attenuated ethanol induced sleep time.

Further support for the role of the GABA-receptor in ethanol induced intoxication is obtained through studies examining the antagonism of ethanol induced behavioral responding by RO15-4513 and other agents which interact with the benzodiazepine receptor site. As mentioned earlier, the GABA-A receptor is a complex unit with receptor sites for benzodiazepines and barbiturates. Both benzodiazepines and barbiturates bind to this complex and indirectly regulate GABA receptor mediated chloride conductance resulting in membrane hyperpolarization (Sieghart 1992). The partial inverse benzodiazepine agonist RO15-4513 has been demonstrated, although the literature is not consistent in this regard (Hatch & Jernigan 1988), to partially antagonize some of ethanol's physiological and behavioral effects, including ethanol induced narcosis (Suzdak, Paul, & Crawley, 1988) and motor incoordination (Hoffman, Tabakoff, Szabo, Suzdak, & Paul, 1987). Further, the effects of ethanol stimulated chloride flux have been selectively

antagonized through the application of RO15-4513 (Ticku 1989; Renolds, Prasad, & MacDonald 1992). These data provide further support for the notion that the GABA-A receptor system may mediate some of ethanol's intoxicating effects.

While the evidence described above, provided substantial support for a role of the GABA-A receptor system in mediating some of ethanol's physically intoxicating effects, there was also some evidence to suggest that the GABA-B receptor may also play a role (Allan & Harris 1989; Martz, Deitrich & Harris 1983; Cott, Carlsson, Engel, & Lindqvist 1976). In particular, phaclofen a specific GABA-B antagonist has been shown to attenuate the effects of ethanol on motor incoordination, locomotor activity and hypothermia (Allan & Harris 1989). baclofen a GABA-B agonist, on the other hand has been shown to potentiate ethanol-induced narcosis and motor incoordination (Martz, Deitrich & Harris 1983). In an earlier study, Cott, Carlsson, Engel, & Lindqvist (1976) demonstrated that pretreatment with the GABA-B agonist baclofen would attenuate the locomotor excitation induced by ethanol in mice.

Thus, the data would clearly suggest that the GABA-A receptor system, and to some extent GABA-B system, may mediate ethanol's intoxicating effects.

GABAergic Manipulations and Ethanol Self-Administration

While voluntary ethanol intake has been demonstrated to be sensitive to the influence of manipulations of the GABAergic system, the effects of these manipulations have, to date, been difficult to interpret. Unlike the bi-directional effects observed with ethanol induced intoxication, produced as a function of GABAergic agonist and antagonist manipulations, voluntary ethanol intake has for the most part been reduced as a function of GABAergic manipulation (e.g. Rassnick, D'Amico, Riley, & Koob 1993; Fadda, Argiolas, Melism, De Montis & Gessa 1983).

Consistent with the effects of GABAergic manipulations of ethanol induced intoxication, the attenuation of GABAergic neurotransmission has also been demonstrated to correlate with a diminished expression of ethanol self-administration. The administration

of the picrotoxin ligand isopropylbicyclophosphate (IPPO), a functional GABA antagonist which is believed to inhibit chloride flux, reduced the intake of ethanol in a 30 minute session of free choice operant responding (Rassnick, D'Amico, Riley, & Koob 1993). Similarly, administration of the benzodiazepine inverse agonist RO15-4513 which has also been described as decreasing the ethanol induced chloride flux, has been reported to decrease ethanol intake in both limited access (McBride, Murphy, Lumeng, & Li 1988; June, Colker, Domangue, Perry, Hicks, June, & Lewis 1992) and operant paradigms (Samson, Haraguchi, Tolliver, & Sadeghi 1989; Rassnick, D'Amico, Riley, & Koob 1993). The attenuating effects of RO15-4513 on ethanol intake have also been replicated with other inverse agonists such as RO19-4603 (Balakleevsky, Colombo, Fadda, & Gessa 1990) and FG 7142 (Samson, Haraguchi, Tolliver, Sadeghi 1989).

On the basis of these data it may be tempting to suggest that the reduction in ethanol intake observed following the general inhibition of GABAergic neurotransmission may represent an attenuation of the reinforcing properties of ethanol (Rassnick, D'Amico, Riley, & Koob 1993). However, any simple mediational role ascribed to GABA may be complicated by findings suggesting that numerous manipulations which increase neurotransmission in the GABAergic systems also reduce voluntary ethanol intake. The inhibitory influence of an increase in GABAergic neurotransmission on ethanol intake has been demonstrated through the administration of the nonspecific GABA agonists Gamma-butyrolactone (Fadda, Argiolas, Melism, De Montis & Gessa 1983) and calcium acetyl homotaurine (Boismare, Daoust, Moore, Saligaut, Lhuintre, Chretien & Durlach 1984). Both of these manipulations were observed to decrease ethanol intake in animals. In the latter study, the effects of calcium acetyl homotaurine were attenuated through the concurrent administration of the GABA-A antagonist bicuculline. This finding lends support to the notion that the reduction in ethanol intake was a specific function of GABAergic influences and not simply and indirect effect. More recently it has been demonstrated that an increase in the synaptic availability of GABA, through the

administration of the GABA transaminase (T) inhibitor gamma-vinyl GABA, decreased voluntary ethanol intake in alcohol preferring AA rats (Wegelius, Halonen, & Korpi 1993). Similarly, the GABA-T inhibitor AOAA was observed to decrease ethanol intake in a group of Long Evans rats which were selected for high ethanol preference (Daoust, Saligaut, Lhuintre, Moore, Flipo, & Boismare 1987).

In contrast to numerous accounts suggesting a reduction in ethanol intake following the enhancement of GABAergic neurotransmission, it has been reported that the administration of THIP, a GABA-A agonist, resulted in an increase in voluntary ethanol intake in an acquisition paradigm (Smith, Robidoux, & Amit 1992). Further support for a role of the GABA-A receptor in regulating ethanol intake, came from recent studies which have indicated that the GABA-A agonist muscimol, when injected into the dorsal raphe region of the brain, increased ethanol intake (Tomkins, Sellers, & Fletcher 1994). It is of interest, that these studies (Smith, Robidoux, & Amit 1992; Tomkins, Sellers, & Fletcher 1994) are some of the very few which report an increase, rather than the ubiquitous decrease, in voluntary ethanol intake following a neuropharmacological manipulation.

Similarly, the GABA-B agonist baclofen has also been demonstrated to increase ethanol intake in an acquisition paradigm (Smith, Robidoux, & Amit 1992). However, in contrast to the effects of the GABA-A agonists, the authors have suggested that baclofen's effects may not be specific to ethanol (Smith, Robidoux, & Amit 1992). Still other research has revealed that baclofen administration decreased ethanol intake in a maintenance paradigm (Daoust, Saligaut, Lhuintre, Moore, Flipo, & Boismare 1987). The apparent discrepancy in the effects of baclofen, observed in these two studies, may have been a function of the differential levels of experience with ethanol inherent in the use of acquisition and maintenance paradigms.

Nonetheless, the nature of the effects of differential GABAergic manipulations on voluntary ethanol intake remain ambiguous. Conceptually, it is difficult to propose a central and specific role for GABA in voluntary ethanol intake when its behavioral effects, i.e.

self-administration, appear to be independent of its neuropharmacological effects (Rassnick, D'Amico, Riley, & Koob 1993; Daoust, Saligaut, Lhuintre, Moore, Flipo, & Boismare 1987).

On the basis of the established role of the GABAergic receptor system in mediating ethanol's intoxicating effects it would be parsimonious to assume that the ambiguity regarding the effects of GABAergic manipulations on voluntary ethanol intake may be in part a reflection of the poor behavioral resolution of traditional intake paradigms. Therefore, in the second phase, the present thesis attempted to demonstrate the utility of the microstructural paradigm through the differentiation of the effects of specific manipulations of the GABAergic system on voluntary ethanol intake. Specifically, the present thesis using the microstructural approach, defined in Phase 1 of this thesis, examined the effects of manipulations of the GABA -A and B receptor systems on the structure and pattern of food, water and ethanol intake.

Prior to the microstructural analysis of the effects of GABAergic manipulations on voluntary ethanol intake, the present thesis examined the influence of the GABA-A agonist THIP, and the indirect GABA-A antagonist picrotoxin on the maintenance of voluntary ethanol intake using a traditional preference paradigm (Experiment 4). Subsequently, experiments 5 through 7 demonstrated the capacity of the microstructural approach to effectively differentiate the behavioral effects of the specific GABA-A receptor agents, THIP, picrotoxin and the GABA-B agent baclofen on the microstructure and temporal pattern of food, water and ethanol intake.

EXPERIMENT 4

AN EXAMINATION OF THE EFFECTS OF GABAergic AGONISTS AND ANTAGONISTS ON THE MAINTENANCE OF VOLUNTARY ETHANOL INTAKE

Thus far, research has failed to clearly identify the nature of the influence of GABAergic manipulations on ethanol intake. Manipulations which potentiate (Fadda, Argiolas, Melism, De Montis & Gessa 1983; Boismare, Daoust, Moore, Saligaut, Lhuintre, Chretien & Durlach 1984) or attenuate GABAergic transmission (Rassnick, D'Amico, Riley, & Koob 1993; McBride, Murphy, Lumeng, & Li 1988; June, Colker, Domangue, Perry, Hicks, June, & Lewis 1992) have both been demonstrated to reduce voluntary ethanol intake.

However, the outcome of preliminary research would suggest that pharmacological manipulations which target specific GABA receptors sites may produce effects on ethanol intake which are more consistent with their neuropharmacological effects.

In this regard, it has been suggested that the GABA-A receptor system may play a role in mediating the expression of voluntary ethanol intake (e.g. Smith, Robidoux, & Amit 1992). Research conducted to date has demonstrated that the GABA-A agonist muscimol increases ethanol intake when injected into the dorsal raphe region of the brain (Tomkins, Sellers, & Fletcher 1994). Similarly, the GABA-A agonist THIP (4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3(2H)-one), a structural analog of muscimol, increases ethanol intake in an acquisition paradigm (Smith, et al. 1992). In contrast, the picrotoxin ligand isopropylbicyclophosphate (IPPO), a functional GABA antagonist reduced the intake of ethanol in a free choice operant paradigm (Rassnick, D'Amico, Riley, & Koob 1993). Taken together, these methodologically diverse studies, would suggest that the GABA-A receptor system may play a role in mediating voluntary ethanol intake.

In an attempt to further clarify the role of the GABA-A receptor system in the mediation of voluntary ethanol intake, the present study examined the influence of the specific GABA-A agonist THIP and the GABA-A Cl^- channel blocker picrotoxin on the maintenance of ethanol intake within a preference paradigm.

METHOD

Subjects

Thirty three male Long Evans new colony rats (Charles Rivers Canada Inc.) weighing between 175-200g were individually housed in stainless steel cages in a room controlled for constant temperature, humidity and a 12 hr light/dark schedule. Food and water were freely available throughout the test period.

Drugs

THIP was dissolved in 0.9% NaCl and injected IP at a dose of 16 mg/kg body weight (b. wt.) in a volume of 1 ml/kg. Similarly, picrotoxin was dissolved in 0.9% NaCl and injected IP at a dose of 2 mg/kg in a volume of 1 ml/kg. Saline was injected IP in a volume of 1 ml/kg. Ethanol solutions, with concentrations which ranged from 2 to 9% (v/v) were prepared from 95% ethanol mixed with tap water.

Procedure

Following a period of acclimatization to their environments, the rats were exposed to a screening procedure during which a sequence of increasing concentrations of ethanol solutions were presented, in glass Richter type tubes mounted on the front of the home cages, in a free choice with water on an alternate day schedule. Beginning with a 2% ethanol solution (v/v) the concentrations were increased after every second ethanol presentation until a 9% concentration was achieved. The position of the ethanol filled tube, in relation to the water filled tube, was altered on successive ethanol presentation days in order to avoid the development of a position bias. During the intervening days both tubes were filled with water.

Following the screening procedure and a subsequent stabilization period, the higher drinking rats were selected and exposed to 5 ethanol presentations at a concentration of 9%, in a free choice with water, on five alternate days. This interval constituted the baseline period.

Beginning with the first alternate day following the baseline period the rats were exposed to a test period in which the effects of the GABAergic manipulations were determined. The test period consisted of 5 additional ethanol presentations on alternate days, in a free choice with water.

During the test period, rats were assigned to groups which received I.P. injections of either 16 mg/kg of THIP (a GABA-A agonist), 2 mg/kg of picrotoxin (a GABA-A chloride-ionophore channel blocker) or saline on the ethanol presentation days. Group selections were made in such a manner that the amount of absolute ethanol consumed during the baseline period was approximately equal for each group. The dose of THIP utilized was selected on the basis of literature reporting that it significantly increased ethanol intake in an acquisition paradigm (Smith, Robidoux, Amit, 1992). Throughout the baseline and test periods ethanol and water scores in addition to body weight were recorded.

RESULTS

In the present experiment, the data on the effects of the GABA-A agonist THIP and the indirect GABA-A antagonist picrotoxin on the intake of ethanol, water and body weight were examined using multiple 3-way ANOVAs (with drug type and the repeated measure variables trial periods and days). The source of interactions within the factorial ANOVAs were established using tests of simple main effects and simple interactions.

The analysis of the effects of the GABAergic manipulations on absolute ethanol intake, presented in Figure 29, indicated a bi-directional effect. The results demonstrated a significant interaction between trial periods and drug groups [$F(2,30)=15.44$ $p< 0.0001$]. In light of the significant 2-way interaction, a test of simple main effects and simple interactions was performed holding drug group variable constant. As may be observed in

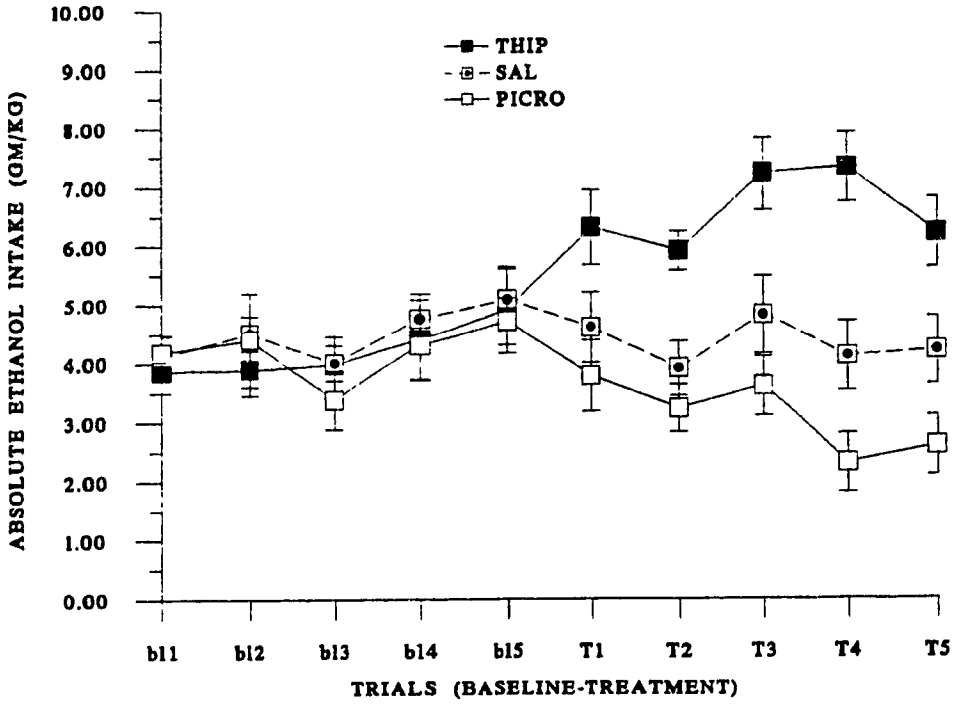

Figure 29. The effects of THIP and Picrotoxin on absolute ethanol intake (gm/kg) across baseline and treatment periods. Vertical lines represent S.E.M.

Figure 29, THIP treatment resulted in an increase in the intake of absolute ethanol during the test trials when compared to values observed during the baseline period [$F(1,30)=27.57$, $p<0.001$]. In contrast, picrotoxin produced a decrease in absolute ethanol intake relative to its baseline values [$F(1,30)=4.88$, $p<0.035$]. Saline treated rats failed to show any significant differences across trial periods [$F(1,30)=.21$, $p<0.64$].

Presented in Figure 30, are the preference ratios for THIP, picrotoxin and saline treated rats during baseline and test trials. The analysis revealed a significant interaction [$F(1,30)=27.64$, $p<0.0001$] between trial periods and drug groups. Subsequent tests of simple main effects and interaction, holding drug groups constant, demonstrated that, consistent with what had been observed with absolute ethanol intake, THIP treatment increased preference ratios [$F(1,30)=40.92$, $p<0.0001$] during the test trial period relative to the baseline period. In contrast, picrotoxin decreased preference [$F(1,30)=14.43$, $p<0.0007$]. No differences in preference ratios between baseline and test trials were observed with saline treatment [$F(1,30)=71$, $p<0.40$].

An analysis of the total fluid intake, which is presented in Figure 31, demonstrated that the drug groups did differ in terms of their overall levels of total fluid intake [$F(2,30)=4.69$, $p<0.016$]. However, there were no significant changes in intake as a function of drug treatment.

Finally, the analysis of body weight values pre nted in Figure 32, revealed a significant three way interaction between drug treatment, trial period and days [$F(8,120)=2.58$, $p<0.0124$]. However, the main effect for drug treatment was not significant. An analysis of simple main effects and simple interactions holding the drug group variable constant indicated that both THIP [$F(1,30)=19.26$, $p<0.001$] and saline [$F(1,30)=52.09$, $p<0.00001$] treated rats exhibited an increase in body weight across trial periods. In contrast, picrotoxin treated rats failed to demonstrate any significant change in body weight across trial periods [$F(1,30)=2.32$, $p<0.138$].

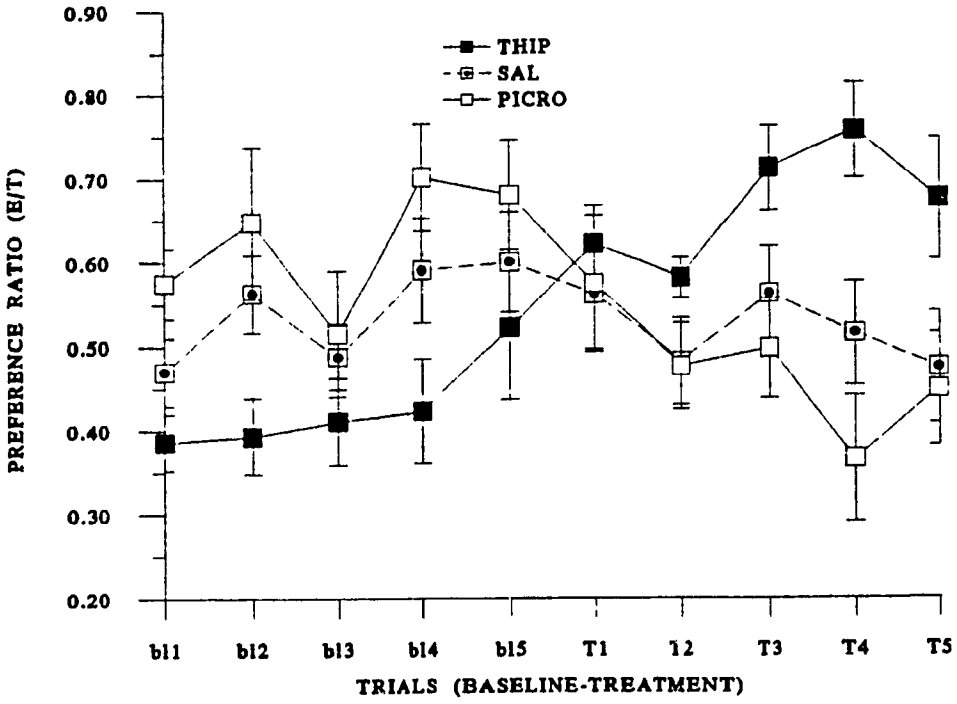

Figure 30. The effects of THIP and Picrotoxin on mean preference ratios across baseline and treatment periods. Vertical lines represent S.E.M.

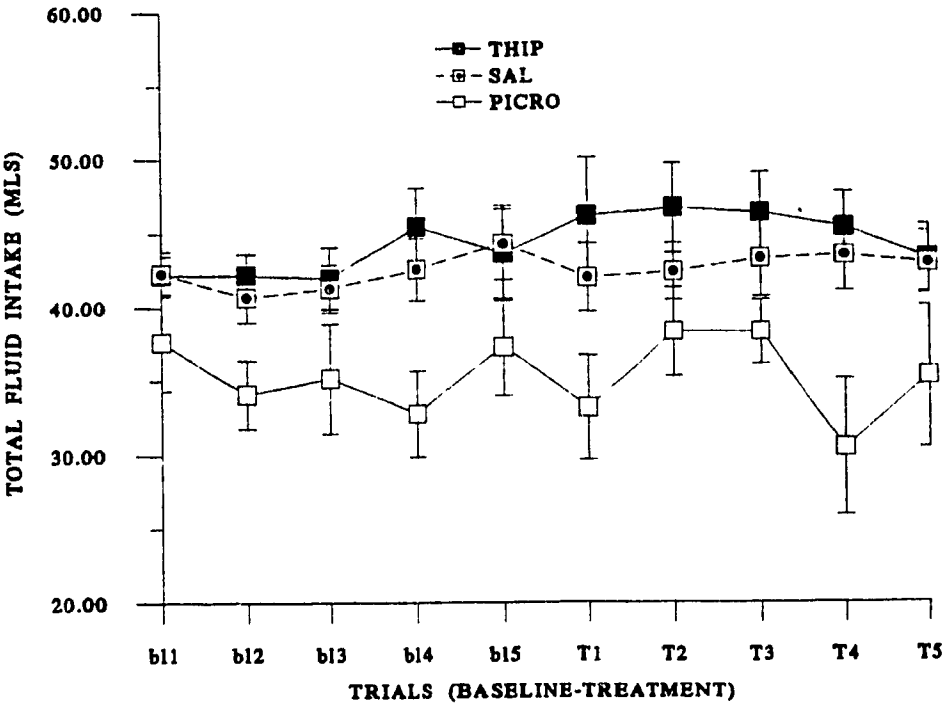

Figure 31. The effects of THIP and Picrotoxin on total fluid intake values (mls) across baseline and treatment periods. Vertical lines represent S.E.M.

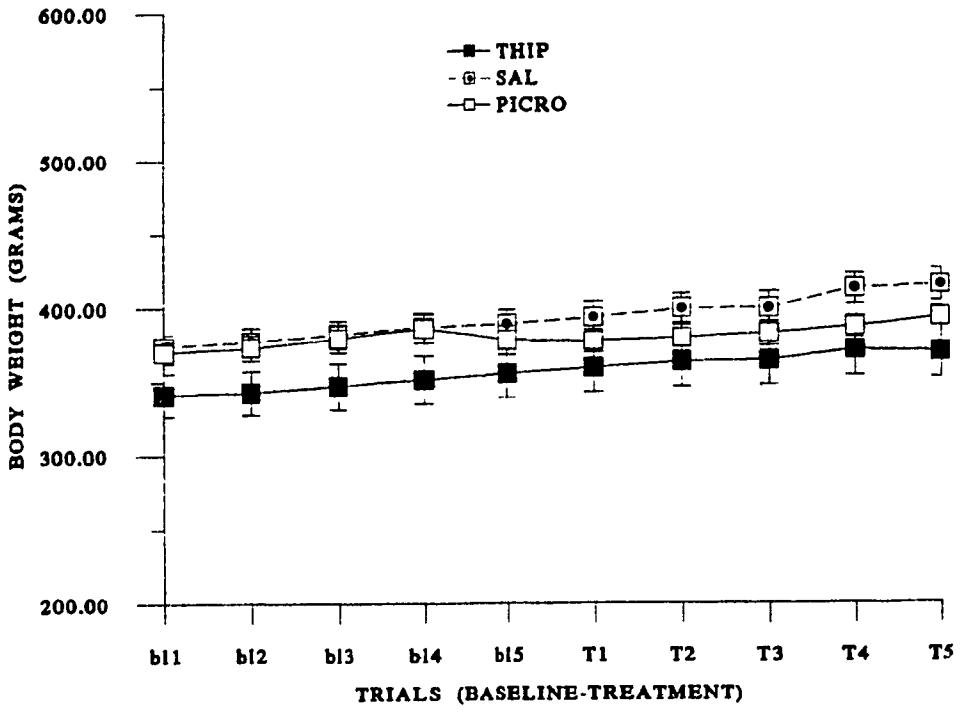

Figure 32. The effects of THIP and Picrotoxin on mean body weight values (grams) across baseline and treatment periods. Vertical lines represent S.E.M.

DISCUSSION

Results of the present study indicated that in a maintenance paradigm, the GABA-A receptor agonist THIP acted to enhance voluntary ethanol intake while the functional GABA-A antagonist picrotoxin decreased ethanol intake. These findings were consistent with previous data which suggested that THIP facilitated ethanol intake within an acquisition paradigm (Smith, Robidoux, Amit, 1992). Over all the results indicated that GABAergic manipulations known to produce opposing effects on Cl^- flux also produced directionally similar effects on ethanol intake and preference. It is worth noting that reports on bi-directional effects of agonists and antagonists of the same system, as observed in the present study, are quite rare. The present findings would therefore argue strongly in favor of a role for the GABA-A receptor in regulating voluntary ethanol intake.

While the GABAergic manipulations produced changes in ethanol intake, their effects on ethanol were not a function of a generalized fluid effect. Total fluid intake for all treatment conditions were unchanged as a function of drug administration.

In contrast, changes in body weight were not consistent across treatment groups. THIP treated subjects exhibited a rate of increase in body weight equivalent to that of saline controls. Changes in the pattern of body weight gain due to drug treatment were observed only in the picrotoxin treated subjects. Specifically, these subjects failed to demonstrate the increase in body weight across trial periods which was observed in both the THIP and saline controls.

While the present findings support the notion that the GABA-A receptor system may contribute to the regulation of the voluntary intake of ethanol, they are inconsistent with a report in the literature which suggested that the GABA-A receptor agonist muscimol was ineffective in modulating ethanol intake (Daoust, et al., 1987). It is unlikely that the discrepancy between these studies is related to the differential use of acquisition or maintenance paradigms, since the GABA-A agonist THIP has now been demonstrated to influence ethanol intake in both maintenance and acquisition (Smith, et al., 1992) studies.

Therefore, the failure of the GABA-A agonist muscimol to influence ethanol intake (Daoust, et al., 1987) may be attributable to methodological issues such as that pertaining to the dose of the drug administered. In particular, the dose of muscimol administered in this particular study (Daoust, et al., 1987) was relatively low. Thus, the failure to observe an effect on ethanol intake could be argued to reflect the use of a dose of muscimol insufficient to produce alterations in behavioral responding. Support for this suggestion is obtained from the failure of the authors (Daoust, et al., 1987) to report any effect of the administered dose on indices of consumatory behavior, such a changes in the pattern of body weight gain or total fluid intake, despite the fact that muscimol like THIP is reported to be an anorectic (Cooper, Howard, White, Soroko, Ingold, & Maxwell 1980). Furthermore, as was mentioned earlier, a more recent study has provided additional evidence in support of a role of the GABA-A receptor system in the regulation of ethanol intake (Tomkins, Sellers, & Fletcher 1994). Specifically, it has been demonstrated that muscimol, when injected into the dorsal raphe region of the brain, resulted in an increase in the voluntary intake of ethanol (Tomkins, Sellers, & Fletcher 1994).

Thus, the bi-directional effects of THIP and picrotoxin on the maintenance of ethanol intake, observed in the present study, provides further support for a role for the GABA-A receptor system in regulating the voluntary intake of ethanol. However, the preference paradigm employed in the present study does not have the inherent fine grain resolution necessary to determine the nature of the behavioral mechanisms which mediate the influence of the GABA-A receptor system on ethanol intake. In order to further define the nature of this interaction, Experiments 5 and 6 examined the effects of GABA-A receptor system manipulations on the microstructure and temporal pattern of food, water and ethanol intake.

EXPERIMENT 5

A MICROSTRUCTURAL ANALYSIS OF THE EFFECTS OF THE GABA-A AGONIST THIP ON VOLUNTARY ETHANOL INTAKE

The outcome of the previous experiment suggested that GABA-A receptor system played a role in regulating voluntary intake of ethanol. Specifically, THIP (4,5,6,7-Tetrahydroisoxazolo(5,4-c)pyridin-3(2H)-one), a structural analog of muscimol was found to potentiate the intake of ethanol. In the present experiment the microstructural approach described in Phase 1 of the present thesis was used to examine the more molecular changes in consumatory behavior which followed the administration of THIP. In this manner, insight into the processes which mediate the effects of THIP on ethanol intake was hoped to be gained.

METHOD

Subjects

Twenty male Long Evans rats (Charles Rivers Canada Inc.) weighing 200-250g 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 hr light/dark cycle. Food and fluids were available ad libitum throughout the test period.

Drugs

THIP was dissolved in 0.9% NaCl and injected IP at a dose of 16 mg/kg b. wt. in a volume of 1 ml/kg. Saline was injected IP in a volume of 1 ml/kg. Ethanol solutions, with concentrations which ranged from 2 to 10% (v/v), were prepared from 95% ethanol mixed with tap water.

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) equipped with feeders which 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 hour period. During a daily 60 min. computer shutdown period, the volume of each fluid tube was recorded and incorporated into the subsequent data analysis. All accumulated raw data were processed in order 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 measures 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 the animals with the food cups and drinking spouts, only those bouts consisting of more than 5 consecutive events were included in the analysis. Complete design specifications for the computerized acquisition system have been previously detailed (Gill, Mundl, Cabilio & Amit 1988).

Procedure

Following a five day period of acclimatization to the operant boxes, the 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, in order 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 4 ethanol presentation sessions.

On those presentation days when ethanol was available, animals received either an injection of THIP (16 mg/kg I.P. (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 which suggested that 16 mg/kg of THIP produced optimal increases in voluntary ethanol intake. Injections were administered during the daily 60 min. computer shutdown which occurred 1 hr prior to the onset of the animal's dark cycle.

RESULTS

The effects of THIP on total food, ethanol and water intake, as well as individual bout parameters were examined. The data were analyzed through the use of multiple two-way ANOVAs (drug groups with repeated measure across ethanol concentrations). The source of interactions within the factorial ANOVAs were established using tests of simple main effects and simple interactions. In order to further characterize the behavioral effects of THIP, the temporal pattern of food, water and ethanol intake was also examined using multifactorial ANOVAs (drug group with repeated measures for temporal period and ethanol concentrations)

Structural Pattern of Intake Following THIP Administration

The effects of THIP on the voluntary intake of ethanol are presented in Figure 33. An analysis of the results revealed that THIP treated subjects maintained higher overall levels of absolute ethanol intake (gm/kg) across ethanol concentrations [$F(1,18)=13.06$, $p<0.002$). Furthermore, the results indicated a significant interaction between drug treatment and days [$F(11,198)=2.39$, $p<0.01$]. A similar increase was also evident in the effects of THIP on the volume of ethanol (mls) consumed, also presented in Figure 33 [$F(1,18)=12.32$, $p<0.003$).

In a pattern consistent with the effects of THIP on the ethanol intake values described above, the present results demonstrated that THIP treated animals maintained consistently higher ethanol preference levels [$F(1,18)=14.99$, $p<0.001$].

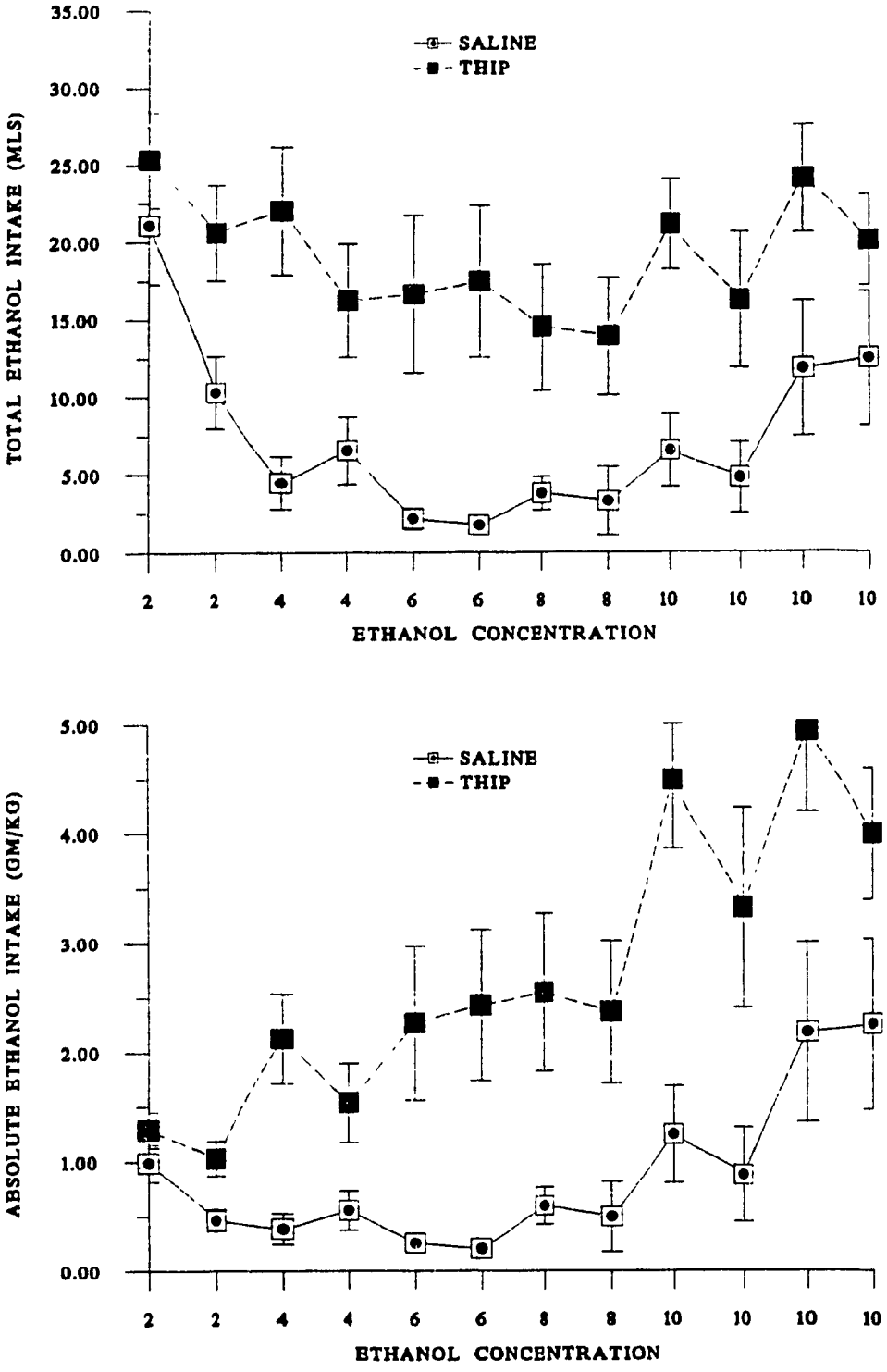

Figure 33. The effects of THIP on mean ethanol (mls) and absolute ethanol (gm/kg) intake values across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

A microanalysis of the increased intake of ethanol reported above revealed significant THIP induced increases in the size [$F(1,18)=7.30$, $p<0.01$] of the ethanol bouts. The effects of THIP on the mean ethanol bout size (mls) are presented in Figure 34. In addition, significant increases were also observed for the duration [$F(1,18)=6.72$, $p<0.01$] (presented in Figure 35) and frequency [$F(1,18)=6.32$, $p<0.02$] of mean ethanol bouts (presented in Figure 36).

While the results suggest that THIP administration influenced multiple bout parameters, the pattern of changes observed in ethanol bout size (Figure 34) appeared to be the most consistent with the pattern of changes observed in total ethanol intake, presented in Figure 33.

The data presented in Figure 37 demonstrated that the increase in ethanol intake induced by THIP was not associated with a comparable increase in the rate of ethanol consumption. An analysis of the rate of ethanol intake per bout (mls/sec) failed to indicate any significant changes as a function of THIP administration. It was apparent that as the size of the ethanol bouts increased, there was a concomitant increase in the duration of ethanol bouts. Neither the main effect for group [$F(1,18)=0.06$ $p<0.81$] or the interaction between ethanol concentration and group [$F(11,198)=1.38$ $p<0.2$] were significant.

Total fluid intake was not significantly influenced by THIP administration [$F(1,18)=1.72$ $p<0.21$]. Water intake, presented in Figure 38, while superficially decreased, was not significantly influenced by THIP administration [$F(1,18)=3.87$ $p<0.065$]. Furthermore, there were no significant interactions between drug treatment and days [$F(11,198)=1.41$ $p<0.18$].

Consistent with the absence of an effect on total water intake, the rate of water intake was not significantly influenced by THIP treatment [$F(1,18)=1.43$ $p<0.25$].

However, Food intake was significantly decreased in THIP treated subjects [$F(1,18)=12.58$, $p<0.002$]. The effects of THIP on food intake, as measured by the total number of food pellets consumed, is presented in Figure 39. The decreased level of total

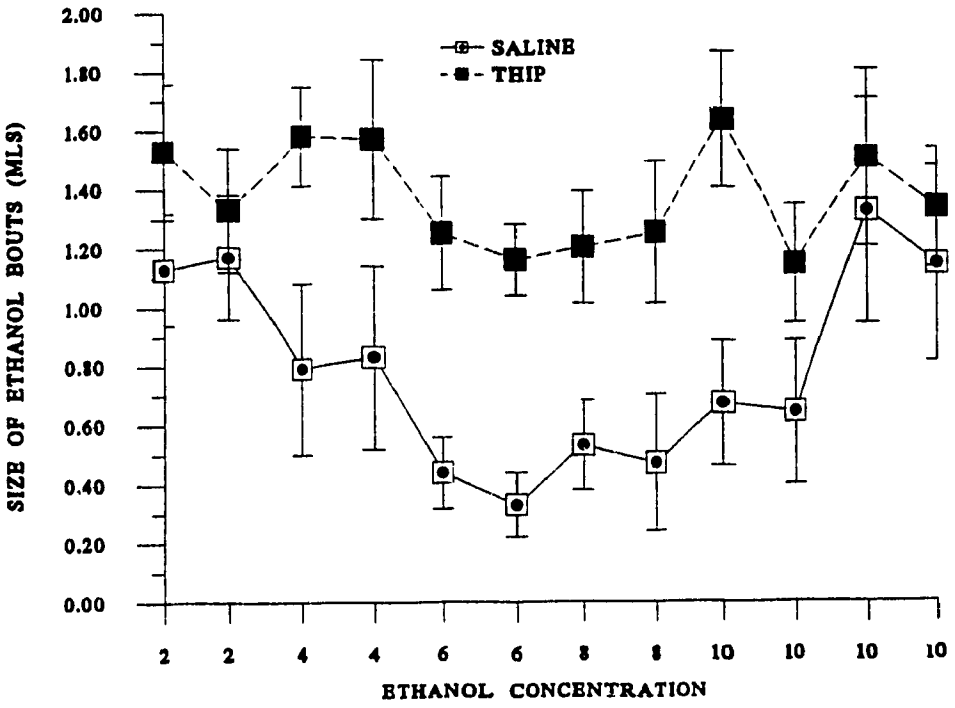

Figure 34. The effects of THIP on size of ethanol bouts (mls) observed across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

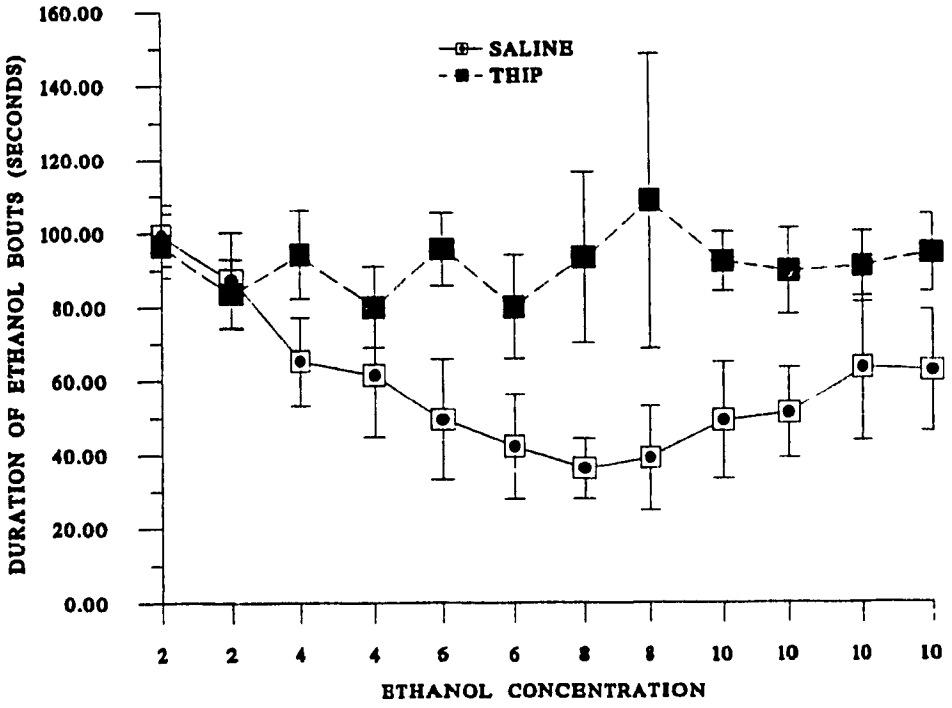

Figure 35. The effects of THIP on mean duration of ethanol bouts (seconds) observed across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

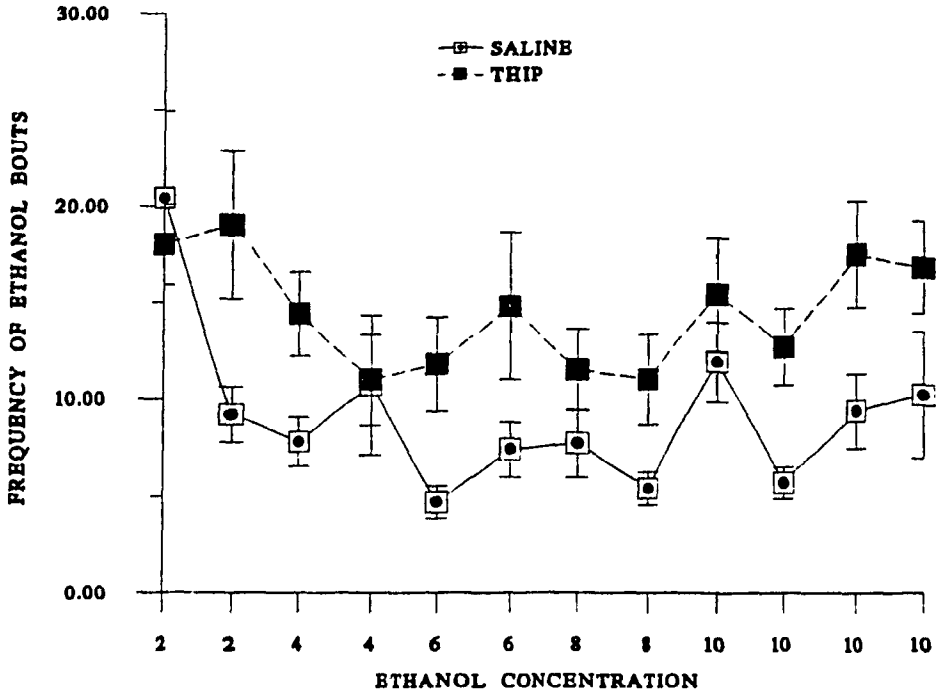

Figure 36. The effects of THIP on frequency of ethanol bouts (seconds) observed across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

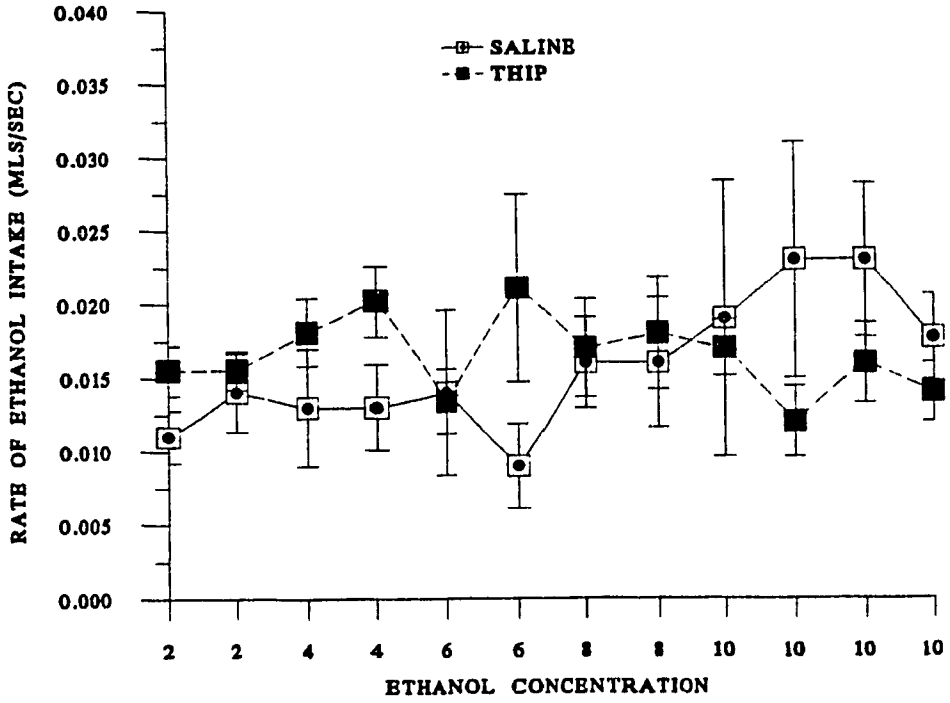

Figure 37. The effects of THIP on rate of ethanol intake (mls/sec) observed across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

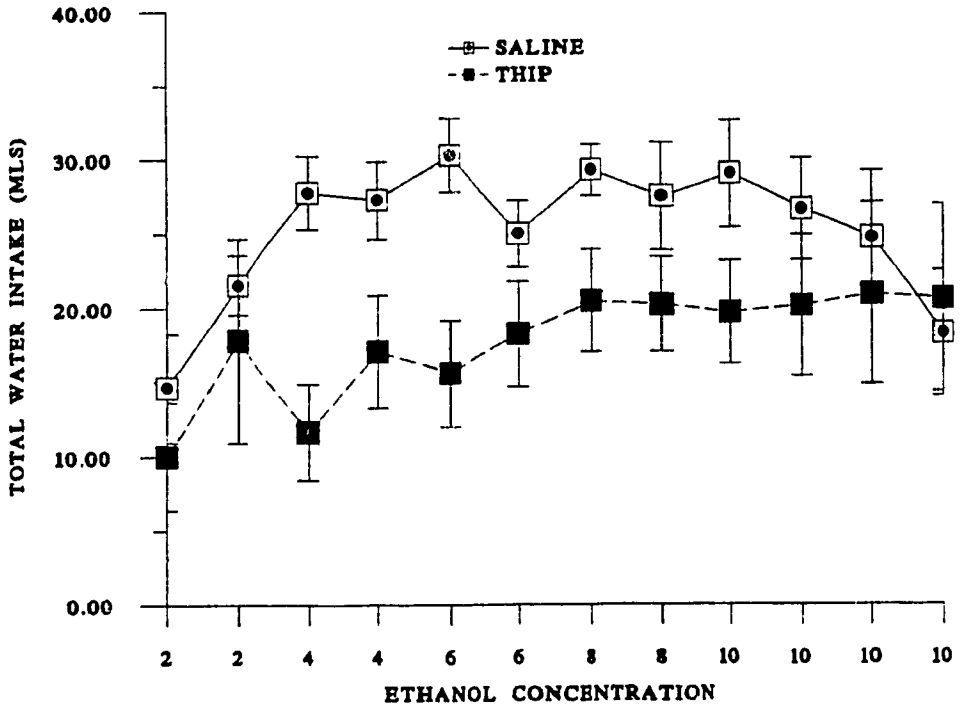

Figure 38. The effects of THIP on otal water intake values (mls) observed across the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

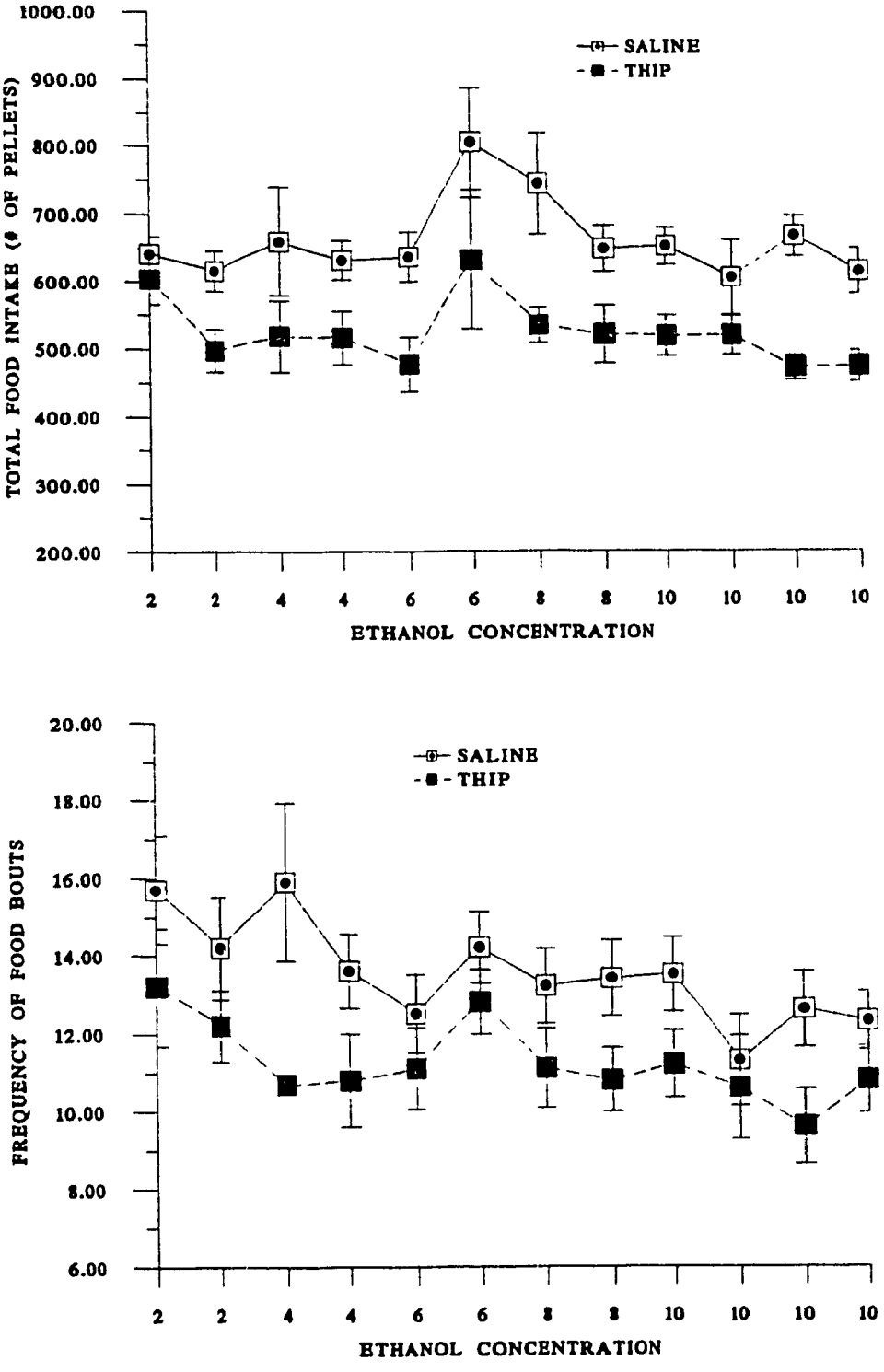

Figure 39. The effects of THIP on food intake (pellets) and food bout frequency values observed during the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

food intake was associated with a slower rate of increase in body weight. An examination of bout parameters suggested that the decrease in total food intake induced by THIP treatment was primarily a function of a decrease in the frequency of food bouts [$F(1,18)=5.7$, $p<0.04$]. The decreased frequency of food bouts in THIP treated subjects may also be observed in Figure 39. Neither the size or duration of food bouts were influenced as a result of THIP treatment.

Temporal Pattern of Intake Following THIP Administration

In order to further characterize the pattern of consumatory behavior, a temporal analysis of representative data was conducted, using the data obtained during the second presentation of each ethanol concentration up to 8%, and all presentations at 10%.

The intake of ethanol across concentrations and temporal periods, for saline and THIP treated subjects, is presented in Figure 40. An analysis of the temporal pattern of ethanol intake indicated a significant interaction between temporal period and drug group [$F(2,36)=8.20$ $p<0.0013$]. A test of simple effects and interactions further demonstrated that ethanol intake was significantly increased following THIP administration throughout both the dark [$F(1,18)=9.82$ $p<0.006$]and light [$F(1,18)=12.37$ $p<0.003$] periods of the day/night cycle. However, there were no differences in the amount of ethanol consumed during the initial period (hour) following the administration of THIP [$F(1,18)=0.55$ $p<0.47$].

In contrast, an analysis of the water intake findings, presented in Figure 41, indicated that THIP administration failed to induce any significant changes in the temporal pattern of intake [$F(2,36)=0.08$ $p<0.9256$].

Similarly, a temporal analysis of food intake revealed that THIP failed to induce any shifts in the pattern of food intake. The results demonstrated that there were no significant interactions between drug groups and temporal periods [$F(2,36)=2.50$ $p<0.0965$], or drug groups, temporal periods and ethanol concentrations [$F(14,252)=0.92$ $p<0.537$]. It would

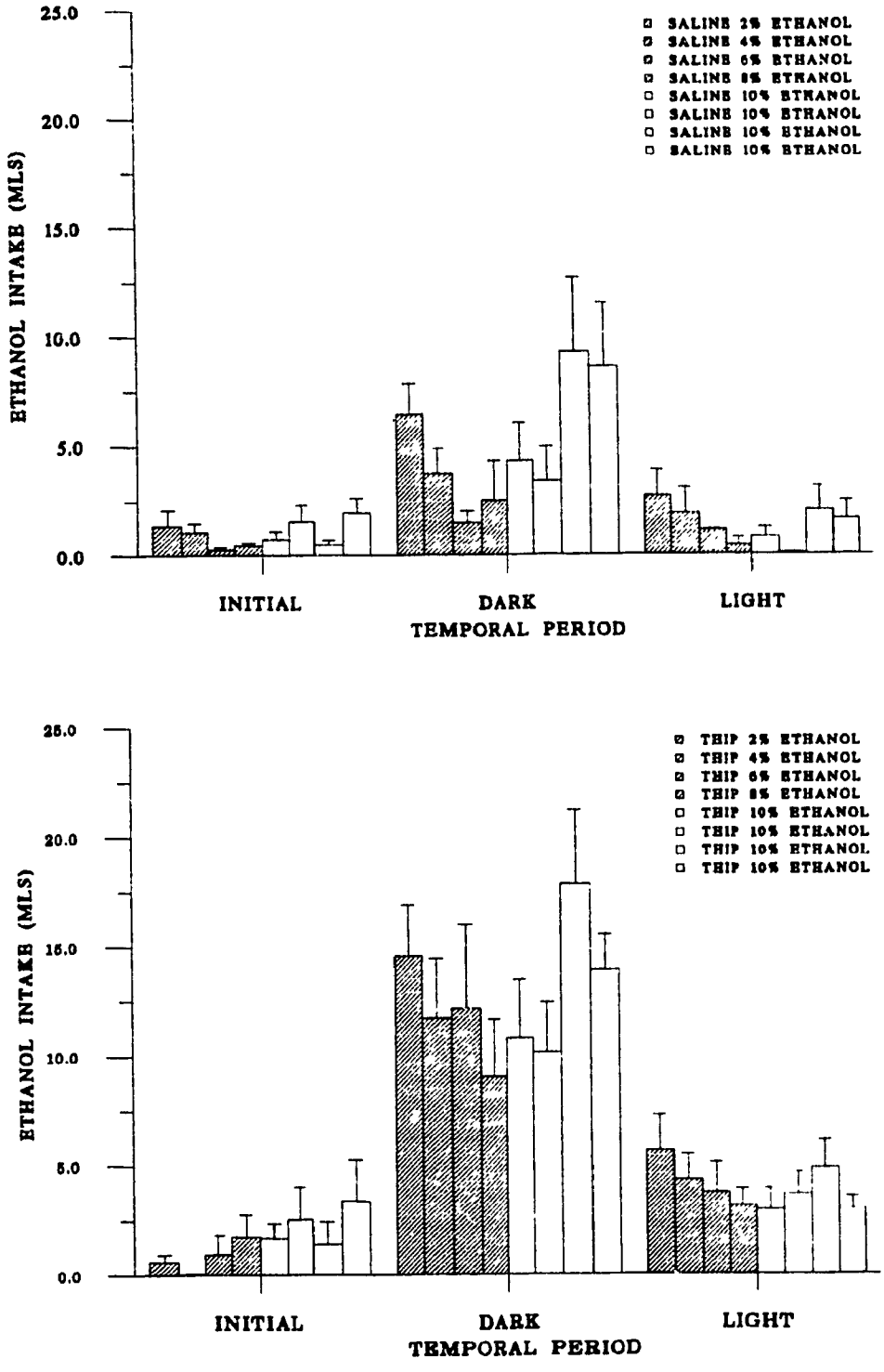

Figure 40. The effects of saline (upper figure) and THIP (lower figure) administration on temporal pattern of ethanol intake (mls) across ethanol concentrations. Vertical lines represent S.E.M.

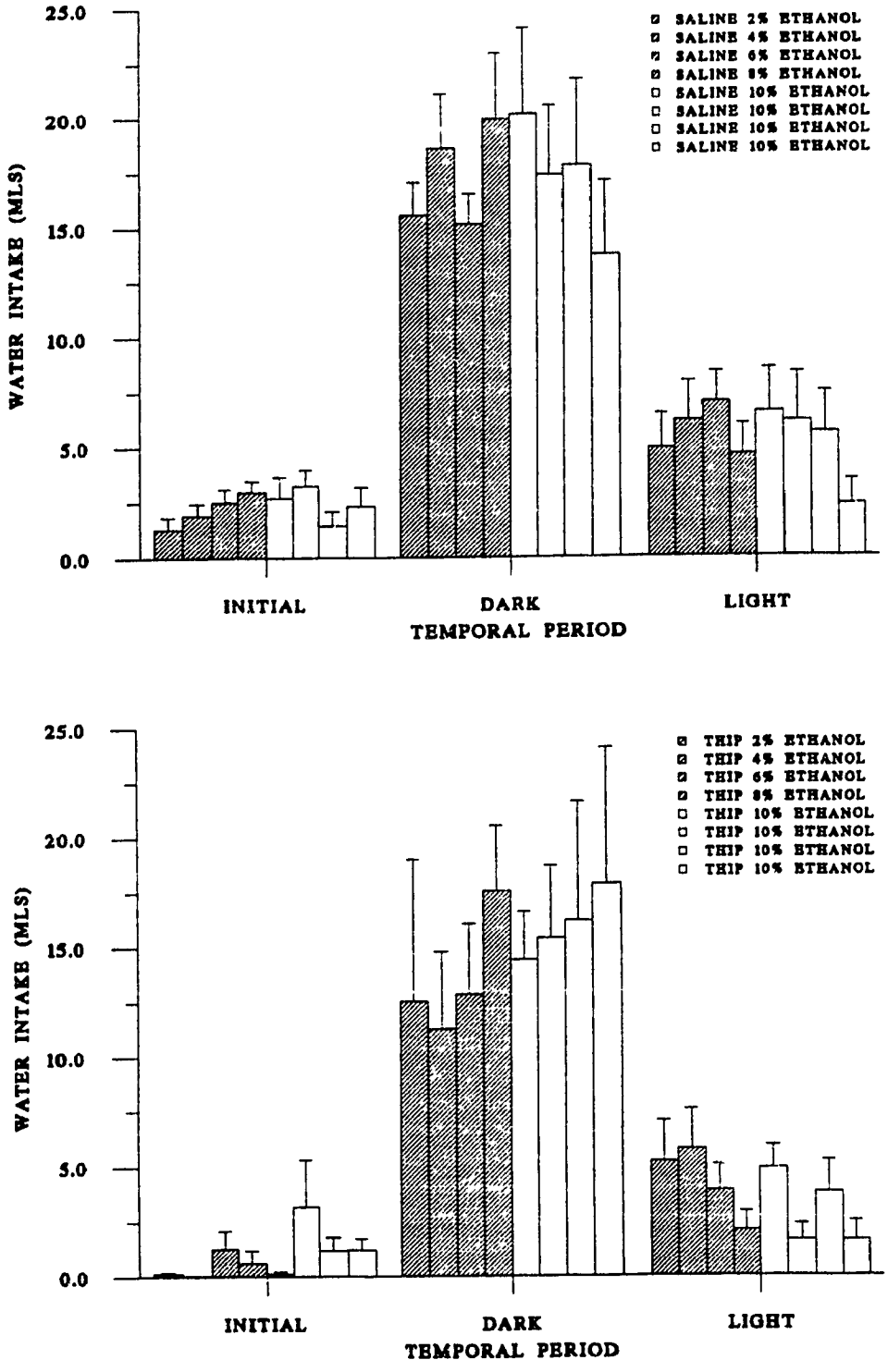

Figure 41. The effects of saline (upper figure) and THIP (lower figure) on temporal pattern of water intake (mls) during the presentation of increasing concentrations of ethanol. Vertical lines represent S.E.M.

appear that the decrease in total food intake was a reflection of a decrease in food intake across all temporal periods.

DISCUSSION

The present study demonstrated an enhanced acquisition of voluntary ethanol intake and preference throughout the range of ethanol concentrations tested following the administration of THIP. These findings were consistent with those of Experiment 4, which indicated enhanced ethanol intake following GABAergic manipulation in a traditional maintenance paradigm. Furthermore, these findings are consistent with other reports on the capacity of GABA-A agonists to enhance ethanol intake (Smith, et al., 1992; Tomkins, Sellers, & Fletcher 1994). Furthermore, 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.

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, the size of ethanol bouts (Figure 34) appeared to account for most of the observed increase in total ethanol intake (Figure 33).

It is interesting to note that while the size of the ethanol bouts were increased, the rate of ethanol intake (mls/sec) remained unchanged. This finding is in contrast to the parallel increases in bout size and rate of intake observed during the acquisition of voluntary ethanol intake in experiment 1. The absence of any change in the rate of ethanol intake may indicate that the mechanisms which mediated the effects of the GABA-A agonist on ethanol intake are different from those which mediate normal acquisition. However, one must also consider the potential vulnerability of measures of rate to nonspecific influences such as sedation. This latter explanation must be considered seriously given the demonstrated potentiating effects of GABAergic manipulations on ethanol induced intoxication (Martz, et

al., 1983; Liljequist & Engel 1982; Hakkinen & Kulonen 1976; Cott, et al., 1976; Martz, Deitrich & Harris 1983; Allan & Harris 1989; Martz, et al., 1983).

The concomitant reduction in total food intake induced by THIP would suggest that THIP administration also produced 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 which have been suggested to act normally to limit the size of meals rather than their frequency (Blundell 1984).

While it is evident that THIP influenced both food and ethanol intake, there nevertheless appears to be a relatively clear dissociation between the effects of THIP on the microstructural pattern of ethanol and food intake. The observed increases in ethanol intake are a reflection of changes in mechanisms regulating both the initiation and the 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 which mediate the initiation or termination of consumatory behaviors, such as those mentioned above, have been suggested to correspond to motivational states mediated by distinct physiological events (Gill, & Amit 1989). The qualitatively different patterns in the microstructure of consumatory behavior of ethanol and food observed following THIP administration would appear to confirm that differential behavioral mechanisms may underlie the effects on food and ethanol observed in the present study.

The following study further examined the influence of the GABA-A receptor system on the voluntary intake of ethanol. In particular, the functional GABA-A antagonist picrotoxin was examined.

EXPERIMENT 6

A MICROSTRUCTURAL ANALYSIS OF THE EFFECTS OF THE FUNCTIONAL GABA-A ANTAGONIST PICROTOXIN ON VOLUNTARY ETHANOL INTAKE

Experiment 4 suggested that picrotoxin produces a significant decrease in voluntary ethanol intake. These findings were consistent with a report in the literature which suggested that the administration of the picrotoxin analogue IPPO resulted in a decrease in voluntary ethanol intake (Rassnick, D'Amico, Riley, & Koob 1993).

In the present experiment the microstructural approach was used to provide a high resolution analysis of the changes in consumatory behavior which followed the administration of the indirect GABA-A antagonist Picrotoxin.

METHOD

Subjects

Twenty male Long-Evans new colony rats (Charles Rivers Canada Inc.) weighing 175 - 200 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 was available ad lib throughout the test period.

Drugs

picrotoxin was dissolved in 0.9% NaCl and injected (IP) at a dose of 2 mg/kg b. wt. in a volume of 1 ml/kg. Saline was injected (IP) in a volume of 1 ml/kg. Ethanol solutions, with a concentration of 10% (v/v). were prepared from 95% ethanol mixed with tap water.

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 which dispensed 45-mg standard Bioserve pellets. The feeders were activated by the interruption

of photo beams resulting from the placement of a rats head into a food cup. Each photo beam interruption resulted in a single pellet being dispensed. In addition, each chamber was equipped with two plastic drinking tubes fitted with steel ball bearing spouts.

All feeding and drinking behavior were monitored continuously over a 23-h period. During a daily 60-minute 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 to have been 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 minutes 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 or drinking spouts, only those bouts consisting of more than five consecutive events were included in the analysis. Complete design specifications for the computer acquisition system have been previously detailed (Gill, Mundl, Cabilio & Amit 1988).

Procedure

Following a 7-day period of acclimatization to the operant boxes, the rats were presented, over 4 consecutive days, with a forced exposure to ethanol in which a 10% (v/v) ethanol solution was the sole fluid available.

Immediately following the period of forced ethanol exposure the rats were placed on a schedule in which 10% ethanol was presented in a free choice with water on alternate days. The position of the ethanol-filled tube was altered on successive days to avoid a position bias. Both tubes were filled with water on intervening days.

After a period of time during which ethanol intake was permitted to stabilize, the temporal and structural pattern of food, water and ethanol intake were recorded in order to establish a baseline level of consumption. The baseline period consisted of 5 consecutive alternate day presentations of ethanol in a free choice with water. The 5 ethanol presentations trials, which followed the termination of the baseline period, constituted the treatment period. It was during the treatment period that the effects of the indirect GABA-A antagonist picrotoxin (a GABA-A chloride-ionophore channel blocker) was evaluated.

On those presentation days when ethanol was available, during the treatment period, animals received either an injection of picrotoxin (2 mg/kg I.P. (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 Experiment 4 in which 2 mg/kg of picrotoxin produced a significant decrease in voluntary ethanol intake. Injections were administered during the daily 60 min. computer shutdown which occurred 1 hr prior to the onset of the animal's dark cycle

RESULTS

The effects of picrotoxin on measures of total intake and bout parameters were examined for food, ethanol and water consumption across baseline and treatment phases. The data were analyzed through the use of multiple three way ANOVAs (the variables consisted of drug group with repeated measures across treatment phases and days). In order to further characterize the effects of picrotoxin on consumatory behavior, the temporal pattern of food, water and ethanol consumption was also examined. The analyses of the temporal data were conducted through the use of multiple 4 way ANOVAs (with the variables consisting of the drug group with repeated measures across treatment phases, days and temporal periods). The source of interactions within the factorial ANOVAs were established using tests of simple main effects and simple interactions.

Structural Pattern of Intake Following Picrotoxin Administration

The administration of the indirect GABA-A agonist picrotoxin failed to significantly influence the consumption of 10% ethanol [$F(1,18)=0.50, p<0.49$] or the level of absolute ethanol (presented in Figure 42) [$F(1,18)=0.60, p<0.46$]. Similarly, the magnitude of the ethanol preference ratio remained unchanged [$F(1,18)=0.42, p<0.53$] following picrotoxin administration.

An examination of ethanol bout parameters revealed that the frequency of ethanol bouts presented in Figure 43, were significantly increased [$F(1,18)=8.35, p<0.01$]. However, most other parameters including the size [$F(1,18)=2.28, p<0.15$], rate [$F(1,18)=1.21, p<0.29$] and duration of ethanol bouts [$F(1,18)=0.08, p<0.79$] were uninfluenced by picrotoxin administration.

Consistent with the observed effects on total ethanol intake, picrotoxin failed [$F(1,18)=0.07, p<0.80$] to influence the total intake of water (Figure 44). Furthermore, there were no observable effects upon size [$F(1,18)=0.02, p<0.89$], frequency [$F(1,18)=1.11, p<0.31$], duration [$F(1,18)=2.49, p<0.14$] or rate of water intake [$F(1,18)=0.49, p<0.50$].

In contrast to the minimal effects on fluids described above, picrotoxin exhibited a potent anorexic effect. The effects of picrotoxin on food intake and body weight are presented in Figure 45. Total food intake was significantly attenuated throughout the picrotoxin treatment phase [$F(1,18)=36.26, p<0.00001$]. Furthermore, an analysis of the body weight data (Figure 45) revealed a significant 3 way interaction (treatment x days x group) [$F(4,72)=2.91, p<0.028$]. Over the course of the 5 day drug treatment phase, the normal gain in body weight observed in controls was attenuated in picrotoxin treated subjects.

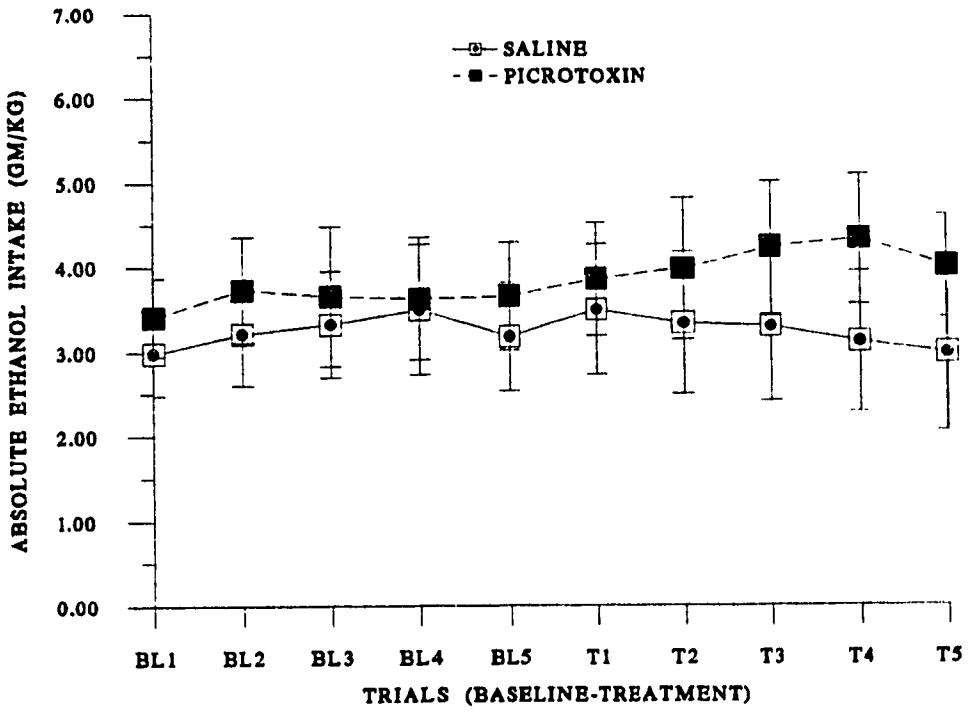

Figure 42. The effects of Picrotoxin on absolute ethanol (gm/kg) intake values observed across baseline and treatment periods. Vertical lines represent S.E.M.

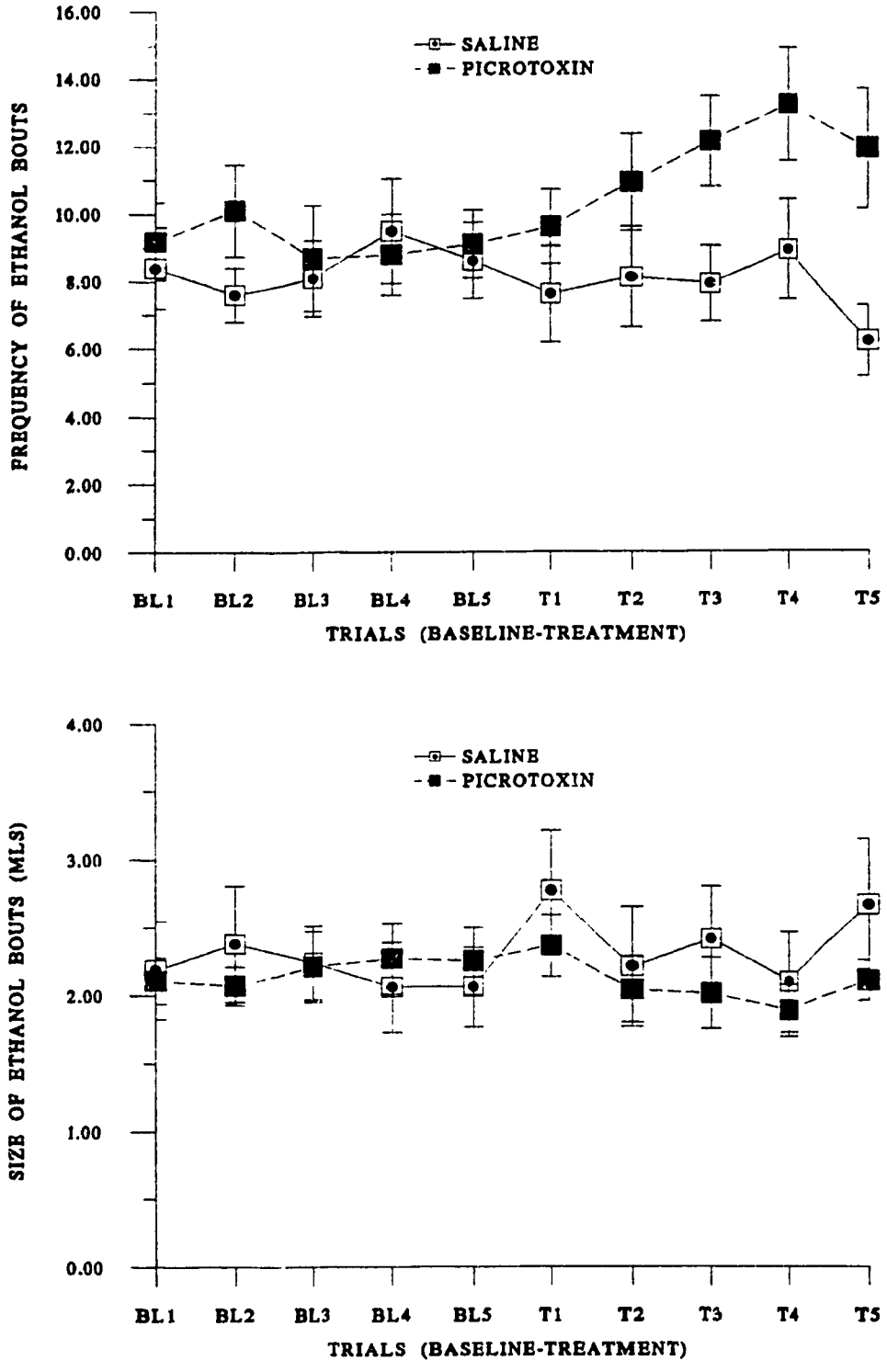

Figure 43. The effects of Picrotoxin on the frequency and size of ethanol bouts across baseline and treatment periods. Vertical lines represent S.E.M.

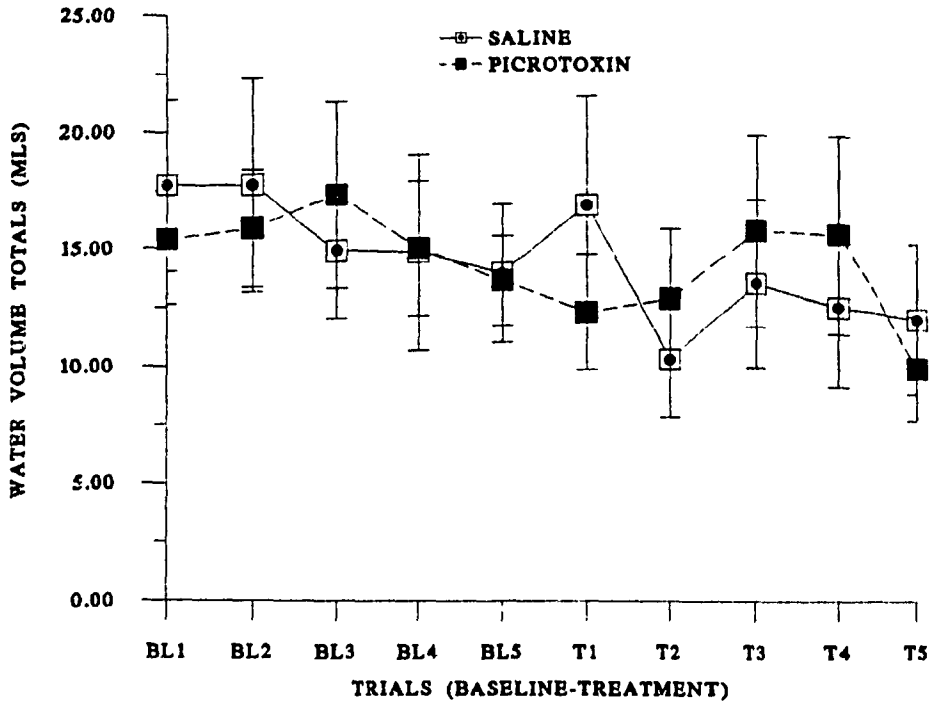

Figure 44. The effects of Picrotoxin on total water intake (mls) across baseline and treatment periods. Vertical lines represent S.E.M.

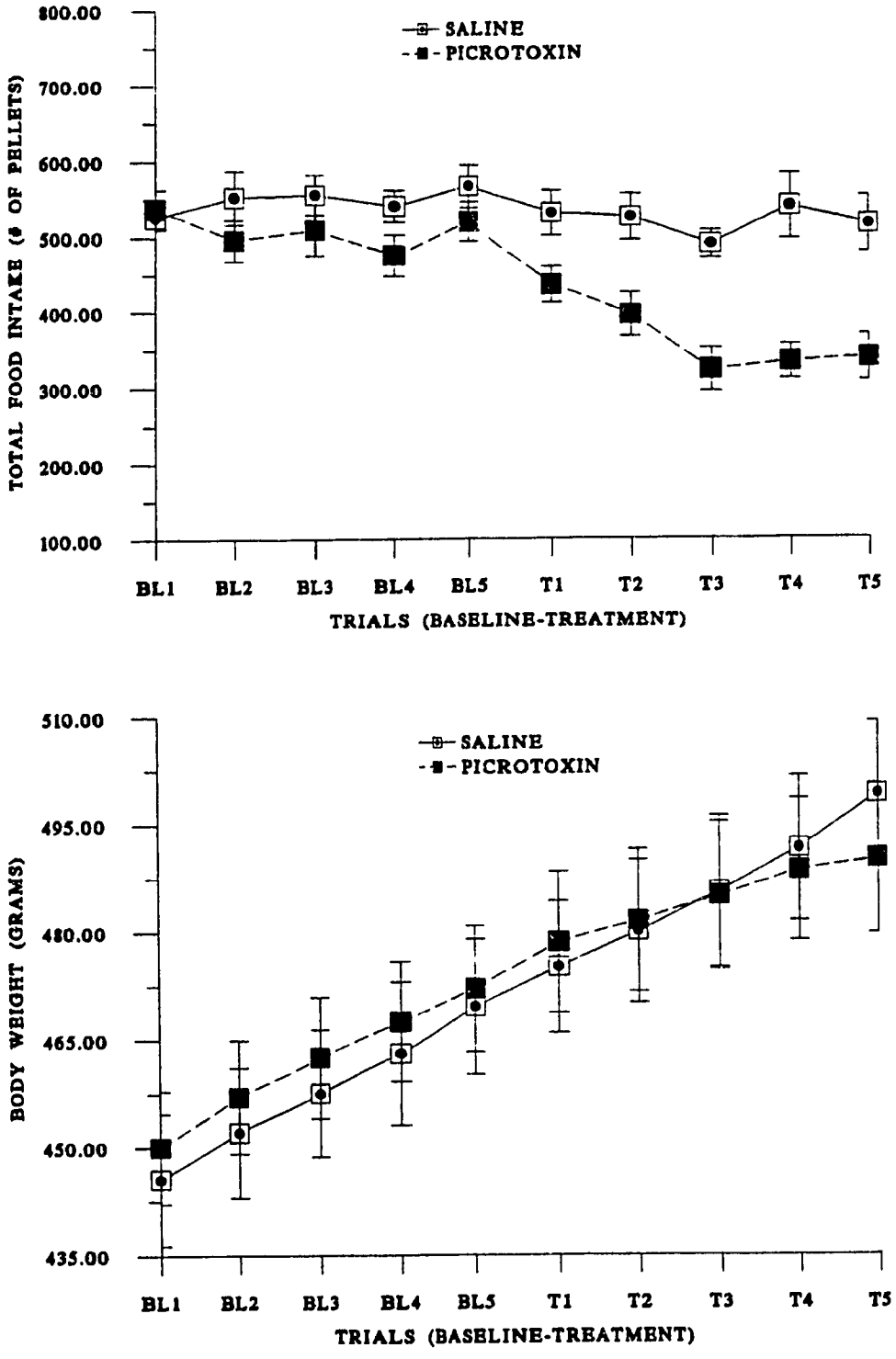

Figure 45. The effects of Picrotoxin on total food intake (pellets) and body weight (grams) values across baseline and treatment periods. Vertical lines represent S.E.M.

The anorexic effects of picrotoxin were specifically associated with significant decreases in the size [$F(1,18)=18.19, p<0.0005$] and duration [$F(1,18)=7.05, p<0.02$] of food bouts. These bout parameters are presented in Figure 46. In contrast, the frequency of food bouts was not observed to be significantly influenced by picrotoxin treatment [$F(1,18)=0.10, p<0.76$].

Temporal Pattern of Intake following Picrotoxin Administration

Although there were no significant effects on total ethanol intake, the increase in the frequency of ethanol bouts induced by picrotoxin suggested that temporal pattern of intake may reflect the change in the number of ethanol bouts. There was no significant effects of picrotoxin on the temporal pattern of intake. Specifically, analyses demonstrated that the interactions for drug x treatment phase [$F(2,36)=1.51, p<0.24$], temporal period x drug [$F(1,18)=0.51, p<0.48$] and treatment x temporal period x drug [$F(2,36)=0.05, p<0.96$] were not significant. These findings would suggest that the increase in the number of ethanol bouts described above may have been distributed fairly equally throughout the light cycle. The temporal pattern of ethanol intake for picrotoxin-treated subjects is presented in Figure 47.

The temporal pattern of water intake was similarly uninfluenced by the administration of Picrotoxin. Interactions at drug x treatment phase [$F(1,18)=0.001, p<0.97$], temporal period x drug [$F(1,18)=0.05, p<0.83$] and treatment x temporal period x group [$F(2,36)=1.55, p<0.23$] were not significant.

The temporal pattern of food intake for picrotoxin treated subjects is presented in Figure 48. Food intake was attenuated within all temporal periods. There were significant interactions between treatment phase and drug [$F(1,18)=1.99, p<0.0002$] as well as temporal period and drug [$F(1,18)=35.41, p<0.00001$].

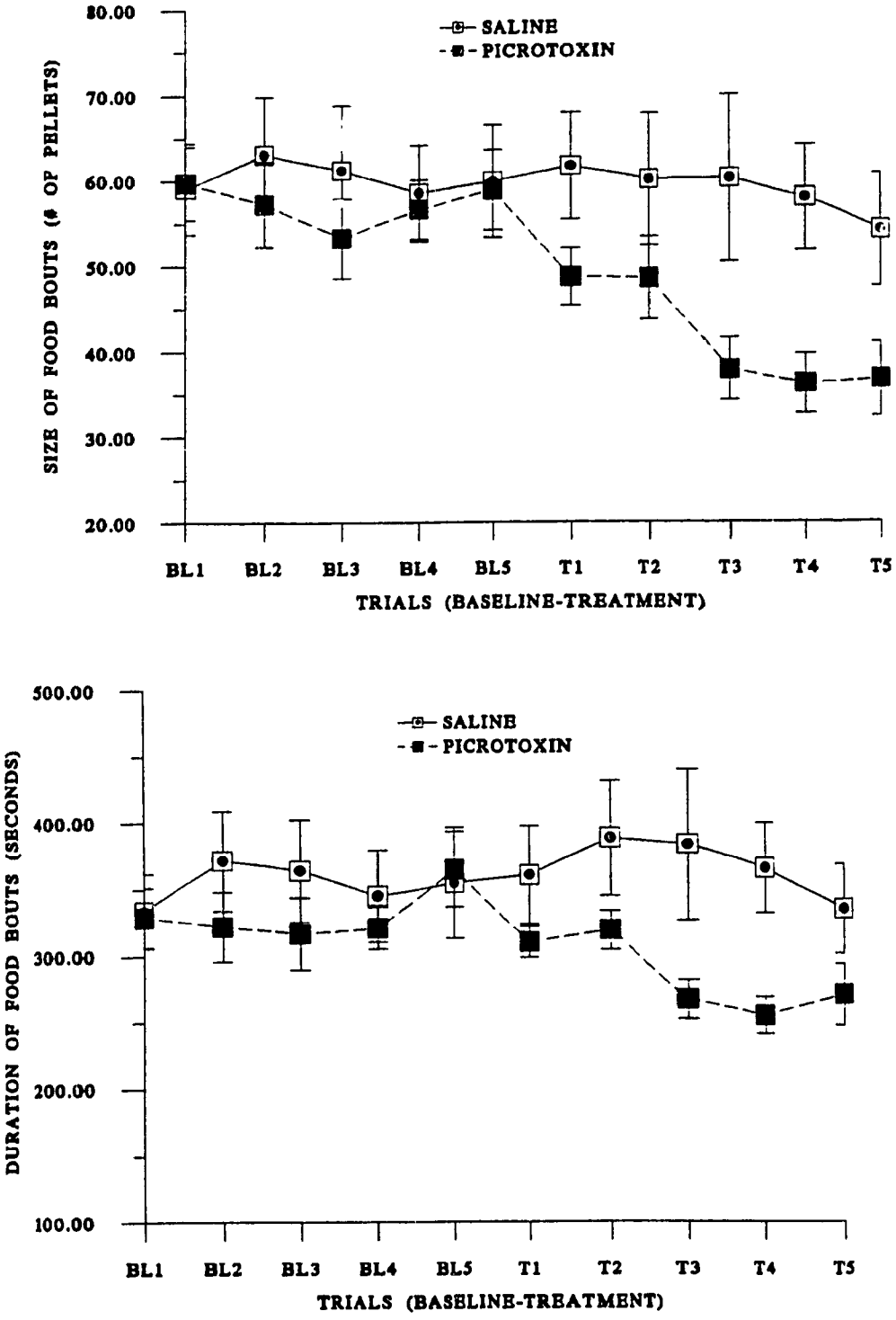

Figure 46. The effects of Picrotoxin on size (pellets) and duration (seconds) of food bouts across baseline and treatment periods. Vertical lines represent S.E.M.

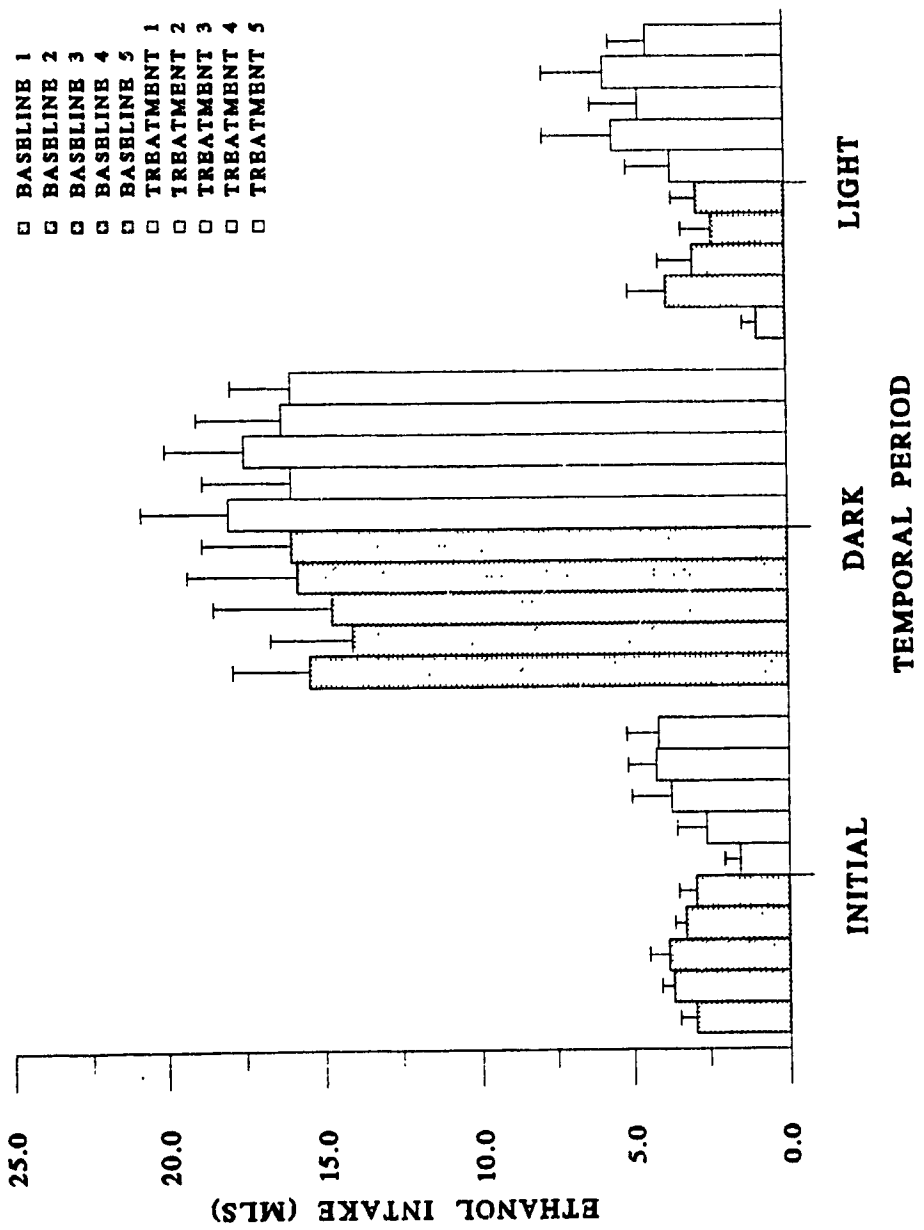

Figure 47. Temporal pattern of ethanol intake (mls) for Picrotoxin treated subjects across baseline and treatment periods. Vertical lines represent S.E.M.

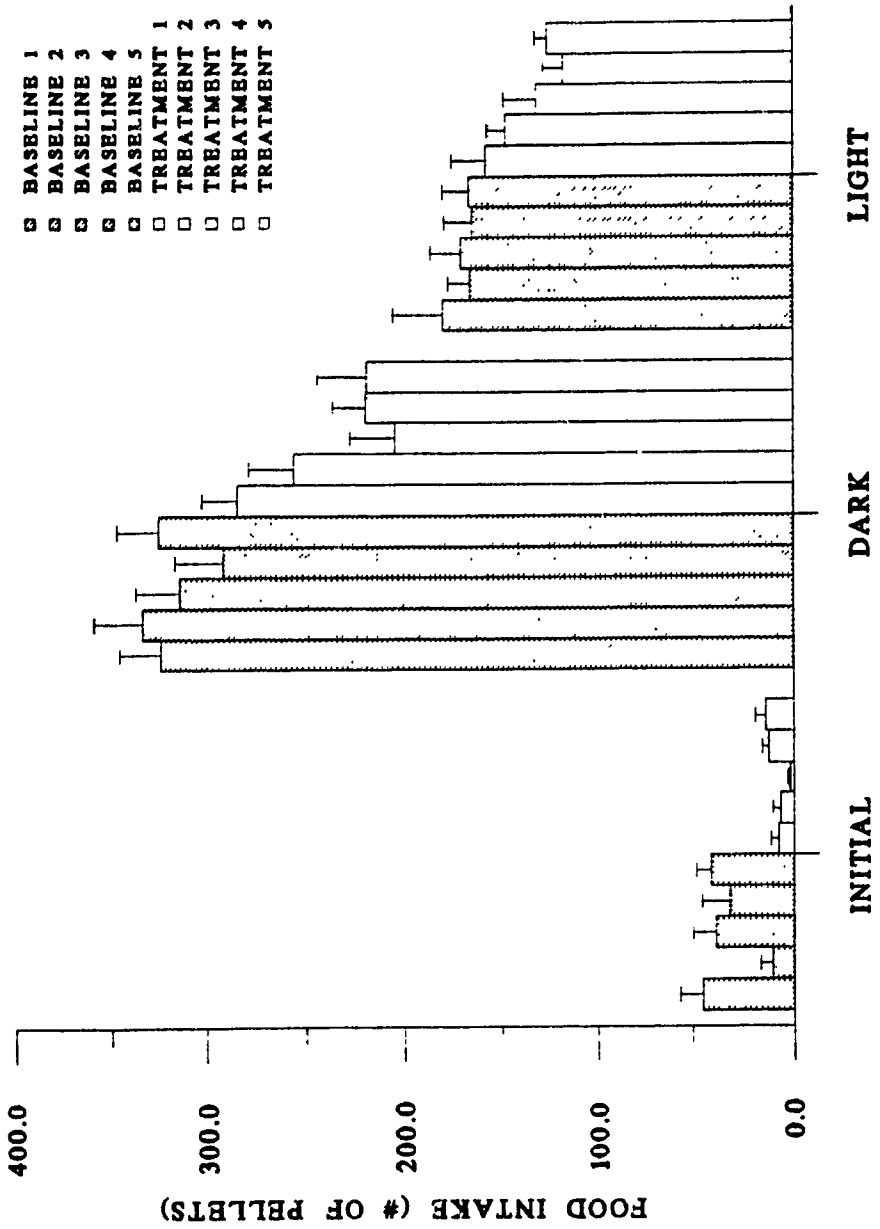

Figure 48. Temporal pattern of food intake (mls) for Picrotoxin treated subjects across baseline and treatment periods. Vertical lines represent S.E.M.

DISCUSSION

In contrast to the decrease in voluntary ethanol intake observed in experiment 4, picrotoxin in the present study failed to decrease ethanol intake. This finding would also appear to be inconsistent with a previous report in the literature which indicated that the picrotoxin ligand isopropylbicyclophosphate (IPPO) decreased the operant self-administration of ethanol (Rassnick, et al. 1993).

Interestingly, however, picrotoxin did exhibit a potent anorexic effect which was consistent with the changes in body weight observed in experiment 4. The decrease in total food intake appeared to be a specific function of a decrease in the size of food bouts.

The inconsistency in the effects of picrotoxin on ethanol intake, in the present thesis, is not easily explained. The observed effects of picrotoxin on ethanol intake reported in experiment 4, may have been indirect and possibly a function of an interaction with the drugs anorexic effects. However, there is no direct evidence to support of such a contention. More likely, the inconsistencies in the effects of picrotoxin may be a function of differences in the experimental environments. However, a significant factor may lie in the differential history of ethanol intake. In particular, out of a concern for the levels of ethanol intake the subjects of the present experiment were exposed to 4 days of forced choice in contrast to those of experiment 4.

Nonetheless, the absence of any effect on the rate of ethanol intake or the size of ethanol bouts, observed in the present study, would clearly suggest that the antagonism of the GABA-A receptor system is not sufficient to induce a reduction of ethanol intake.

EXPERIMENT 7

A MICROSTRUCTURAL ANALYSIS OF THE EFFECTS OF THE GABA-B AGONIST BACLOFEN ON VOLUNTARY ETHANOL INTAKE

In recent years reports in the literature has suggested that the GABA-B receptor may also play a role in the mediation of some of ethanol's behavioral effects (Allan, Harris 1989; Martz, Deitrich, Harris 1983). Moreover, it has been suggested that activation of the GABA-B receptor may be required for the expression of an ethanol induced enhancement of GABA-A receptor-chloride flux (Allan, Burnett, Harris 1991).

However, few data are available which support, in any consistent fashion, the notion of a major contribution of GABA-B receptors in the mediation of voluntary intake of ethanol. Just as an illustration, the GABA-B agonist baclofen has been reported to both increase (Smith, et al., 1992) or alternatively decrease (Daoust, Saligaut, Moore, Flipo, Boismare 1987) the maintenance of voluntary ethanol intake.

In order to clarify and expand our understanding of the role of GABA-B receptors in the regulation of voluntary ethanol intake, the present study examined the effects of baclofen on the microstructure and pattern of voluntary ethanol intake utilizing a maintenance paradigm.

Baclofen (beta-(Aminomethyl)-4-chlorobenzenepropanoic acid) is a GABA-B agonist (The Merck Index Online(SM)). When orally administered, baclofen is distributed throughout the body and has been reported to have a serum half-life of 2.5-4 hours ((c)1994 Amer.Soc.of Hosp. Pharmacist Inc). It was also suggested that only small amounts of the drug, take orally, cross the blood-brain barrier. However, in sufficiently large doses baclofen produces generalized CNS depression (e.g. sedation, somnolence, ataxia, respiratory and cardiovascular depression) ((c)1994 Amer.Soc.of Hosp. Pharmacist Inc).

METHOD

Subjects

Twenty male Long Evans new colony rats (Charles Rivers Canada Inc.) weighing 175 - 200 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 was available ad lib throughout the test period.

Drugs

Baclofen was dissolved in 0.9% NaCl and injected IP at a dose of 10 mg/kg b. wt. in a volume of 1 ml/kg. Saline was injected IP in a volume of 1 ml/kg. Ethanol solutions, with a concentration of 10% (v/v), were prepared from 95% ethanol mixed with tap water.

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 which dispensed 45-mg standard Bioserve pellets. The feeders were activated by the interruption of photo beams resulting from the placement of a rats head into a food cup. Each photo beam interruption resulted in a single pellet being dispensed. In addition, each chamber was equipped with two plastic drinking tubes fitted with steel ball bearing spouts.

All feeding and drinking behavior were monitored continuously over a 23-h period. During a daily 60-minute 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 thc bouts of feeding and drinking responses. A bout of activity was considered to have been 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 minutes 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 or drinking spouts, only those bouts consisting of more than five consecutive events were included in the analysis. Complete design specifications for the computer acquisition system have been previously detailed (Gill, Mundl, Cabilio & Amit 1988).

Procedure

Following a 7-day period of acclimatization to the operant boxes, the rats were presented, over 4 consecutive days, with a forced exposure to ethanol during which a 10% (v/v) ethanol solution was the sole fluid available.

Immediately following the period of forced ethanol exposure the rats were placed on a schedule of 10% ethanol presented in a free choice with water on alternate days. The position of the ethanol-filled tube was altered on successive days to avoid a position bias. Both tubes were filled with water on intervening days.

After a period of time, during which ethanol intake was permitted to stabilize, the temporal and structural pattern of food, water and ethanol intake were recorded in order to establish a baseline level of consumption. The baseline period consisted of 5 consecutive alternate day presentations of ethanol in a free choice with water. These 5 alternate day ethanol trials presented following the termination of the baseline period constituted the treatment period. It was during the treatment period that the effects of the GABA-B agonist baclofen were evaluated.

On those presentation days when ethanol was available, throughout the treatment period, animals received either an injection of baclofen 10 mg/kg I.P. (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 the experiment which demonstrated that 10 mg/kg of baclofen produced a significant increase in voluntary ethanol intake. Injections were

administered during the daily 60 min. computer shutdown which occurred 1 hr prior to the onset of the animal's dark cycle.

RESULTS

The effects of baclofen on total intake and bout parameters were examined for food, ethanol and water consumption across baseline and treatment phases. The data were analyzed through the use of multiple three way ANOVAs (the variables consisted of drug group with repeated measures across treatment phases and days). In order to further characterize the behavioral effects of baclofen, the temporal pattern of food, water and ethanol consumption was also examined. The temporal analyses were conducted using multiple 4 way ANOVAs (with the variables consisting of the drug group with repeated measures across treatment phases, days and temporal periods). The source of interactions within the factorial ANOVAs were established using tests of simple main effects and simple interactions.

Structural Pattern of Intake Following Baclofen Administration

The administration of the GABA-B agonist baclofen significantly increased both the intake of absolute ethanol [$F(1,18)=7.08$, $p<.0.02$] and the relative preference for ethanol [$F(1,18)=4.85$, $p<.0.041$] (Figure 49). Similarly, the volume of 10 % ethanol solution consumed was also increased [$F(1,18)=7.67$, $p<.0.013$].

The increase in total ethanol intake following baclofen administration was primarily attributable to an increase in the frequency of ethanol bouts. The frequency of ethanol bouts (Figure 50) increased significantly during the phase in which baclofen was administered [$F(1,18)=8.4$, $p<.0.0096$]. However, as may also be seen in Figure 50, the size of ethanol bouts did not change as a function of baclofen administration [$F(1,18)=1.0$, $p<0.34$]. Consistent with this finding, the level of absolute ethanol intake consumed per bout was not observed to be significantly influenced by baclofen treatment [$F(1,18)=0.63$, $p<.0.44$].

These effects on ethanol intake were nor accompanied by any significant changes in the rate at which ethanol was consumed during the bouts [$F(1,18)=1.73$, $p<0.21$].

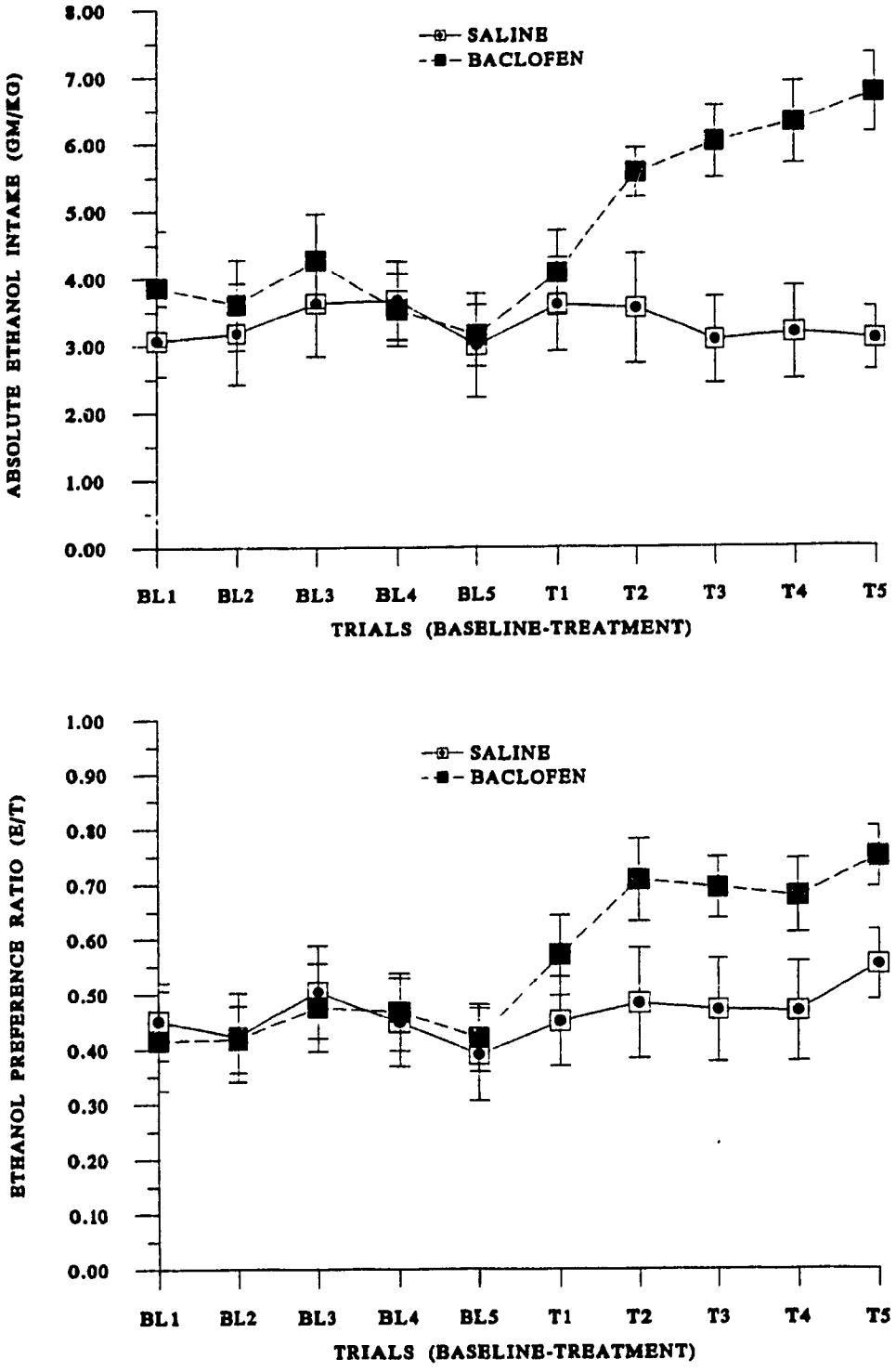

Figure 49. The effects of Baclofen on absolute ethanol intake (gm/kg) intake and preference ratios across baseline and treatment periods. Vertical lines represent S.E.M.

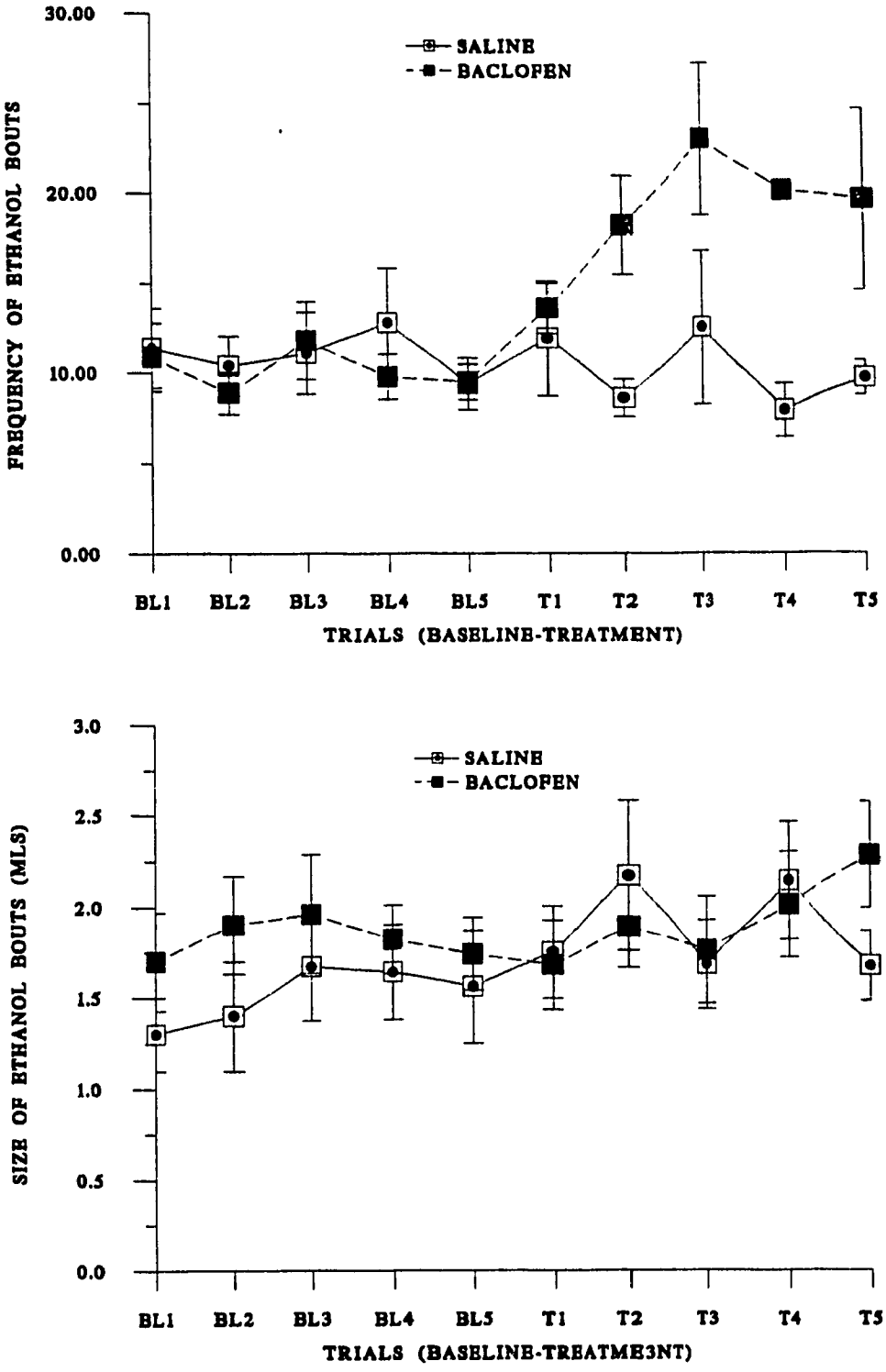

Figure 50. The effects of Baclofen on frequency and size of ethanol bouts (mls) across baseline and treatment periods. Vertical lines represent S.E.M.

In contrast to baclofen's influence on total ethanol intake, total water intake (Figure 51) did not change as a function of baclofen treatment [$F(1,18)=2.05$, $p<0.17$]. However, an analysis of the microstructure of water intake (Figure 51) revealed that the size of the water bouts were significantly reduced as a function baclofen treatment [$F(1,18)=7.46$, $p<0.02$]. The effects of baclofen were not evident following an examination of other bout parameters such as the frequency of water bouts [$F(1,18)=1.09$, $p<0.31$] or the rate of water intake [$F(1,18)=1.02$, $p<0.33$].

An analysis of the total fluid intake demonstrated a significant three-way interaction for group, treatment phase and days ($F(4,72)=3.91$, $p<0.0063$). The subsequent analysis of simple main effects and interactions revealed that baclofen treated subjects significantly increased their total fluid intake across treatment days [($F(4,72)=2.85$, $p<0.03$].

In the present study baclofen also exhibited a potent anorexic effect. Total food intake, as presented in Figure 52 was significantly reduced as a function of baclofen treatment [$F(1,18)=87.72$, $p<0.00001$]. Furthermore, as a consequence of the potent suppression of food intake, baclofen-treated rats did not exhibit the normal pattern of weight gain, which was characteristic of saline treated subjects during the treatment phase [$F(1,18)=10.05$, $p<0.006$].

An analysis of the microstructure of food intake, presented in Figure 52, suggested that the decrease in total food intake was primarily a function of a decrease in the size of food bouts [$F(1,18)=43.18$, $p<0.000001$]. Reflecting the decreased amount of food consumed during food bouts, the time spent eating within a bouts was decreased during the treatment period [$F(1,18)=6.34$, $p<0.022$]. The frequency of food bouts were not significantly influenced by baclofen administration [$F(1,18)=1.75$, $p<0.21$].

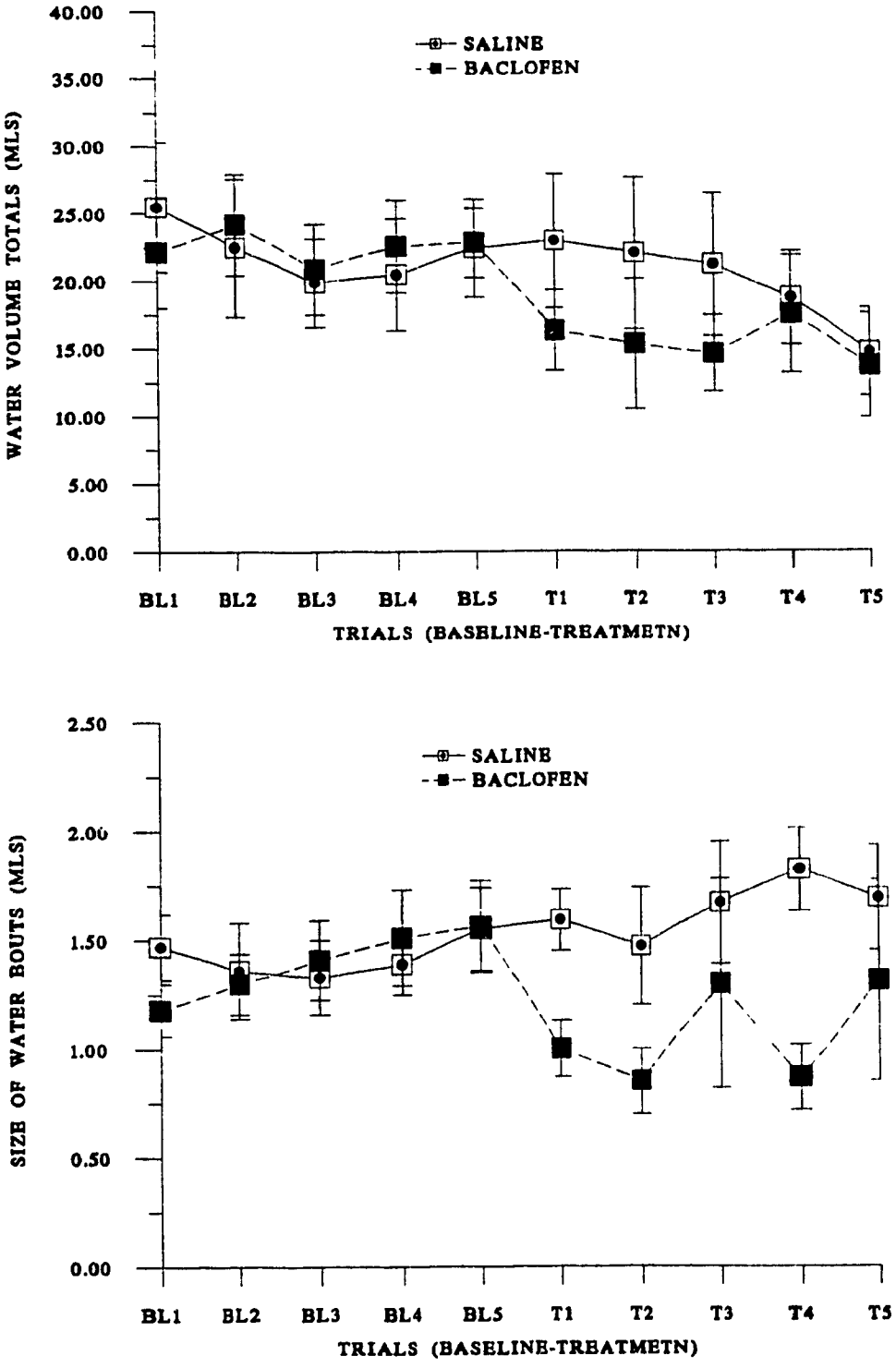

Figure 51. The effects of Baclofen on total water intake (mls) and size of water bouts (mls) across baseline and treatment periods. Vertical lines represent S.E.M.

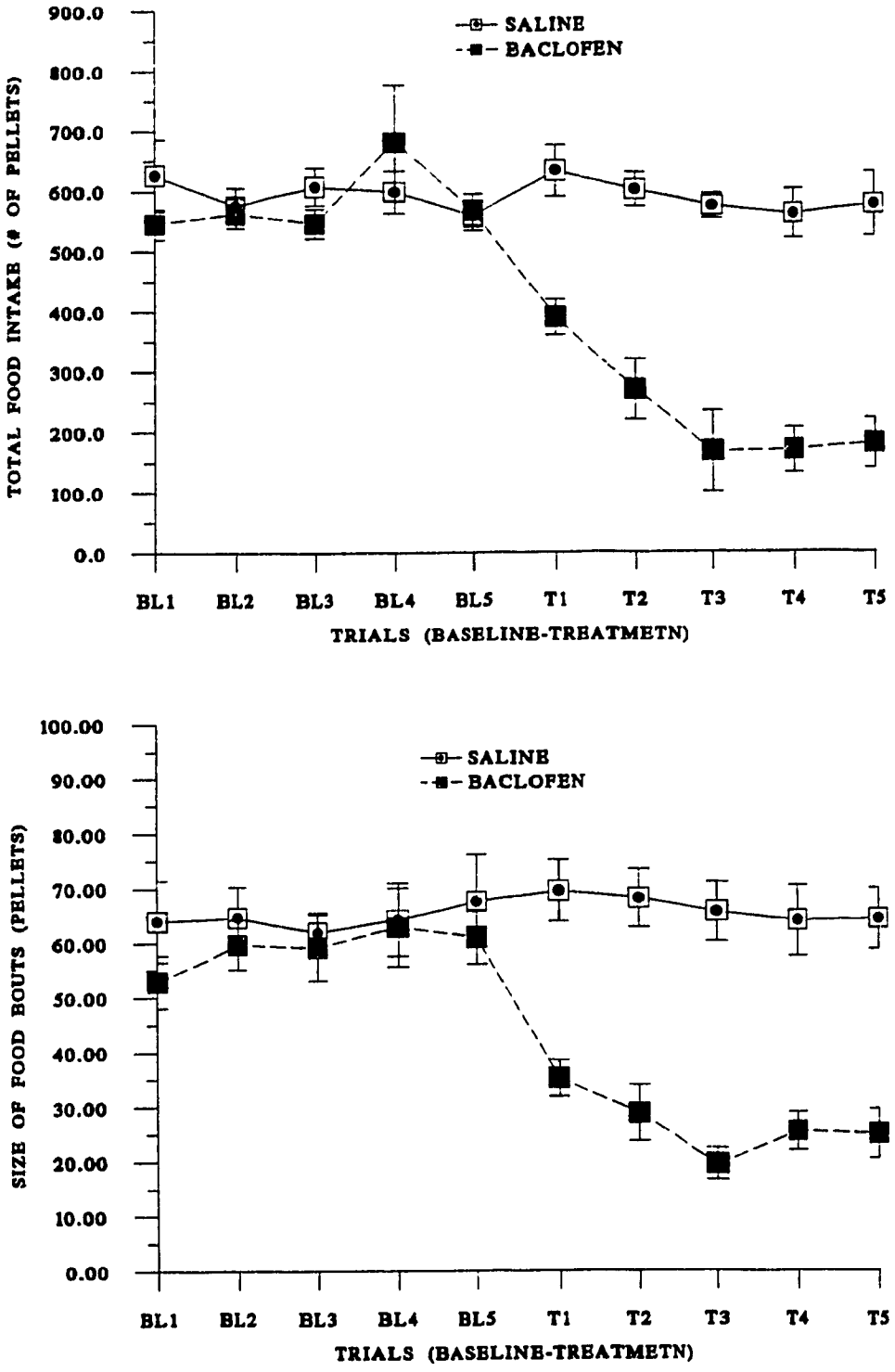

Figure 52. The effects of Baclofen on total food intake (pellets) and size of food bouts (pellets) across baseline and treatment periods. Vertical lines represent S.E.M.

Temporal Pattern of Intake following Baclofen Administration

In order to further characterize the pattern of consumatory behavior, temporal analyses were conducted for food, water and ethanol intake, across baseline and treatment phases .

The temporal pattern of ethanol intake for baclofen and saline treated subjects across treatment phases is presented in Figure 53. An analysis of the results revealed a significant three way interaction between drug group, treatment phases and temporal periods [$F(2,36)=17.96$, $p<0.0001$]. A test of simple effects and interactions indicated that ethanol intake was significantly reduced when compared to both baseline [$F(1,18)=24.19$, $p<0.0001$], and control levels [$F(1,18)=23.37$, $p<0.0002$], during the initial hour following baclofen administration.

In contrast to this initial suppression, ethanol intake was subsequently increased from baseline and control levels, as a function of baclofen treatment, during both the dark [$F(1,18)=24.75$, $p<0.00001$; $F(1,18)=15.48$, $p<0.0015$] and light portions of the day/night cycle [$F(1,18)=57.45$, $p<0.0001$; $F(1,18)=10.28$, $p<0.005$].

The effects of baclofen on ethanol intake did not appear to be a phenomenon which generalized to other fluids. Consistent with the absence of any effect on total water intake, baclofen failed to effect any changes in the temporal pattern of water intake.

While the observed effects on the temporal pattern of ethanol intake described above were biphasic in nature, the directionality of baclofen's effects on food intake, as presented in Figure 54, were consistent across the temporal periods. Temporal analysis indicated a significant three way interaction between drug group, treatment phases and temporal periods [$F(2,36)=19.94$, $p<0.0001$]. Specifically, an analysis of simple main effects and interactions demonstrated that food intake was reduced from both baseline and control levels throughout the initial [$F(1,18)=9.32$, $p<0.0068$; $F(1,18)=20.44$, $p<0.0003$], dark [$F(1,18)=117.06$, $p<0.00001$; $F(1,18)=46.9$, $p<0.00001$] and light portions [$F(1,18)=20.87$, $p<0.0002$; $F(1,18)=21.06$, $p<0.0002$] of the day/night cycle.

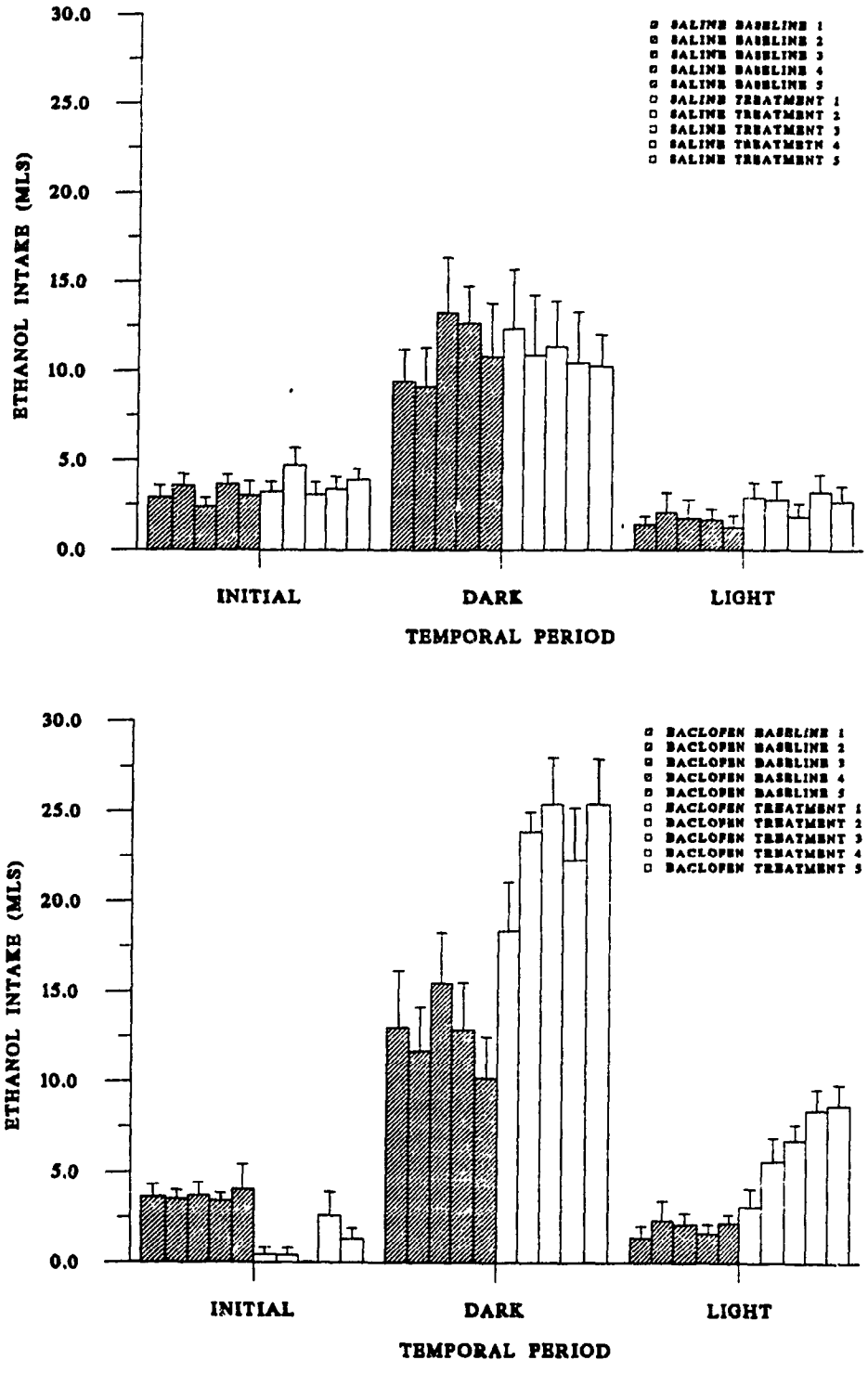

Figure 53. The effects of the administration of saline (upper figure) and Baclofen (lower figure) on temporal pattern of ethanol intake across baseline and treatment periods. Vertical lines represent S.E.M.

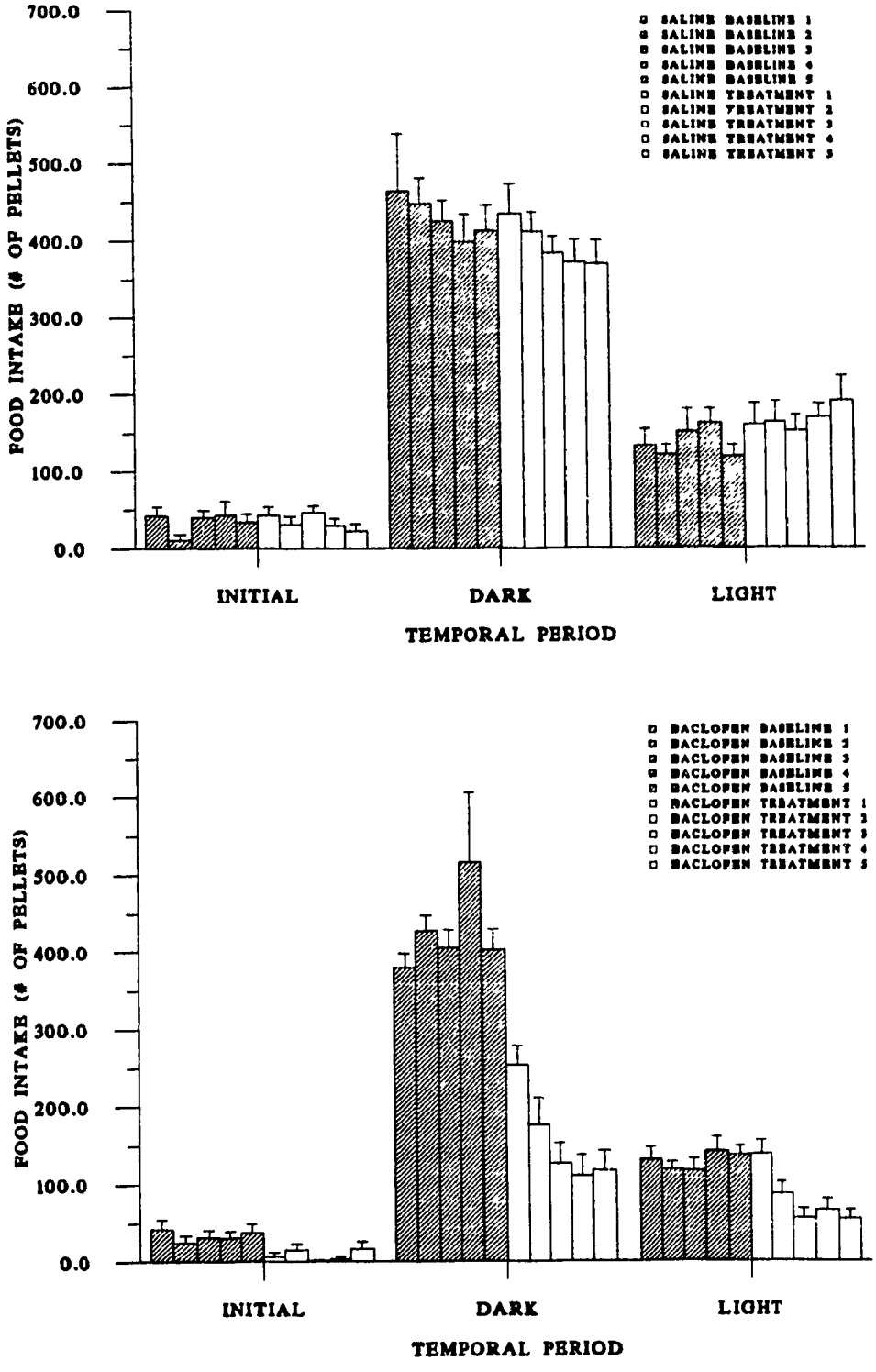

Figure 54. The effects of the administration of saline (upper figure) and Baclofen (lower figure) on temporal pattern of food intake across baseline and treatment periods. Vertical lines represent S.E.M.

DISCUSSION

Consistent with a previous report in the literature (Smith, et al., 1992), the present study found that baclofen increased voluntary ethanol intake. Microstructural analysis of ethanol intake revealed that the changes in ethanol intake induced by baclofen were exclusively a function of an increase in the frequency of ethanol bouts.

In addition, the present findings would suggest that the effects of baclofen are not specific to ethanol. This finding is reflected by the observed increase in total fluid intake during the treatment period. These findings would support a previous report in the literature which suggested that baclofen administration resulted in generalized fluid effects, as demonstrated by an increase in total fluid intake (Smith, et al., 1992).

Interestingly, the temporal analysis of consumatory behavior demonstrated a clear dissociation in the effects of baclofen on food and ethanol intake throughout the treatment period. Food and ethanol intake were both observed to be suppressed during the first hour following the administration of baclofen. However, while food intake remained suppressed throughout the duration of the day/night cycle, ethanol intake, while initially suppressed, increased above baseline values throughout the dark and light portions of the day/night cycle.

Thus, the lack of specificity of the effects of baclofen observed in the present study would suggest that the GABA-B receptor system may not play a central role in the mediation of voluntary ethanol intake.

GENERAL DISCUSSION PHASE 2

Consistent with reports in the literature (Smith, Robidoux, & Amit 1992), the present thesis provided evidence that the GABA-A receptor system may play a significant role in the mediation of voluntary ethanol intake. However, the present examination suggested that the administration of a GABA-A agonist and antagonist do not inevitably produce bi-directional effects on ethanol intake or preference, despite their inverse effects on the GABA-A receptor Cl^- ion flux.

THIP administration produced a specific increase in the self-administration of ethanol within both acquisition and maintenance paradigms. This finding is consistent with reports in the literature, regarding the influence of GABA-A agonists (Smith, Robidoux, & Amit 1992; Tomkins, Sellers, & Fletcher 1994). Critically, a microstructural analysis suggested that the effects of THIP on ethanol intake were primarily, although not exclusively, a function of an increase in the size of ethanol bouts. Interestingly, these effects upon the size of ethanol bouts were comparable with the changes observed during the normal acquisition of ethanol intake in Phase 1 of the present thesis. This finding would imply that processes mediating the increase in ethanol intake observed following the administration of a GABA-A agonist, are consistent with those observed in the regulation of ethanol intake during the normal acquisition of voluntary ethanol intake in pharmacologically unmanipulated animals.

However, in contrast to the effects of the GABA-A agonist THIP, the effects of picrotoxin on ethanol intake appeared to be inconsistent and paradigm specific. The results of the present thesis suggested that the antagonism of the GABA-A receptor system was not sufficient to induce a change in voluntary intake of ethanol. The inconsistency in the effects of picrotoxin on ethanol intake observed in the present thesis may have been a function of differences in the experimental environments or history of ethanol intake in the rats used.

Contrary to the specificity of the GABA-A agonist THIP, administration of the GABA-B agonist baclofen resulted in a nonspecific increase in fluid intake. The lack of

specificity regarding baclofen's action is consistent with a previous report in the literature (Smith, Robidoux, & Amit 1992).

A microstructural analysis of the effects of baclofen on ethanol intake suggested that the increase in ethanol intake was exclusively a function of an increase in the frequency of ethanol bouts. This effect on ethanol bouts was not consistent with the processes which appear to regulate ethanol intake in pharmacologically unmanipulated animals, as observed in Phase 1 of the present thesis. Thus, the lack of fluid specificity coupled with the observed effects upon the frequency of ethanol bouts suggested that the GABA-B receptor system may not play a direct role in the mediation of normal voluntary ethanol intake.

It is evident that THIP and baclofen influenced both food and ethanol intake. However, there appeared to be a relatively clear dissociation between the effects of these pharmacological agents on the microstructural pattern of ethanol and food intake. The. qualitatively different patterns would suggest that differential mechanisms underlie the effects of these manipulations on food and ethanol observed in the present study.

While it is argued that the microstructural pattern of food and ethanol intake are differentially influenced by GABA manipulations, there is an alternative interpretation of the results which must be considered. In particular, this interpretation is based upon the common notion that ethanol may be consumed for its caloric value (Dole, Ho, & Gentry, 1985; Lester, & Freed, 1973).

This notion would suggest that the increase in ethanol intake observed following GABAergic manipulations in the context of the present findings could be interpreted in terms of a compensatory mechanism resulting from the loss of calories following a GABAergic induced decrease in food intake. In essence, one source of food calories is substituted for another. This interpretation would by necessity suggest that the GABAergic manipulations resulted in a decrease in the perceived palatability or rewarding value of a particular food type, precipitating as a consequence an increase in preference for ethanol, a source of liquid calories.

However, the present thesis provided data which clearly fail to indicate that a manipulation which decreases food intake necessarily results in a compensatory increase in ethanol intake. For example, all three GABAergic manipulations utilized in the present study were demonstrated to possess significant anorectic properties. However, the pharmacological agents were characterized by differential effects on total ethanol intake. Most significantly, the administration of picrotoxin produced a reduction in food intake without any significant effect on ethanol intake.

Similarly, reports are found in the literature which are inconsistent with a calorie based theory of ethanol consumption. For example, 5-HT reuptake inhibitors, such as Sertraline or Zimeldine, have been demonstrated to produce significant decreases 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 fact reduced the intake of ethanol (Gill, Filion, & Amit, 1988; Gill & Amit 1989).

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.

In summary, the results of Phase 2 of the present thesis suggest that the potentiation of the GABA-A receptor system will enhance the voluntary intake of ethanol. Furthermore, the behavioral mechanisms mediating the effects of the GABA-A agonist THIP appear to be consistent with those which normally act to regulate ethanol intake in pharmacologically unmanipulated animals. However, the inverse pharmacological manipulation involving the antagonism of the GABA-A receptor system was not sufficient to reduce ethanol intake.

GENERAL DISCUSSION

The present thesis represents an attempt to demonstrate the utility of the microstructural approach in providing fine detail and critical information concerning the nature of the behavioral processes mediating voluntary ethanol intake. Furthermore, the microstructural approach provided the means to differentiate the apparently similar effects of divergent manipulations on ethanol intake. By way of illustrating this point, the present thesis has demonstrated that specific increases in ethanol intake induced by behavioral and pharmacological manipulations appear to be primarily mediated by increases in the size of individual ethanol bouts. Clearly, the increases in voluntary ethanol intake, observed as a function of the use of different acquisition techniques as well as GABA-A agonist administration, were found quite consistently to produce increases in ethanol bout size.

In contrast, manipulations which resulted in non-specific fluid effects, such as general increases in total fluid intake, were not observed to influence ethanol bout size. For example, the effects of the GABA-B agonist baclofen, which increased the total intake of fluid, was characterized exclusively by an increase in the frequency but not the size of ethanol bouts.

The relationship, described above, between the changes observed in bout size and capacity of manipulations to specifically influence ethanol intake are supported by the outcome of other studies reported in the literature using a microstructural approach (Gill et al. 1987; Higgins et al. 1992). For example, the analysis of the influence of serotonergic reuptake inhibitors on consumatory behavior revealed nonspecific fluid effects on ethanol intake (e.g. Gill & Amit 1989; Montgomery, & Burton 1986). In fact most studies which have examined serotonergic reuptake inhibitors, such as sertraline (Higgens et al. 1992), zimeldine (Gill et al. 1987) and dexfenfluramine (Higgens et al. 1992) have demonstrated that these agents decreased ethanol intake through a decrease in the frequency of bouts. Only one inconsistent finding suggested that the serotonin reuptake inhibitor sertraline decreased the size of ethanol bouts (Gill et al. 1988).

Nonetheless, it is important to note that while the present findings suggested that manipulations which produced specific effects on ethanol intake did so as a function of changes in the size of ethanol bouts, the precise mechanisms mediating these changes remain uncertain. However, it has been suggested that the rate and potentially the magnitude of the acquisition of voluntary ethanol intake may be a function of the extent to which a rat is able to make an association between the drinking behavior and the perception of ethanol's central reinforcing properties (Amit, & Smith, 1991; Smith, Segal, & Amit, 1989; Boyle, Smith & Amit 1992). Manipulations which enhance this association would be predicted to facilitate the acquisition of ethanol intake. Thus, it could be argued that the acquisition techniques, used in Phase 1 of the present thesis, and the administration of THIP, in Phase 2, both increased the size of ethanol bouts through an enhancement of the association of the drinking behavior with ethanol's central pharmacological effects.

Special Nature of Ethanol Reinforcement

The focus on the significance of the association between the behavior of drinking and ethanol's central reinforcing properties stems from the special nature of ethanol reinforcement. As it has been suggested with food reinforcement (Wise 1987), ethanol reinforcement, as it is traditionally studied, is qualitatively different from the reinforcement derived from other drugs of abuse. However, the qualitative nature of ethanol reinforcement is to a large extent a function of the route of self-administration. In animal research, ethanol is primarily self-administered orally. In contrast, research examining the self-administration of most other drugs of abuse utilize techniques which are characterized by a more rapid and direct delivery of drug. For example, intravenous self-administration will introduce a drug directly into the bloodstream potentially resulting in a very rapid onset of central pharmacological effects.

Oral ethanol self-administration, perhaps most significantly, has been characterized by a significant delay in the onset of this drug's central pharmacological effects. In this regard, microdialysis revealed that the latency to the onset of detectable brain levels of

ethanol following gastric intubation in Wistar rats was approximately 4 min., with maximal level occurring at 30 min. (Nurmi, Kiianmaa & Sinclair 1994).

The significance of this particular latency is emphasized by the observation that, from Experiments 1 and 5 of the present thesis, the average duration of an ethanol bout was approximately 60 to 80 seconds. This would imply that the average ethanol bout was terminated prior to the onset of detectable and pharmacologically meaningful brain levels of ethanol. It would appear therefore, that the acquisition of ethanol intake requires that an association be formed between the immediate consequence of ethanol intake, namely the presentation of a fluid with specific taste and smell, for example, and the subsequent perception of ethanol's central pharmacological effects.

The functional significance of this association, which results from the temporal dissociation between the drinking behavior and the experience of ethanol's central pharmacological effects has been demonstrated with Sprague-Dawley (Rowland & Barnett 1992) and ethanol preferring P rats (Rowland & Morian 1994) in a restricted access paradigm. Specifically, when experienced animals from both of these strains had been deprived of the post ingestive pharmacological effects of ethanol during ethanol consumption, as a function of an open gastric fistula, one failed to observe any compensatory changes in ethanol intake across an intermediate time period (Rowland & Morian 1994; Rowland & Barnett 1992).

These findings would suggested that the temporal dissociation between the performance of the drinking behavior and subsequent perception of reinforcement must have resulted in the sensorial properties of ethanol (e.g. taste etc.) becoming potent secondary reinforcers. The question remains, however, as to how the divergent acquisition techniques observed in Phase 1 of the present thesis and the administration of a GABA-A agonist (in Phase 2) can both act to enhance this association and consequently increase ethanol intake?

Ethanol Acquisition: The Role of Aversive Influences and Conditioned Stimuli

Secondary only to the central role of ethanol's pharmacological effects, one factor which has been shown to influence the acquisition of ethanol intake has been taste. An effect of taste factors on ethanol ingestion is perhaps not surprising. It has been known for many years that consumatory behavior in mammals is influenced by the predisposition towards sweet substances which suggest the possession of caloric value, and inversely away from substances which are bitter and might suggest plant poison (Garcia & Hankins 1975). It is argued in the present discussion that while the influence of taste on ethanol intake is potentially significant, it is indirect and modulatory in nature.

Recently, the significance of taste issues in the study of ethanol intake have become manifest in research which suggested that individual differences in saccharin (a non-nutritive sweetener) preference were positively correlated with preference for ethanol (Overstreet, Kampov-Polevoy, Rezvani, Murrelle, Halikas, & Janowsky 1993; Sinclair, Kampov-Polevoy, Stewart, & Li 1992). Similarly, it has been demonstrated that differences in saccharin preference may be related to the rats initial selection of ethanol (Kampov-Polevoy, Kasheffskaya, Sinclair 1990).These data would imply that sensitivity to the taste of ethanol (i.e. preference for sweet tastes) may exert a degree of control over its intake.

Similarly, there are also data showing that taste factors may exert an inhibitory control over the consumption of ethanol. Several recent studies have provided evidence to suggest that the taste of ethanol changes with increases in its concentration. It has been proposed that while low concentrations of ethanol are perceived as "sweet by animals", concentrations of ethanol above 5% are perceived as being both "sweet and bitter" (Kiefer, Bice, Orr, & Dopp 1990; Bice, Kiefer, & Elder 1992; DiLorenzo, Kiefer, Rice & Garcia 1986). It has often been suggested that the unwillingness of the rat to consume higher concentrations of ethanol may be a function of the innate aversion to the bitter sensory properties of the ethanol solutions (Kahn & Stellar 1960; Erikson 1968; Myers 1968). In

point of fact, manipulations which altered the influence of factors such as taste are clearly and consistently associated with changes in ethanol intake (Lester 1966; Royer 1972; Bice and Kiefer 1990). For example, when the sense of taste is dulled, via anesthesia, ethanol intake has been observed to increase (Lester 1966; Royer 1972). Furthermore, there are data to suggest that the expression of voluntary ethanol intake obtained as a function of selective breeding may be a function of differences related to taste (Bice and Kiefer 1990). Specifically, differences in the level of ethanol intake obtained with the selectively bred lines of preferring (P) and non-preferring (NP) rats have been associated with differences in the habituation to the aversive taste of ethanol (Bice and Kiefer 1990). NP rats, in contrast to P rats, maintain very low levels of ethanol intake and are characterized by the failure to exhibit an habituation to the aversive taste of ethanol.

Therefore, these findings would support the conclusion that taste factors would exert regulatory control of ethanol intake. However, there is also evidence to suggest that the influence of taste factors such as those described above may, in fact, be indirect. While manipulation of the aversive taste of ethanol has been demonstrated to influence ethanol intake, there is evidence that the reactivity to the taste of ethanol does not always correlate with subsequent oral ethanol consumption (Bice, Kiefer, & Elder 1992). Furthermore, the authors (Bice et al. 1992) concluded that taste factors may be reflective of processes different from those that regulate ethanol intake.

The literature on feeding provides us with an example of the manner in which taste may exert an influence on behavior secondary to that of reinforcement. It has been demonstrated that while food deprived animals are attracted to different types of sweet solutions, such as those containing sucrose and saccharin, the maintenance of intake is dependent on the post-ingestive effects of these solutions. Specifically, food deprived rats have been shown to increase their consumption of sucrose, while in contrast, their intake of the non-nutritive sweetener saccharin extinguished (LeMagnen 1954).

Similarly, it is suggested that while taste factors related to ethanol can exert inhibitory effects on ethanol consumption, this inhibitory influence is primarily expressed in the absence of ethanol's central pharmacological effects. It is argued that once the animal experiences ethanol's central reinforcing properties as a consequence of drinking, the taste of the solution is transformed from that of a taste signal and an inhibitor of ethanol intake to a stimulus which signals the imminent onset of ethanol's central effects. Therefore, it appears that through conditioning, the taste of the ethanol solution acquires the status of a potent secondary reinforcer.

Furthermore, manipulations such as the traditional acquisition techniques utilized in Phase 1 of the present thesis are thought to increase ethanol intake through both a reduction in the influence of ethanol's aversive taste and through the pairing of the taste stimuli with ethanol's central effects. It would appear that a critical function of divergent induction techniques is the maintenance of sufficient levels of ethanol consumption to increase the probability of an animal experiencing ethanol's central pharmacological properties. In this manner, manipulations of taste potentially act to facilitate the association between the intake of ethanol and its subsequently experienced reinforcing properties.

This suggestion is, in fact, consistent with what was observed in experiment 1 of the present thesis. As the concentration of ethanol was increased from 2 to 6%, the combination of the size of the ethanol bouts and the low concentration of the available ethanol resulted in absolute levels of ethanol intake which were below that known to produce significant blood levels or central effects (Gill et al. 1986). In the absence of significant central ethanol effects, it is argued that intake was subject primarily to the influence of palatability of the solution. The observed outcome was a gradual decrease in total ethanol intake and preference, as the concentration of ethanol increased and its palatability decreased.

The gradual increase in ethanol concentrations from 2%, however, potentially acted to habituate the animals to the inhibitory influence of ethanol taste factors, thereby preserving

the ethanol bout size during the presentation of ethanol concentrations up to 6%. As a result, when the ethanol concentrations were increased above 6%, the absolute level of ethanol consumed increased significantly. It would appear that a comparable effect is differentially achieved by the adulteration of ethanol solutions with sucrose in the sucrose fading technique (e.g. Samson 1986; Grant & Samson 1985). In this manner the traditional acquisition paradigm appeared to increase the probability of animals experiencing ethanol's central pharmacological properties by preserving the ethanol bout size.

Consequently, as the ethanol concentrations were increased above 6%, the conditioned association of the taste of the ethanol solution (and presumably olfactory stimulation) with its subsequent pharmacological effects was established and enhanced. Accordingly, ethanol intake was observed to increase as the ethanol concentrations were increased from 6 to 10%.

The enhanced levels of ethanol intake observed in the present thesis following forced ethanol exposure (Experiment 2 and 3) could be similarly interpreted in terms of the influence of multiple factors relating to taste. In particular, exposure to ethanol, as a sole source of fluid, seems to result in the consumption of a concentration of ethanol which would otherwise be avoided in naive rats (Erikson 1968; Myers 1968). The implications of these observations were several fold. First, the forced exposure may have potentially speeded up and magnified the process of habituation to ethanol's aversive properties, a process which normally occurred to some degree during the exposure to ethanol (e.g. Kiefer & Dopp 1989). Secondly, and perhaps most importantly, the forced consumption of ethanol provided the opportunity for the animal to consume ethanol in large quantities and at concentrations which potentially produce significant central pharmacological effects. The coupling of these factors may explain the higher levels of intake subsequently observed during free choice ethanol intake than are generally reported in the same strain of animals using traditional preference paradigms (experiment 1 for example).

It would appear that the divergent induction techniques used to induce animals to consume greater amounts of ethanol do so indirectly by the conditioned association of ethanol intake, with all of its accompanying stimuli such as taste, to its central reinforcing effects. A critical element in this process is the induction of sufficient amounts ethanol intake to produce significant pharmacological effects. In part, this is achieved through a reduction in the influence of ethanol's aversive taste.

GABA-A Agonists: Potentiating the Saliency of Ethanol's Reinforcing Effects

While it has not been demonstrated that GABA-A agonists altered the perception of ethanol's sensorial properties such as taste, it has been argued that these pharmacological agents may act to increase ethanol intake through a more direct enhancement of the association between drinking behavior and ethanol's central pharmacological effects (Amit, & Smith, 1991; Smith, Segal, & Amit, 1989).

In the present thesis, the administration of the GABA-A agonist THIP resulted in the maintenance of large ethanol bouts throughout an acquisition procedure. It appeared that THIP administration eliminated both the decrease in ethanol intake and bout size which normally characterize an ethanol acquisition curve. Across the presentation of the lower ethanol concentrations (2-6%) THIP treated animals maintained an average ethanol bout size (approximately 1.5 mls) which was only achieved by animals in a traditional acquisition paradigm during the presentation of much higher ethanol concentrations. The changes in the microstructure of ethanol intake induced by THIP may be indicative of a THIP-induced alteration of the stimulus properties of ethanol.

It is not evident, at this time, that THIP enhanced ethanol intake through the potentiation of those processes which directly mediated ethanol's reinforcing properties. However, it has been proposed that GABA-A agonists may have, in fact, acted to facilitate the acquisition of voluntary ethanol intake as a function of the potentiation of ethanol induced intoxication (Amit, & Smith, 1991; Smith, Segal, & Amit, 1989). Clearly, as suggested earlier, GABA agonists potentiated the intoxicating effects of ethanol as

assessed, for example, through the measurement of sleep time and tilted plane performance (Martz, Deitrich & Harris 1983; Liljequist & Engel 1982; Hakkinen & Kulonen 1976; Cott, Carlsson, Engel, & Lindqvist 1976; Martz, Deitrich & Harris 1983; Allan & Harris 1989; Martz, Deitrich & Harris 1983).

It has been suggested that ethanol may possess properties, such as its physical intoxicating effects, which are closely associated with its reinforcing effects (Amit, & Smith, 1991; Smith, Segal, & Amit, 1989). It was suggested that these central properties are perceived by the animals as introceptive cues and function to enhance the association between ethanol consumption and subsequent reinforcement. The magnitude of these perceived cues have been suggested to have an influence on the extent to which animals will acquire the ethanol drinking behavior. The GABAergic manipulations which result in a potentiation of ethanol's intoxication (e.g. Martz, Deitrich & Harris 1983; Liljequist & Engel 1982; Hakkinen & Kulonen 1976; Cott, Carlsson, Engel, & Lindqvist 1976) would, therefore, act to enhance the discriminative stimuli associated with ethanol. In this manner, the GABA-A agonists may potentially increase the saliency of ethanol's central effects and facilitate the acquisition of ethanol drinking.

The present thesis and the data collected in this laboratory generally supported this interpretation (Amit, & Smith, 1991; Smith, Segal, & Amit, 1989) of the role of GABA-A receptor system in voluntary ethanol intake. It was suggested in an earlier discussion, that throughout the presentation of concentrations below 6% in a traditional acquisition paradigm, ethanol intake was predominantly driven by taste factors due to the absence of the influence of central pharmacological effects. The data in the present thesis would support the notion that the administration of THIP reduced the threshold concentration at which ethanol's central reinforcing effects were perceived, as a function of an increase in the saliency of ethanol's pharmacological signals. In this manner, THIP administration would act to enhance the conditioned association between ethanol intake and ethanol's central effects. This shift in the perception of ethanol's pharmacological effects to the left,

may potentially account for the THIP induced increases in ethanol intake observed during both the acquisition and maintenance paradigms in the present thesis.

Summary and Conclusions

It has been suggested that traditional research paradigms lack the behavioral resolution necessary to differentiate the processes which regulate voluntary ethanol intake. The microstructural data presented in this thesis have provided a high resolution descriptive analysis of the processes which act to regulate ethanol intake. In this regard, it was demonstrated that the acquisition of voluntary ethanol intake was characterized by a clear dissociation in the microstructure of ethanol intake above and below the concentration of 6% ethanol. These results suggested that the acquisition of voluntary ethanol intake was mediated by differential, concentration dependent, behavioral mechanisms. As a result inferences drawn regarding the mechanisms regulating ethanol self-administration, must take into account the concentration of ethanol which is self-administered.

Furthermore, the present thesis emphasized the significance of the size of the individual ethanol bouts in the regulation of ethanol intake. Pharmacological (GABA-A agonist) and nonpharmacological manipulations alike were demonstrated to increase ethanol intake primarily through an influence on the size of ethanol bouts. Additionally, while the size of ethanol bouts was suggested to mediate the observed increases in ethanol intake, an analysis of the duration of these same bouts suggested that the average ethanol bout was terminated prior to the point when one would expect the onset of detectable brain ethanol levels of ethanol (Nurmi, Kiianmaa & Sinclair 1994). On the basis of this lag in the onset of ethanol reinforcement following drinking, it was reasoned that the acquisition of voluntary ethanol intake may be a function of the magnitude of association between the drinking behavior and the perception of ethanol's central reinforcing properties (Amit, & Smith, 1991; Smith, Segal, & Amit, 1989; Boyle, Smith & Amit 1992).

It was expressly argued that increases in ethanol intake induced by divergent acquisition techniques and GABA-A agonist administration were a function of an increase

in the strength of the conditioned association between ethanol drinking behavior and the experience of ethanol's central pharmacological effects. Multiple factors, such as taste and the expression of ethanol intoxication, were proposed as some of the factors which contributed to the establishment of ethanol intake.

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