# ABSTRACT

Gagan K. Singh.  **ATTENUATION OF ALCOHOL CONSUMPTION BY FG 5893 IN CYANAMIDE TREATED RATS:  CHRONIC AND ACUTE STUDIES.** (Under the direction of Dr. Gerhard W. Kalmus) Department of Biology, East Carolina University, July 1992.

An abundance of research has implicated the involvement of the neurotransmitter serotonin, and its receptor subtypes, in one of the underlying biochemical causes of alcohol addiction.  This investigation was undertaken to determine the effect of the novel compound FG 5893 (2-[4-[4,4-bis(4-Fluorophenyl)butyl]-1-piperazinyl]-3-pyridinecarboxylic acid methyl ester) on the volitional drinking of alcohol in calcium cyanamide treated Sprague-Dawley rats.  Cyanamide treatment was administered to each subject in a dose of 10 mg/kg twice daily for three days.  Each rat was offered water and its preferred concentration of ethyl alcohol (based on a 3-30% alcohol screening procedure), using a three bottle free choice paradigm.  After a 3 day pre-drug control test, either a saline control vehicle or FG 5893 was administered subcutaneously at 1600 and 2200 for 3 consecutive days.  FG 5893 was administered in a dose of 0.5, 1.0 or 2.5 mg/kg. Alcohol consumption was significantly reduced by all three doses, as measured by both absolute g/kg and proportion of alcohol to total fluid intake.  Alcohol consumption of the saline control animals was not affected. Neither body weight

or food intake was affected by any dose of FG 5893 during or post treatment. Because FG 5893 possesses both $5-HT_{1A}$ agonist and $5-HT_2$ antagonist properties, it is envisioned that this anxiolytic/antidepressant drug acts on central serotonergic synapses thought to be involved in the addictive liability of alcohol consumption.

In addition, amperozide (FG 5606) was perfused in the rat nucleus accumbens to determine its effects on monamine concentration and turnover. The nucleus accumbens is believed to be one of the structures of the limbic mid- and forebrain system which is involved in drug reward motivational behavior.

ATTENUATION OF ALCOHOL CONSUMPTION BY

FG 5893 IN CYANAMIDE TREATED RATS: CHRONIC

AND ACUTE STUDIES

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# THE ATTENUATION OF ALCOHOL CONSUMPTION BY

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## INTRODUCTION

As a result of significant strides made towards the understanding of alcoholism over the past two decades, the etiology of this intriguing disorder is slowly emerging. Several classical studies have implicated a biological basis to this illness. No longer is the disease of alcoholism thought to stem solely from deep-seated psychological problems. In fact, the nature of this addiction is multi-fold. Along with the sociocultural, personal and anxiety related stresses which might induce one to drink, research indicates that certain individuals have a propensity to abuse alcohol because of an imbalance in their brain, at the level of certain neurotransmitters (Myers, 1982). Evidence gathered from human population studies support underlying genetic factors in the predisposition towards alcoholism. Longitudinal adoption studies of half-siblings, identical and fraternal twins all indicate that the susceptibility towards abusing alcohol appears to be consanguineous in nature. Children born of alcoholic parents, but raised without any knowledge of their parents disorder, still display a heightened risk to abuse alcohol themselves (Cloninger and Sigvardsson, 1981).

Because of alcohol's profound pharmacological effects on the brain, it is essential to understand what role the

central nervous system and its individual nerve cells play in this addictive disease.

By sending and receiving messages from neighboring nerve cells and the environment, each neuron plays an intrinsic role in determining one's mental and emotional state, thereby governing behavior. Neuronal transmission is mediated by the release of specific chemical agents known as neurotransmitters which can be either excitatory or inhibitory in nature, causing an increase and decrease in neuronal firing, respectively (Bloom, 1990). Each neurotransmitter has specific classes of receptors on both presynaptic and postsynaptic cell membranes (Bloom, 1990). These receptors ordinarily react only with their specific neurotransmitter or with a drug that is similar in structure. An abundance of research has implicated the involvement of two neurotransmitters, serotonin and dopamine, and their receptor subtypes, in the underlying biochemical cause of alcoholism (Myers, 1978).

**MULTIPLE METABOLITE THEORY -- TIQ/THBC STUDIES**

A "multiple metabolite theory" has been proposed, suggesting that the endogenous formation of pharmacologically active aldehyde metabolites in the brain perpetuates the craving "to drink" (Davis and Walsh, 1970). The theory is as follows: Acetaldehyde, a metabolite of alcohol, may alter

the metabolic patterns of both serotonin and dopamine. First, acetaldehyde has been reported to shift the metabolic pattern of serotonin from the oxidized metabolite (5-hydroxyindoleacetic acid) to the reduced metabolite (5-hydroxytryptophol) (Brien and Loomis, 1983). Secondly, acetaldehyde is oxidized to acetate by the enzyme aldehyde dehydrogenase (AlDH). This enzyme is also responsible for the degradation of dopaldehyde (dihydroxyphenlyacetaldehyde) into dihydroxyphenylacetic acid (Brien and Loomis, 1983). It is envisioned that during normal metabolic processes, the primary function of AlDH activity is neurotransmitter degradation. However, because there is a concurrent demand for this enzyme in the presence of high levels of alcohol, the activity may now be shifted towards the break-down of the toxic acetaldehyde (Myers, 1985). This results in a subsequent accumulation of biogenic aldehydes which may undergo a condensation reaction with their unconverted parent biogenic amines, or an aldehyde itself, to yield amine-aldehyde adducts. For instance, tetrahydroisoquinolines (TIQ) such as salsolinol and tetrahydropapaveroline (THP) arise from the condensation of dopamine plus aldehyde and dopamine plus dopaldehyde, respectively (Sjoquist, 1985). Another class of adducts, tetrahydro-ß-carbolines (THBC) arise from a tryptamine/aldehyde condensation (Susilo and Rommelspacher, 1985). Although alcohol does not have an affinity for pre- or

post-synaptic receptors in the neurons in the brain, these metabolites are able to bind to dopaminergic, serotonergic and opiate receptors in the brain, thus acting as false transmitters. Herein lies one possibility for the compulsive nature of alcoholism (Blum et al, 1980).

An extensive investigation of these endogenously formed metabolites began, in order to establish their involvement in the underlying addictive mechanisms. In a 1977 study by Myers and Melchior, THP or salsolinol was infused in the cerebral ventricles (ICV) of non-alcohol drinking rats. In a situation where both water and alcohol were constantly available, the animals exposed to THP exhibited a sharp increase in alcohol consumption, even in noxious concentrations. The infusion of THBC produced a similar elevation in alcohol ingestion. Testing shows that these metabolites do not interfere with the animals' taste discrimination, which could alter their preference for a bad-tasting fluid such as alcohol (Myers and Melchior, 1977). Furthermore, a dose dependant relationship between the quantity of metabolite infused and the degree of alcohol consumed was observed, and this preferential shift appeared to be irreversible (Myers and Melchior, 1977).

The study of human alcoholic patients lends further evidence to the "Multiple Metabolite Theory". Alcoholic patients exhibit heightened levels of THBC and TIQ in their cerebrospinal fluid (CSF), as well as in their urine. This

elevation is seen not only when alcohol is present in their system, but for some time after the alcohol has been metabolized (Myers, 1982; Hirst et al, 1985).

## A POSSIBLE ALCOHOL-OPIATE LINK

A controversial hypothesis linking ethanol and opiates was proposed by Davis and Walsh in 1970. In order to lend support to this hypothesis, certain pharmacological criteria must be met (Blum et al, 1980). First, after exposure to alcohol, isoquinolines must be able to react with biological tissue (i.e., they must be able to bind to certain receptors in the brain). Secondly, TIQs must exhibit opiate-like qualities, such as physical tolerance and dependence, and the addictive behavior should be ameliorated by drugs which relieve opium dependence (Blum, et al. 1980). The "link hypothesis" is substantiated by certain facts. Tetrahydropapaveroline (THP) is the biological precursor to morphine, which is manufactured by the opium poppy plant (Papaver somniferum). In a quantitative test, radioactively labeled THP was injected into rats. The results showed that almost half of the THP was converted into opiate compounds, such as normorphine, morphine, norcodeine and codeine (Davis and Walsh, 1970). Also, a parallel is seen between rats experiencing withdrawal symptoms from alcohol to those withdrawn from morphine (Myers and Oblinger, 1977). This

research strongly implicates the connection between alcohol and addictive opiate compounds. Clinical support is seen by opiate addicts who often substitute alcohol in lieu of opium type compounds.

**PHARMACOLOGICAL THERAPIES**

In light of the above research, several pharmacological agents have been tested in attempts to restore the normal milieu in the brain of the alcoholic. Rats of various strains were first induced to drink by chronic infusions of tetrahydropapaveroline (a TIQ) at birth (Critcher et al, 1983). Once the animals were 120-180 days of age, an alcohol screening procedure was performed. Those exhibiting a predilection for alcohol were chosen to be treated with either naloxone (an opiate antagonist) or morphine (an opiate agonist) (Myers and Critcher, 1982; Critcher et al, 1983). The results showed that both naloxone and morphine attenuated alcohol consumption in a dose dependent manner (Critcher et al, 1983). However, these two drugs were not without side effects in that both naloxone and morphine suppressed food intake, which resulted in a reduced intake of calories. Since morphine and naloxone may act on different opiate receptor sub-populations, they could occupy receptors identical or similar to those to which a TIQ binds (Critcher et al, 1983). A similar, but much longer acting opiate

receptor antagonist, naltrexone, attenuates the voluntary consumption of alcohol in the TIQ treated rat (Critcher et al, 1983) and macaque monkey (Myers et al, 1986).

Because of the implication of serotonergic neurons in the role of alcoholism, drugs affecting the release or re-uptake of the neurotransmitter serotonin (5-HT) have also been investigated in their relation to drinking behavior. Studies in which the serotonergic neurons in the rat brain are lesioned show an enhancement of alcohol intake in a free choice situation (Myers and Melchior, 1975). Additional studies, where either 5-HT, a 5-HT agonist (zimeldine), or a 5-HT precursor (5-hydroxytryptophan) were injected into the ventricles of rats, all showed a decrease in voluntary alcohol consumption (Myers et al, 1972; Gill et al, 1985). Serotonin reuptake inhibitors possessing antipsychotic and antidepressant properties (i.e. clomipramine, doxepine and fluoxetine), which essentially decrease 5-HT turnover, were also shown to reduce the intake of alcohol (Daoust et al, 1984).

It has been found that the indoleamine-aldehyde condensation tetrahydro-ß-carboline (THBC) evokes an acute fear-like response upon infusion into the rat's hippocampus (Huttunen and Myers, 1986). Infusion of THBC into anatomically homologous sites in the dorsal hippocampus also result in the voluntary intake of alcohol (Huttunen and Myers,

1987). The anxiolytic properties of ethanol might induce the animal to drink even larger amounts to overcome their fearful state. As a result, anxiolytic and antipsychotic drugs were then tested in their ability to suppress alcohol drinking. Buspirone, a 5-HT receptor agonist exhibiting anti-aggressive, anti-conflict and anxiety-relieving properties, produced a significant decline in the alcohol drinking of THP treated rats and monkeys, and acted in a similar manner as opiate antagonists in the attenuation of alcohol drinking (Privette et al, 1988; Collins and Myers, 1987).

## PURPOSE OF THIS INVESTIGATION

Because of the involvement of serotonin in various psychiatric disorders, many neuroleptic drugs possess an affinity for 5-HT receptors. A novel antipsychotic drug, amperozide (FG 5606; a diphenylbutylpiperazinecarboxamide derivative possessing $5-HT_2$ antagonist properties), has been shown to exhibit potent antipsychotic, anxiolytic, antiaggressive and antidepressant-like properties without any resulting impairment in motor control or sedation (Christensson and Björk, 1990). As a result of this drug's unique profile, amperozide has been tested for its ability to attenuate alcohol consumption in rats treated with the aldehyde dehydrogenase inhibitor, calcium cyanamide (Myers et

al, 1992). Amperozide exerts its effect on the same structures within the limbic system as does THP and THBC (Myers et al, 1992), and acts on central serotonergic synapses thought to be involved in alcohol consumption (Myers et al, 1991).

The present study was undertaken to investigate the effects of FG 5893 (2-[4-[4,4-bis(4-Fluorophenyl)butyl]-1-piperazinyl]-3-pyridinecarboxylic acid methyl ester), a second generation amperozide compound, on the voluntary consumption of alcohol in Sprague-Dawley rats induced to drink by the systemic administration of cyanamide. FG 5893 is known to be a $5-HT_1$ agonist and $5-HT_2$ antagonist, thereby exhibiting characteristics of both buspirone and ritanserin. This compound also possesses antipsychotic, anti-aggressive and anxiolytic properties, similar to those of amperozide (Pettersson et al, 1992).

Chemical structure of FG 5893
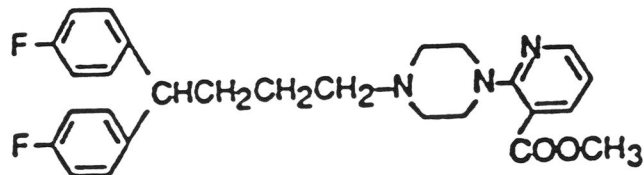


In addition, in vivo studies of amperozide (FG 5606) and its effects on monoamines in the nucleus accumbens were examined. The nucleus accumbens is a structure which lies below the caudate putamen and contains serotonergic and

dopaminergic cells. It is believed to be one of the structures of the limbic mid- and forebrain system which is involved in regulating motivated behavior and the phenomenon associated with the rewarding effects of an addictive drug (Myers, 1990).

## MATERIALS AND METHODS

### I. ANIMALS

Both male and female rats (n=25) of the Sprague-Dawley strain were used in this study. Six of the animals weighing between 80-100 g, were 30 days old at the beginning of the experiment. The remaining 19, (350-450 g), were 100 days old at the onset of the study. The animals were housed in individual, stainless steel wire cages and placed on a 12 hour light-dark cycle (lights on at 0500 hrs). The temperature of the colony room remained between 21 and 23 °C. A diet of Purina rat chow (20-50g per day), tap water (80 ml per day) and a dilution of 95% ethyl alcohol (50 ml per day) were provided to the rats on each day. The daily intakes of food, water and ethanol levels, as well as body weight of each rat, were recorded between 1000 and 1100.

### II. SYSTEMIC CYANAMIDE TREATMENT

Each rat was given a series of subcutaneous injections of calcium cyanamide in a dose of 10 mg/kg. This inhibitor of aldehyde dehydrogenase was administered twice daily at 1000 hr and 1600 hr, for three consecutive days according to the procedures of Critcher and Myers (1987). When alcohol is not available for the animal to drink, calcium cyanamide induces a significant increase in an animal's preference for ethanol. The solution of cyanamide was prepared daily in 0.9% saline

and kept covered on ice. In order to avoid the potential toxicity of the drug, the animals were treated in the absence of alcohol. Food and water intakes, as well as body weights were recorded daily for five days after drug treatment. Each animal was closely monitored during its treatment for signs of a toxic build-up of acetaldehyde. If the animal exhibited visible adverse side-effects, only one injection of cyanamide was administered on that day at 1000 hr.

### III. ALCOHOL SCREENING

After a period 25 days, the animals were placed on an ethanol screening test, based on a three bottle, two choice method previously described by Myers and Holman (1966). Three 100 ml Kimax tubes were affixed equidistantly on the front of each individual cage. The three tubes, which contained either an ethanol solution, water, or remained empty, were rotated daily. Because rats have a tendency to develop left or right paw position habits, the bottles were rotated daily to eliminate any potential bias in the data. Certain animals prefer one concentration of ethanol over another, and in order to ensure that each animal received its preferred concentration, an 11 day alcohol screening test was conducted. The concentration of ethanol was increased on each day after the 1000 hr reading as follows: 3%, 4%, 5%, 7%, 9%,

11%, 13%, 15%, 20%, 25% and 30%.

The animals' optimal concentration was chosen as being that at which the absolute amount and proportion of ethanol consumed was higher than all other concentrations. The amount of ethanol ingested was expressed in term of grams of ethanol consumed per kilogram of body weight. The animals preference for ethanol over water was expressed in terms of a proportion, which is the amount of ethanol consumed divided by the total amount of fluid ingested each day. For example, if an animal drank equal amounts of ethanol and water, the proportion would be 0.5, whereas if the animal drank only ethanol and no water, the proportion would be 1.0. Once the ideal concentration of maximal intake was determined, this concentration was offered to the animal in the presence of water until a stable level of alcohol consumption was reached. This conditioning period ensured that the appropriate concentration was selected for each individual and also provided baseline data on fluid intake. Thus, each animal could serve as its own control once drug testing had begun.

## IV. PREPARATION OF FG 5893

The 1.0 mg/kg dose of FG 5893 was prepared as follows: 60 mg of the drug was slowly dissolved in 200 $\mu$l of 0.1 N acetic acid and 100 $\mu$l of water to which 5.7 ml of water at

a temperature of approximately 60 °C was gradually added. A volume of 54 ml of distilled water was then added to the solution to give a final concentration of 60 mg FG 5893 /60 ml solvent, or 1.0 mg/ml, (pH = 2.33). Thus, if the animal weighed 500 g, 0.5 ml of the drug solution would be administered for a total dose of 1.0 mg/kg.

The 2.5 mg/kg dose was prepared according to the same procedures described above, with one exception: 18 ml of distilled water was added in lieu of 54 ml, to give a final concentration of 60 mg FG 5893/24 ml solvent, or 2.5 mg/ml. Once again, if the animal were to weigh 500 g, 0.5 mls of this solution would deliver a total dose 2.5 mg/kg.

The 0.5 mg/kg dose was prepared by diluting the 2.5 mg/ml stock solution in a ratio of 1:5. In other words, 1.0 ml of the stock solution was added to 4.0 ml of distilled water for a total concentration of 0.5 mg/ml.

The solvent control vehicle was prepared by adding 200 $\mu$l of 0.1 N acetic acid to 5.7 ml distilled water, and 54 ml of 0.9% saline, for a total volume of 60 ml with a final pH of 3.41.

## V. ACUTE TREATMENT WITH FG 5893

Once a stable level of ethanol consumption had been reached, each animal received subcutaneous injections of either FG 5893 (Kabi Pharmacia Therapeutics, Malmö, Sweden) or

a saline control vehicle twice daily at 1600 and 2200 for three consecutive days. Three doses of FG 5893 were administered as follows: 0.5 mg/kg (n = 5 males, 1 female), 1.0 mg/kg (n = 5 males, 1 female), 2.5 mg/kg (n = 5 males, 1 female) and saline (n = 5 males, 1 female). All animals remained on their preferred concentration of ethanol during the time of their treatment as well as for a 40-day post drug period. Ethanol, water, food and body weights were recorded daily for each animal.

## VI. CHRONIC TREATMENT WITH FG 5893

Osmotic mini pumps (ALZET 2001) were implanted in six males, weighing between 500-650 g. The pumps are designed to deliver 24 $\mu$l per day of a drug in solution at a steady rate for seven days. A dose dependant analysis of the results of the acute treatments of the male rats revealed that 1.0 mg/kg twice daily was the most efficacious dose to load in the pumps. Similar to the acute testing procedures, the animals remained on their preferred concentration of ethanol during the course of osmotic pump treatment, as well as for a 4, 30, and 60 day post drug period.

## (A) OSMOTIC PUMP PREPARATION

Because of the 150 g range in body weights, two separate stock solutions were prepared to ensure that the pump was

administering the correct dose of 2.0 mg/kg daily to each animal. The subjects were divided into two groups: a high weight group whose average weight was 625 g and a low weight group averaging 525 g. According to these two weights, the two solutions were prepared as follows: for the low group, 45.8 mg of FG 5893 was dissolved in 200 $\mu$l of glacial acetic acid, 200 $\mu$l of propyleneglycol and 600 $\mu$l of warm water, for a total concentration of 45.8 mg/ml, or 1.1 mg drug/24 $\mu$l. For the high dose, 52.1 mg of FG 5893 was dissolved in 200 $\mu$l of glacial acetic acid, 200 $\mu$l of propylene glycol and 600 $\mu$l warm water, for a total concentration of 52.1 mg/ml or 1.25 mg/24 $\mu$l. Approximately 200 $\mu$l of the respective solution was loaded into the reservoir of each pump. A flow moderator, consisting of a 21 gauge stainless steel tube with a plastic end-cap, was inserted into each pump for drug delivery.

## (B) IMPLANTATION OF OSMOTIC PUMPS

Each rat was placed in a dessicator jar containing methoxyfluorane just long enough to lightly anesthetize the animal. The area caudal to the shoulders was shaved and cleaned with 0.1% iodine solution. After a longitudinal incision was made intrascapularly, the pump was inserted subcutaneously. Wound clips were used to close the incision, and then the area of the incision was covered with (Bacitracin-Neomycin-Polymyxin-B) antibiotic ointment.

## VII.  SURGICAL PROCEDURES

Each rat (n=4) was given an intraperitoneal injection of a sodium pentobarbital solution (60 mg/kg) according to body weight.  A methoxyflurane overlay was administered upon requirement during surgery.  After the animals' heads were shaved and cleaned with iodine, it was placed in a Kopf stereotaxic instrument and a craniotomy hole was drilled in the calvarium above the following coordinates: AP 1.6 mm and LAT 1.40 mm  (Paxinos and Watson, 1986).  A 23 ga stainless steel cannula contained within a pedestal was lowered through dura, so that the tip of the cannula extended 4.0 mm below the dura and the pedestal was flush with the calvarium.  Four anchor screws were positioned in the calvarium and a cranioplastic cement was packed around the cannula/pedestal and screws.  A 27 ga stylet inserted into each guide tube and a protective cap served to prevent foreign material from entering the cannula.

Throughout the procedure, the animal's colonic temperature was monitored regularly with a thermistor probe, and maintained between 33 to 35 °C (Myers, 1977) using a heating pad during surgery and post operatively.  A postoperative recovery period of one week elapsed before any further experimentation began.  During this time, food/water intakes, as well as body weights were monitored.

## VIII.  PUSH-PULL PERFUSIONS OF AMPEROZIDE

For each rat, a series of perfusions were performed at four distinct anatomical regions within the nucleus accumbens (NAC), according to the following depths below the dura mater: 6.0 mm (dorsal edge of NAC), 7.0 and 8.0 mm (upper and lower core of NAC), and 9.0 mm (shell of NAC) (Paxinos and Watson, 1986).   Both a high (5 $\mu$g/$\mu$l) and a low (1.7 $\mu$g/ul) concentration of amperozide was perfused at the four sites. A concentric push-pull needle was lowered through the guide tube to the predetermined depth (Myers, 1986).    The outermost pull cannula, made from 23 ga stainless steel, thin-walled tubing, was attached to a  Hamilton microliter syringe by PE 50 tubing (Myers, 1986).   The inner push cannula, made from 28 ga stainless steel tubing, was connected to a Hamilton microliter syringe by PE 20 tubing (Myers, 1986).   Both the push  syringe containing the amperozide solution and the pull syringe containing artificial CSF (Myers, 1972) and 95mg/500ml sodium metabisulfite were mounted on a multi-channel infusion-withdrawal pump, set at a rate of 15 $\mu$l/min.  The duration of each perfusion was 6.0 minutes.  Pre and post controls were performed by perfusion of an artificial CSF solution alone. The 6.0 minute samples of perfusate were collected from the pull tubing, placed in centrifuge vials containing 5 $\mu$l of 0.1M HCl and then spun for 20 seconds. The extracted supernate was then put in microvials containing 0.5 $\mu$l of 3,4-dihydroxy-

benzylamine (DHBA) standard and stored in a freezer at -80 °C.
All syringes, PE tubing, and push-pull needles were stored in
70% ethanol between each experiment.

## (A) PREPARATION OF SOLUTIONS

The high dose amperozide solution was prepared by mixing
50 mg of drug in 10 ml of distilled water. The low dose
solution was prepared with 16.7 mg amperozide in 10 ml
distilled water. The artificial CSF was prepared by adding
the following to 500 ml of distilled water: 5.0 mg of $Na_2S_2$
$O_5$, 3.73 g NaCl, 95 mg KCl, 70 mg CaCl and 95 mg $MgCl_2 \cdot H_2O$.
Prior to use, all solutions were passed through a 0.2 $\mu$m
acrodisc filter apparatus.

## (B) HPLC ANALYSIS OF PERFUSATES

Each 120 $\mu$l sample of perfusate was stored in a mini-
vial containing 5 $\mu$l of PCA (.1 M) and 5 $\mu$l of the internal
standard DHBA (3,4-dihydroxy-benzylamine). The presence of
monamine neurotransmitters and their metabolites in the
perfusate were detected using an HPLC Autosampler 460 equipped
with pre and post- column carbon filters. A Fisher recorder
and Compaq 286e computer attached to the detector facilitated
the identification and subsequent plotting of the peaks. The
mobile phase (pH 3.5) contained 6.9 g $NaH_2PO_4$ $H_2O$, 0.212 g

$CH_3(CH_2)_6SO_3Na$ $H_2O$, and 0.08 g EDTA added to 900 ml distilled $H_2O$ and 30 ml $CH_3OH$.


## IX.  HISTOLOGICAL ANALYSIS

At the conclusion of the experiments, each animal was given a 60 mg/kg overdose of sodium pentobarbitol by the intraperitoneal route.  Each rat was perfused transcardially with 100 ml of 0.9% saline followed by 100 ml of 10% formalin solution.  The rat was then decapitated, the brain removed and stored in 10% formalin.  After 10 days, each brain was transferred to a 25% sucrose solution for an additional week.  Sections were cut at 100 $\mu$m in the coronal plane and then mounted on slides. The sections were then stained using a standard cresyl violet staining procedure (Wolf, 1971).

## X.  STATISTICAL ANALYSIS

Statisitcal analysis of the alcohol studies was done between groups of data using a one way analysis of variance (ANOVA).  Comparisons were made between pre-control drinking levels verses all post-control levels for g/kg and proportion of alcohol intake.  A p value of $<0.05$ was considered statistically significant.

# RESULTS

Both acute and chronic treatments of FG 5893 markedly altered the volitional consumption of alcohol in a dose dependent manner. As shown in Fig. 1, the low and high doses of FG 5893 administered twice daily showed an initial reduction in the g/kg intake of alcohol, as compared to the pre-control levels. One way analysis of variance (ANOVA) tests show that the decline in drinking from the pre-control levels was significant ($F_{1,35} = 11.95$, p < 0.005, $F_{1,35} = 16$, p < 0.001 respectively). Although a slight decrease in mean g/kg intake of alcohol (18.5 ± 7.8%) was produced by the intermediate dose of 1.0 mg/kg (b.i.d.) initially, this reduction was not statistically significant.

The proportions of alcohol to total fluid consumed were similarly reduced below that of the pre-control levels by both low and high doses of FG 5893 ($F_{1,35} = 7.36$, p = 0.01, $F_{1,35} = 10.53$, p < 0.005, respectively). Once again, the intermediate dose of the drug caused an initial reduction in the proportion of alcohol consumed; however, the results were not statistically significant.

During the four day post period, alcohol consumption remained attenuated by the low and intermediate doses. This was observed by a concurrent decrease in both g/kg alcohol

intake ($F_{1,41}$ = 13.23, p < 0.005 and $F_{1,41}$ = 10.78, p < 0.005 respectively), and proportion ($F_{1,41}$ = 7.1, p = 0.01, $F_{1,41}$ = 11.2, p < 0.005 respectively), as compared once again to the pre-control drinking levels. Significant decreases during the 4 day post treatment period in both g/kg and proportion of alcohol consumed were also seen by the 2.5 mg/kg dose of FG 5893 ($F_{1,41}$ = 6.61, p = 0.01, $F_{1,41}$ = 5.61, p = 0.01, respectively). Saline control animals showed no significant changes in either g/kg or proportion of alcohol intake during and following injections. As presented in Fig. 2, the same general trends of FG 5893 were seen by all three doses (i.e. the greatest effect of the drug occurred by the sixth injection, or following the third day of treatment; in all three cases, a tendency to rebound occurred at the beginning of the 4 day post period).

A composite analysis of the mean effects of all three doses of FG 5893 on food, water and alcohol intakes, as well as on body weights during treatment is shown in Table 1. Both during and 4 days following FG 5893 treatment, no significant effects were produced by the drug with regard to weight, food or water intakes. The increase in mean body weight seen during the 40 day post period was due likely to the normal development of the rats, rather than any effect of the drug.

Table 2 depicts the mean percent decline from the 3 day

pre-drug baseline in both g/kg and proportion of alcohol to total fluid consumed for the 3 days of FG 5893 treatment and for 4 and 40 days post drug. Although the low dose produced the greatest reduction in the absolute g/kg intake of alcohol (37.2 ± 7.8%), this decrease was transient. The intermediate dose of FG 5893 caused a continuous percent decline at the 4 and 40 day post in both g/kg and proportion.

Figures 3, 6 and 10 depict the mean consumption of alcohol over 16 consecutive days (pre, during and 10 days post) and after 40 days for 0.5, 1.0 and 2.5 mg/kg respectively. In each Figure, the top graph depicts the mean proportion of alcohol consumed as compared to total fluid (i.e. alcohol plus water) intake. The bottom graph represents the grams of alcohol consumed per kilogram of body weight. For all three doses, the greatest decline in alcohol drinking from the pre-control baseline level was evident by the third day. A decrease in drinking at the 40 day post period was statistically significant as compared to pre-treatment baseline levels, for the intermediate dose, in both g/kg ($F_{1,41}$ = 22.63, p <0.001) and proportion ($F_{1,41}$ = 17.29, p <0.001). A similar decline occurred in the high dose at 40 days, in g/kg ($F_{1,41}$ = 9.73, p < 0.005) and proportion ($F_{1,41}$ = 13.1, p <0.005). For the low dose treatment, a significant decrease in absolute g/kg intake occurred at 40 days ($F_{1,41}$ = 14.81, p <0.001),

however, no significant changes from pre-control levels occurred in the proportion values. Although the 40 day g/kg post value was significant for the 0.5 mg/kg dose, the percent decline in g/kg and proportion by 40 days was low. This could mean that the effect of the drug was diminishing, since more animals had returned to their normal basal levels by this point.

## INDIVIDUAL DIFFERENCES IN RESPONSE TO FG 5893

In order to evaluate the effects of FG 5893 on individual rats, the mean percent change from the 3 day pre drug baseline for g/kg and proportion of alcohol consumed was determined for the 3 days of treatment and 4 days post drug. Figures 4, 7 and 11 all indicate how the doses affected each rat differentially.

In Fig. 4, all animals treated with 0.5 mg/kg FG 5893 showed a decrease in g/kg during treatment and 2 of the 6 declined further during the 4 day post. Five of 6 animals showed a decrease in proportion during treatment, while only one continued to show a decline during the 4 day post. Four of 6 animals treated with the intermediate dose (Fig. 7) showed a drop in g/kg consumption of alcohol, and all 6 animals showed a decline in proportion during treatment. During post treatment, 5 of 6 animals exhibited a further reduction from the treatment levels in both g/kg and

proportion, while 1 exhibited an increase. In Fig. 11, the percent values in terms of both g/kg and proportion, declined in 5 of 6 animals during 2.5 mg/kg treatment, and remained reduced following treatment. The animal showing an increase in both g/kg and proportion was a female.

Individual animal cases (both males and females) for all three doses are represented by Figs. 5, 8, 9, 12 and 13. Such profound effects of drug treatment that might not be as apparent in group mean graphs, as are seen in these individual representations. For example, Fig. 5 depicts 2 males that received the same dose; however, a distinct difference in response to the drug was seen in both cases. Similarly, Figs. 8, 9, 12, and 13 represent subjects' distinct reactions to treatment with FG 5893.

## CHRONIC (OSMOTIC MINI-PUMP) TREATMENT

Because only male rats were used in the chronic studies, a separate evaluation of the effects of all three doses on male subjects only was done to determine the most efficacious dose to load in the mini-pumps. Figure 14 depicts the percent change from baseline during and 4 days following (POST) injections. The intermediate dose of 1.0 mg/kg (b.i.d) produced a continual decrease in alcohol consumption. As a result this dose was chosen (2.0 mg/kg daily) for use in the osmotic pumps.

As shown in Figs. 15 and 16, a significant decrease in alcohol consumption by chronic administration of FG 5893 occurred in both g/kg and proportion, as compared to pre-control drinking levels during ($F_{1,59}$ = 15.5, p < 0.01, $F_{1,59}$ = 23.1, p <0.01, respectively) and 4 days post treatment ($F_{1,41}$ = 16.7, p < 0.01, $F_{1,41}$ = 15.0, p < 0.01, respectively). Although chronic administration of FG 5893 produced a significant decline in g/kg at 30 and 60 days post ($F_{1,41}$ = 7.4, p < 0.01, $F_{1,41}$ = 41.0, p < 0.01, respectively), a similar decrease (compared to pre-levels) in proportion during these post periods was not significant. The drastic rebound drinking observed following acute treatment was not observed during or after chronic treatment.
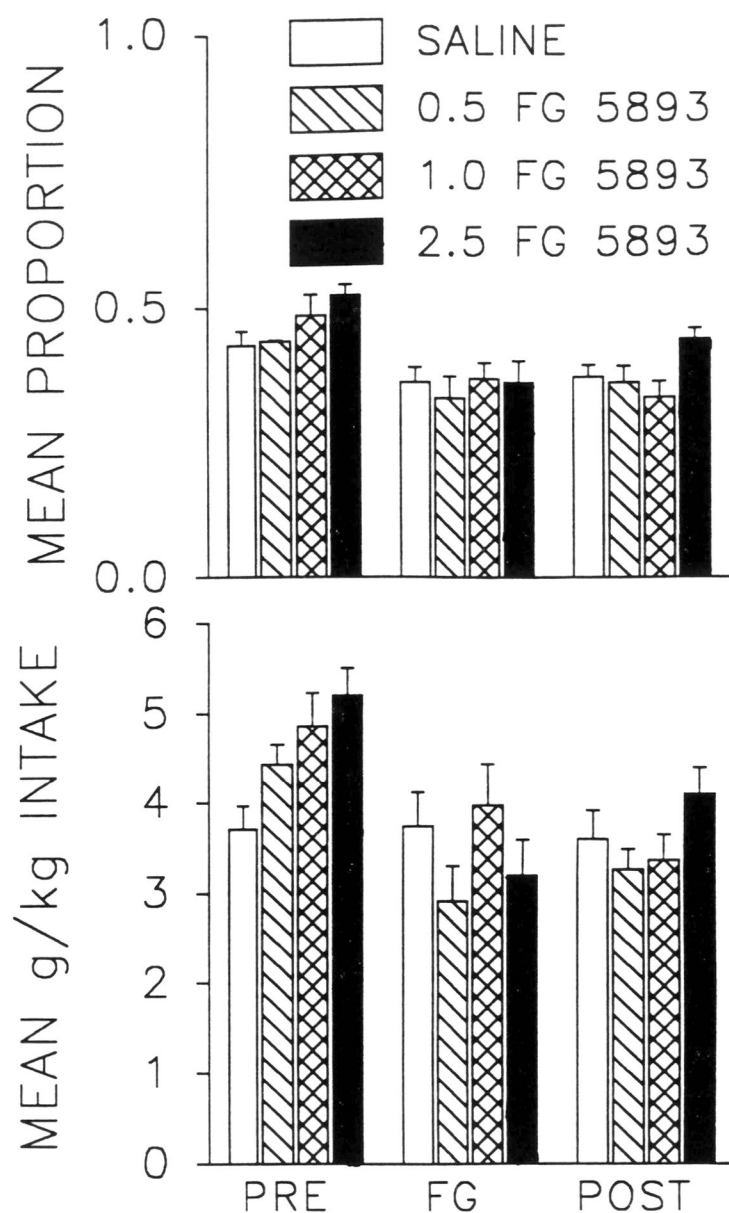
Figure 1. Combined alcohol consumption behavior data for animals
that received either saline control, 0.5 mg/kg, 1.0 mg/kg,
or 2.5 mg/kg FG 5893 subcutaneous injections (b.i.d.) -- pre
for 3 days, drug for 3 days and post for 4 days. The top
graph shows the mean proportion of alcohol consumed divided
by total fluid (i.e. alcohol plus water) intake for each
group. The bottom graph shows the grams of alcohol consumed
per kilogram of body weight pre, during and post treatment.
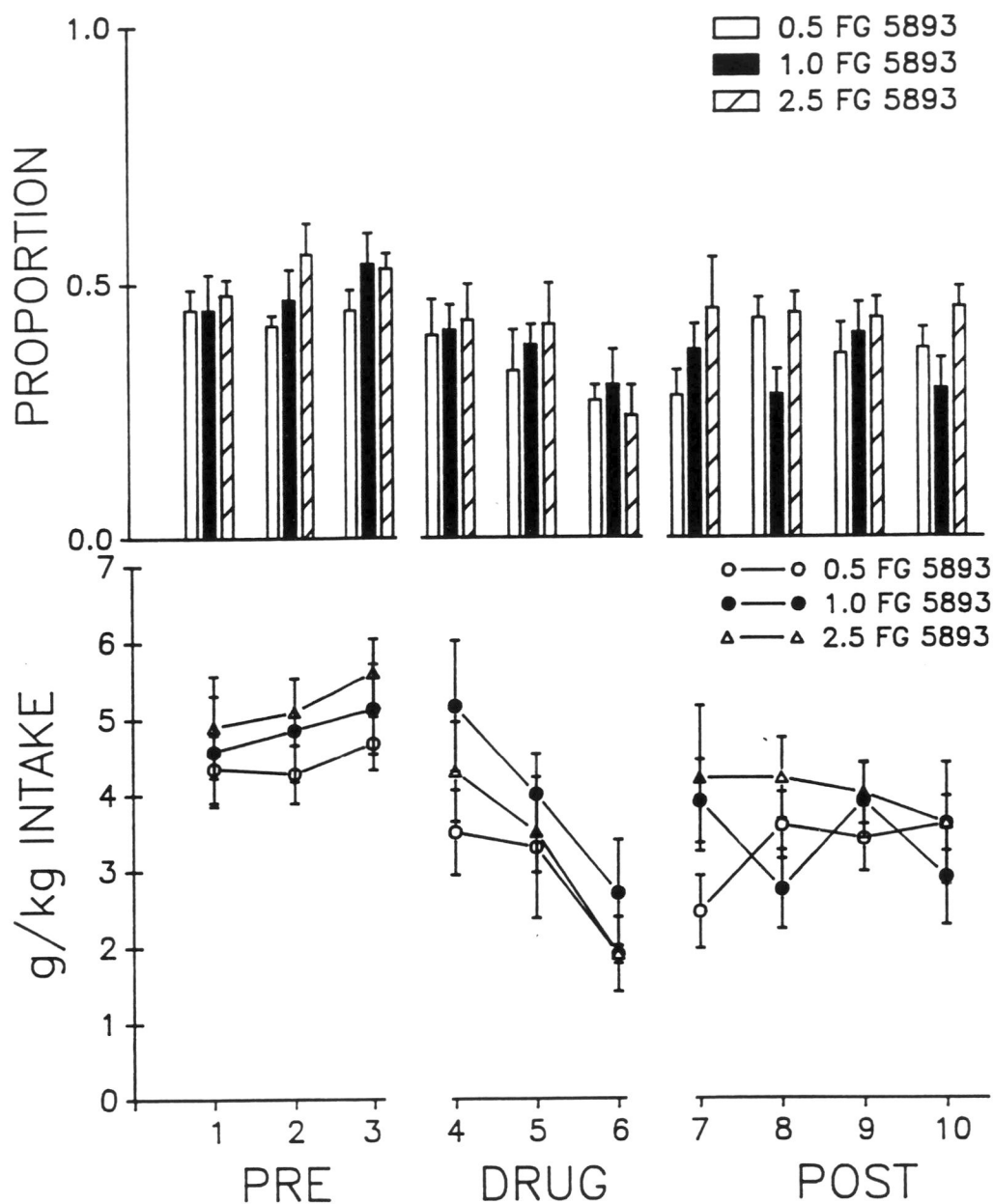N=6 for each group.

Figure 2.    Mean daily alcohol consumption data for animals that received 0.5 mg/kg, 1.0 mg/kg and 2.5 mg/kg FG 5893 (b.i.d.) -- pre for 3 days, FG 5893 for 3 days and post for 4 days. The top graph shows the mean proportion of alcohol consumed divided by total fluid (i.e. alcohol plus water) intake for each group.  The bottom graph shows the grams of alcohol consumed per kilogram of body weight pre, during and post treatment for each group.

Table 1

**MEAN ± S.E. EFFECTS ON FOOD, WATER, ETOH INTAKES AND BODY WEIGHT OF MALE AND FEMALE RATS INJECTED SUBCUTANEOUSLY WITH 0.5, 1.0 OR 2.5 MG/KG FG 5893 OR SALINE VEHICLE ADMINISTERED TWICE DAILY. PRE-CONTROL FOR 3 DAYS, INJECTIONS FOR 3 DAYS, AND POST-CONTROL FOR 4 DAYS. (N = NUMBER OF RATS)**

|  | FOOD (g) | WATER (ml) | ETOH (ml) | WEIGHT (g) |
|---|---|---|---|---|
| **LOW DOSE (N = 6)** | | | | |
| PRE | 19.2 ± .47 | 27.6 ± 1.9 | 20.8 ± 1.0 | 532.9 ± 29 |
| 0.5 mg/kg | 19.6 ± .34 | 26.4 ± 1.8 | 14.1 ± 2.3 | 533.8 ± 29.4 |
| 4 Day Post | 20.2 ± .18 | 28.3 ± 1.7 | 15.6 ± 1.2 | 535.8 ± 25.2 |
| 40 Day Post | 19.9 ± .16 | 28.4 ± 1.8 | 18.3 ± 1.3 | 573.2 ± 39.3 |
| **INTERMEDIARY DOSE (N = 6)** | | | | |
| PRE | 17.2 ± .55 | 20.6 ± 1.5 | 19.4 ± 1.6 | 441.1 ± 24.1 |
| 1.0 mg/kg | 18.2 ± .65 | 26.4 ± 2.3 | 16.3 ± 1.9 | 438.4 ± 24.2 |
| 4 Day Post | 20.2 ± .35 | 26.9 ± 1.2 | 14.2 ± 1.4 | 443.8 ± 21.2 |
| 40 Day Post | 19.5 ± .30 | 29.0 ± 1.7 | 12.3 ± 1.4 | 482.5 ± 22.6 |
| **HIGH DOSE (N = 6)** | | | | |
| PRE | 19.3 ± .46 | 21.9 ± 2.0 | 23.1 ± 1.7 | 475.2 ± 19.4 |
| 2.5 mg/kg | 19.4 ± .39 | 26.3 ± 2.6 | 13.9 ± 1.7 | 475.5 ± 19.4 |
| 4 Day Post | 19.9 ± .41 | 23.5 ± 1.6 | 18.1 ± 1.5 | 474.8 ± 16.3 |
| 40 Day Post | 19.5 ± .36 | 26.8 ± 1.9 | 18.6 ± 1.8 | 512.3 ± 17.3 |
| **SALINE CONTROL (N = 6)** | | | | |
| Pre | 16 ± .46 | 20.4 ± 1.4 | 16.1 ± 1.5 | 422.5 ± 16.5 |
| Saline | 17.9 ± .42 | 27.5 ± 2.0 | 16.1 ± 1.8 | 421.1 ± 16.1 |
| 4 Day Post | 19.4 ± .30 | 25.5 ± 1.4 | 15.6 ± 1.3 | 426.9 ± 13.8 |

TABLE 2. MEAN ± S.E. PERCENT DECLINE FROM BASELINE INTAKES OF ALCOHOL IN g/kg AND PROPORTION OF ALCOHOL TO TOTAL FLUID DURING 0.5, 1.0 AND 2.5 mg/kg  FG 5893 GIVEN B.I.D. FOR 3 DAYS (INJECTION) AND 4 AND 40 DAYS FOLLOWING (POST) ITS ADMINISTRATION

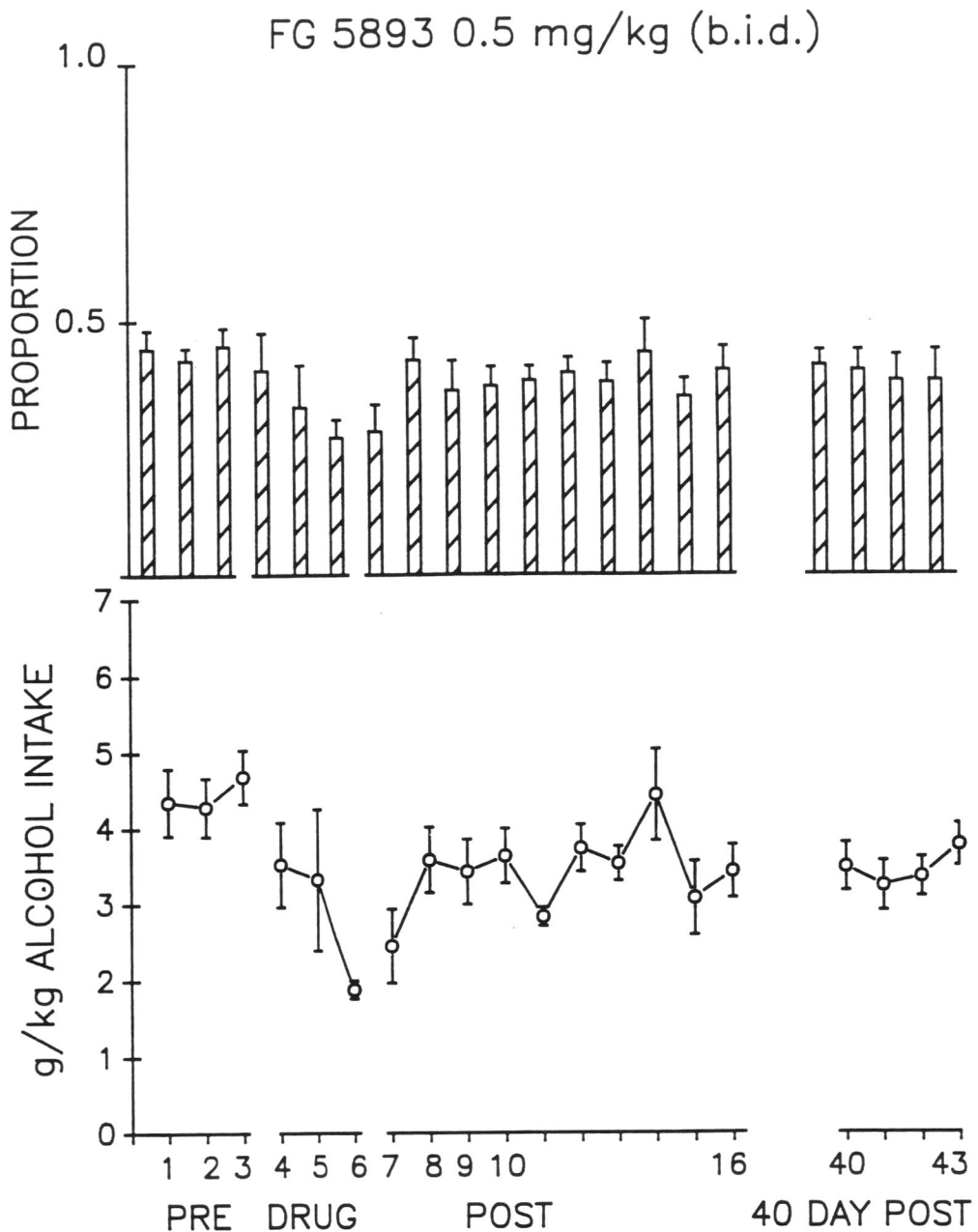| | G/KG | | | PROPORTION | | |
|---|---|---|---|---|---|---|
| DOSE OF FG 5893 | INJECTION | 4 DAY POST | 40 DAY POST | INJECTION | 4 DAY POST | 40 DAY POST |
| 0.5 mg/kg (N = 6) | 37.2 ± 7.8% | 24.7 ± 6.0% | 18.5 ± 8.9% | 24.6 ± 8.8% | 18.3 ± 5.4% | 10.2 ± 9.1% |
| 1.0 mg/kg (N = 6) | 18.5 ± 9.2% | 30.0 ± 8.3% | 39.3 ± 9.5% | 25.4 ± 6.1% | 32.8 ± 6.5% | 37.5 ± 9.7% |
| 2.5 mg/kg (N = 6) | 30.0 ± 16.8% | 18.2 ± 10.1% | 23.5 ± 6.7% | 31.3 ± 9.4% | 16.2 ± 7.2% | 22.3 ± 4.9% |

Figure 3. Mean daily alcohol consumption data for animals (N=6) that received subcutaneous injections of FG 5893 at a dose of 0.5 mg/kg (b.i.d.) -- pre for 3 days, FG 5893 for 3 days, and a 10 and 40 day post period. The top graph depicts the mean proportion of alcohol consumed divided by total fluid (i.e. alcohol plus water) intake for each animal. The bottom graph shows the grams of alcohol consumed per kilogram of body weight.
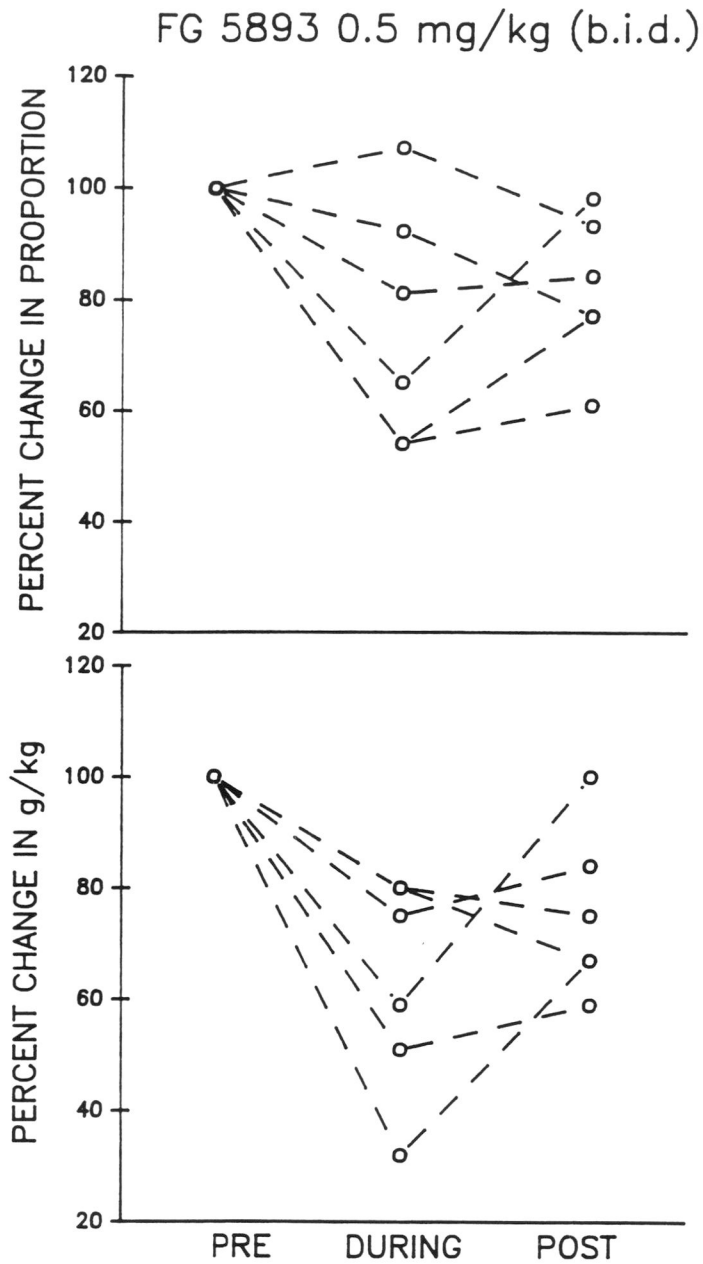
Figure 4.    Change in baseline alcohol consumption for each
animal that received 0.5 mg/kg FG 5893 (b.i.d.) as measured
by percent changes in proportion and g/kg -- pre for 3 days,
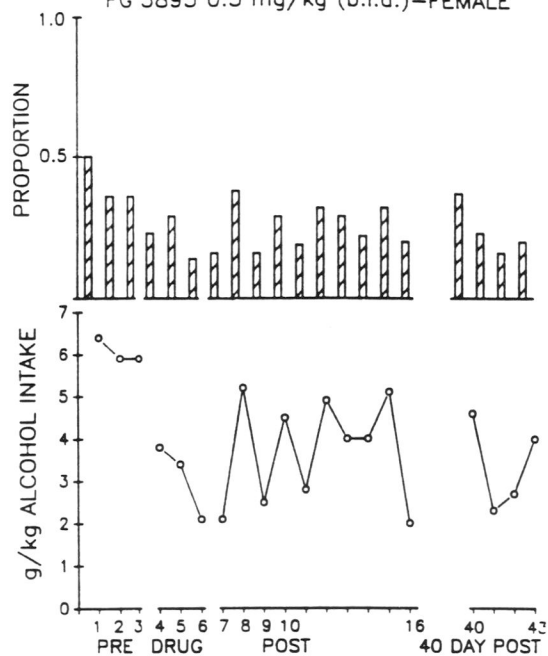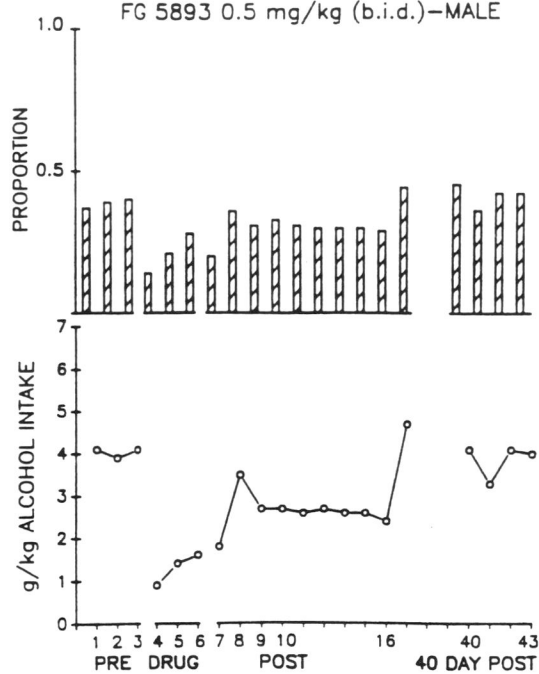drug for 3 days and post for 4 days.

Figure 5.    Daily alcohol consumption data for individual animals (male and female) that received 0.5 mg/kg FG 5893 (b.i.d.) -- pre for 3 days, FG 5893 for 3 days, and a 10 and 40 day post period.   The top and bottom graphs have the same format as those described in fig. 3.
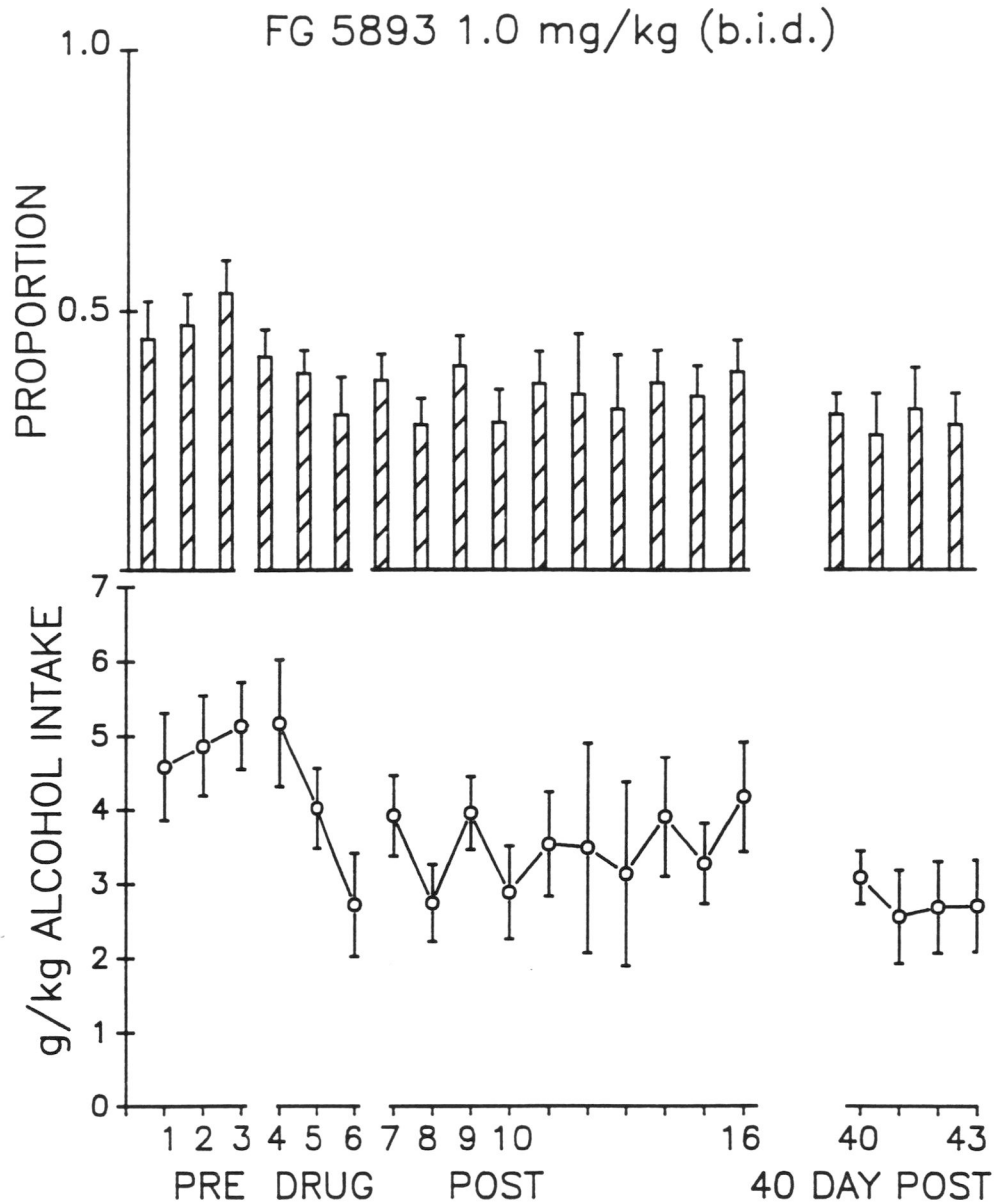
**Figure 6.** Mean daily alcohol consumption data for animals (N=6) that received subcutaneous injections of FG 5893 at a dose of 1.0 mg/kg (b.i.d.) -- pre for 3 days, drug for 3 days and a 10 and 40 day post period. The top and bottom graphs have the same format as fig. 3.
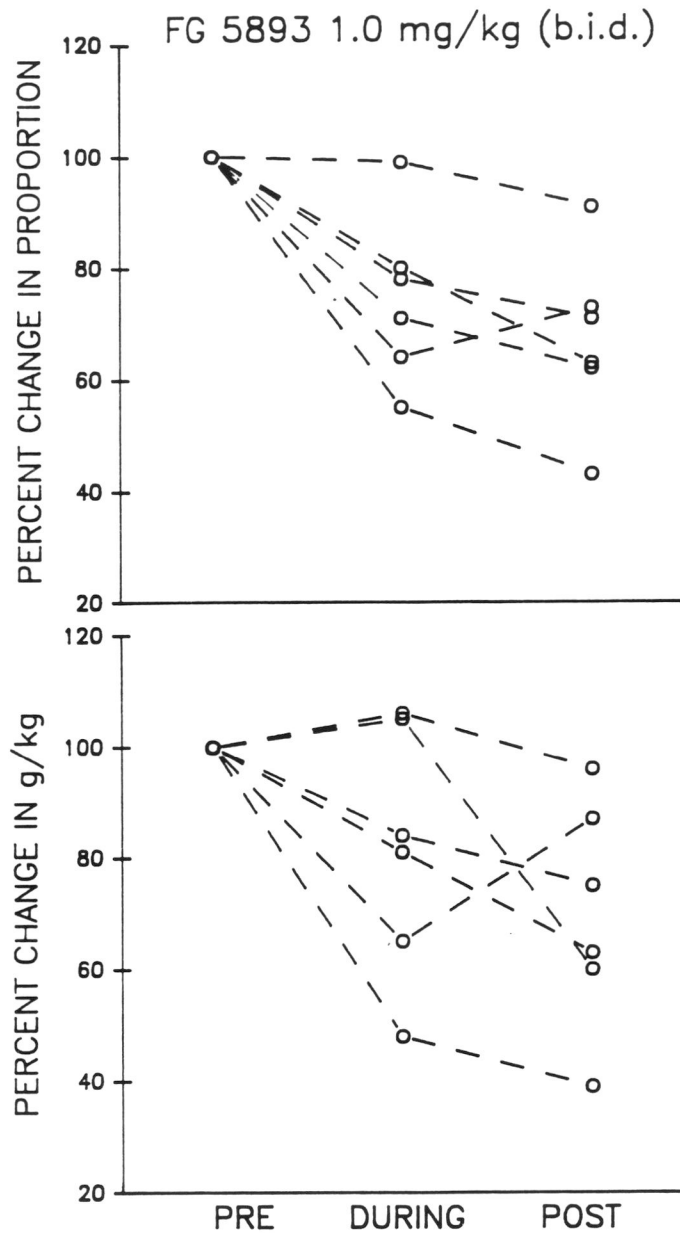
Figure 7.    Change in baseline alcohol consumption for each animal that received 1.0 mg/kg FG 5893 (b.i.d.) as measured by percent changes in proportion and g/kg -- pre for 3 days, FG 5893 for 3 days and post for 4 days.

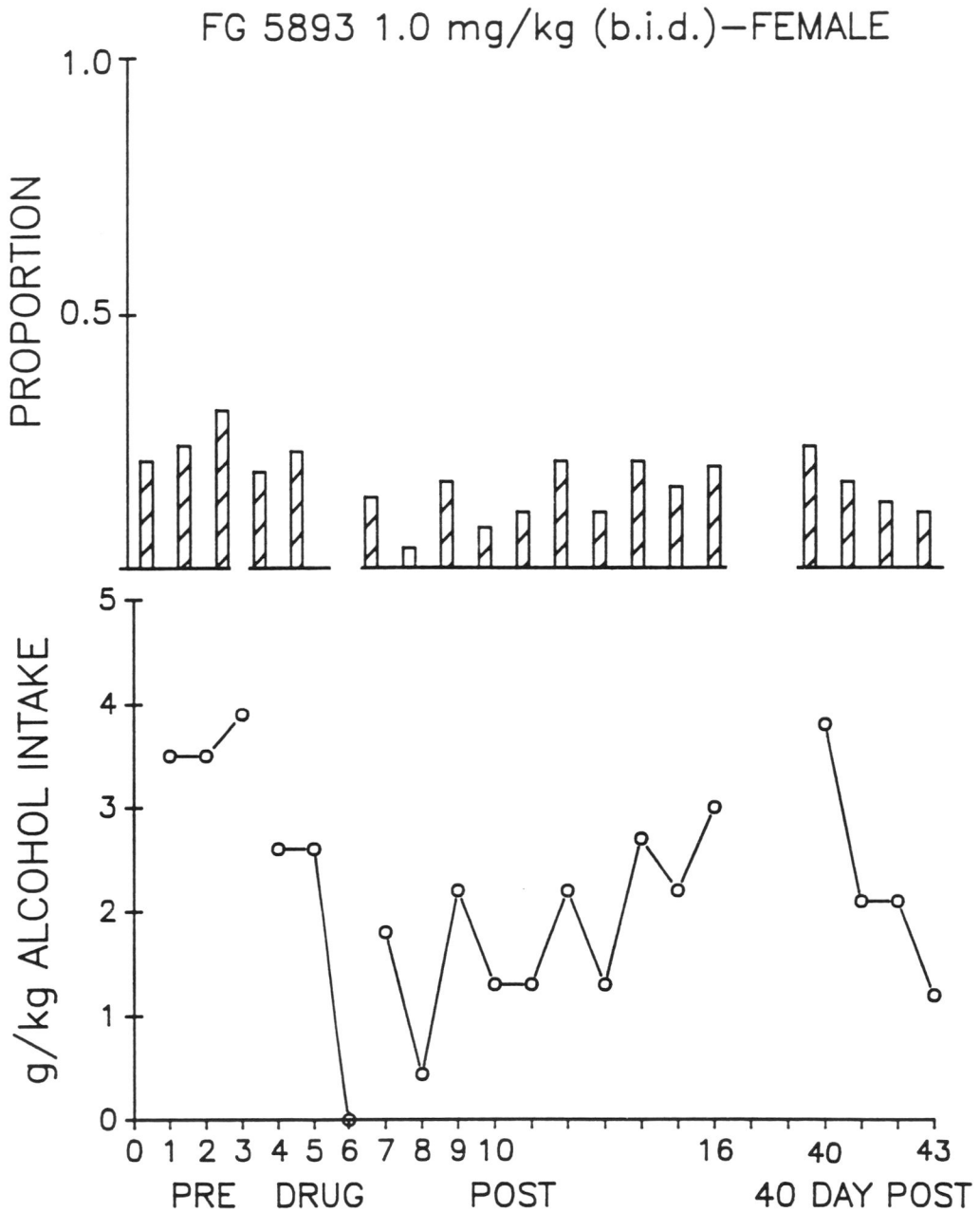## FG 5893 1.0 mg/kg (b.i.d.)—FEMALE



Figure 8.    Daily alcohol consumption data for an individual female that received 1.0 mg/kg FG 5893 (b.i.d.) -- pre for 3 days, drug for 3 days, and a 10 and 40 day post period.  The top and bottom graphs have the same format as fig. 3.

Figure 9. Daily alcohol consumption data for two males that received 1.0 mg/kg FG 5893 (b.i.d.) -- pre for 3 days, drug for 3 days and a 10 and 40 day post period. The top and bottom graphs have the same format as fig. 3.

Figure 10. Mean daily alcohol consumption data for animals (N=6) that received subcutaneous injections of FG 5893 at a dose of 2.5 mg/kg (b.i.d.) -- pre for 3 days, drug for 3 days and a 10 and 40 day post period. The top and bottom graphs have the same format as fig. 3.

Figure 11. Change in baseline alcohol consumption for each animal that received 2.5 mg/kg FG 5893 (b.i.d.) as measured by percent changes in proportion and g/kg -- pre for 3 days, drug for 3 days and post for 4 days.

Figure 12.    Daily alcohol consumption data for an individual female
              that received 2.5 mg/kg FG 5893 (b.i.d.) -- pre for 3 days,
              drug for 3 days and a 10 and 40 day post period.  The top and
              bottom graphs have the same format as fig. 3.
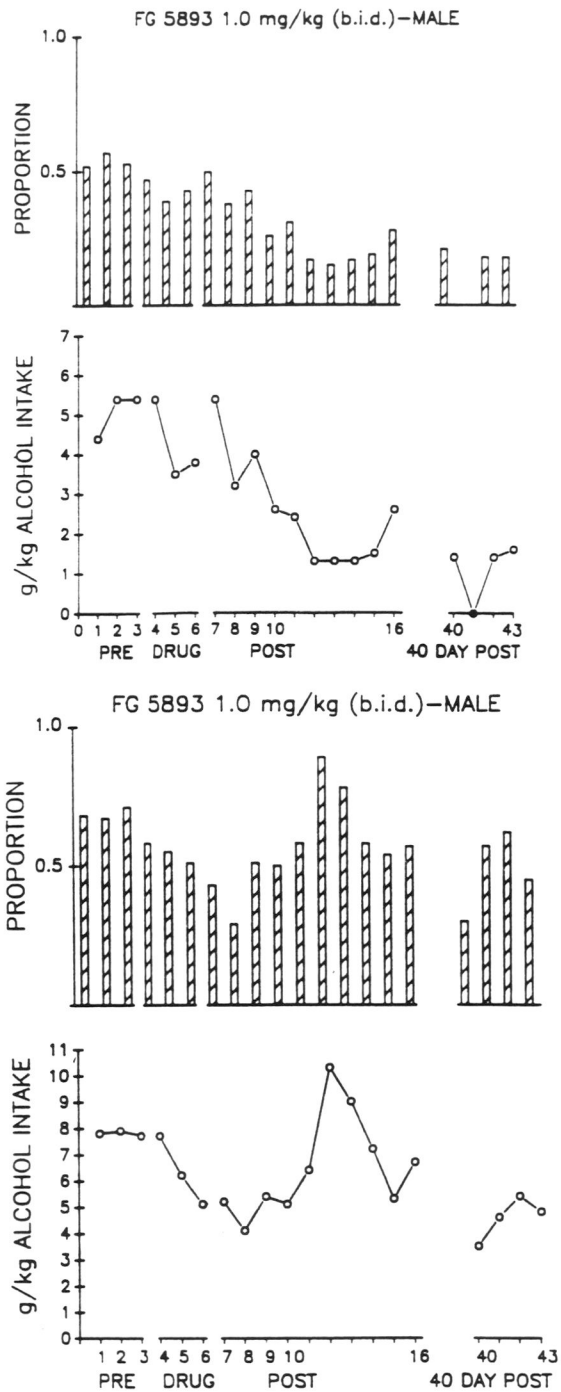
Figure 13. Daily alcohol consumption data for two males that received
2.5 mg/kg FG 5893 (b.i.d.) -- pre for 3 days, drug for 3
days and a 10 and 40 day post period. The top and bottom
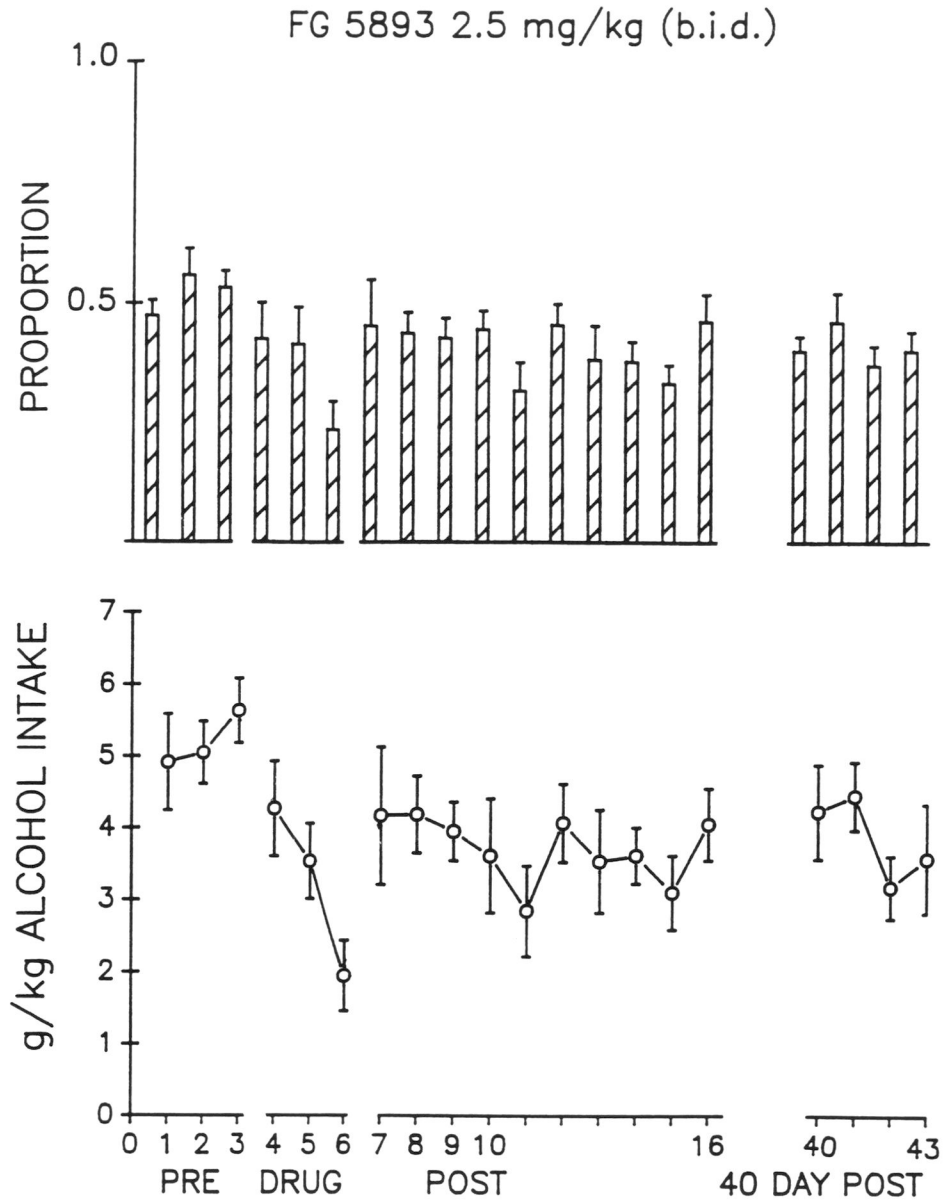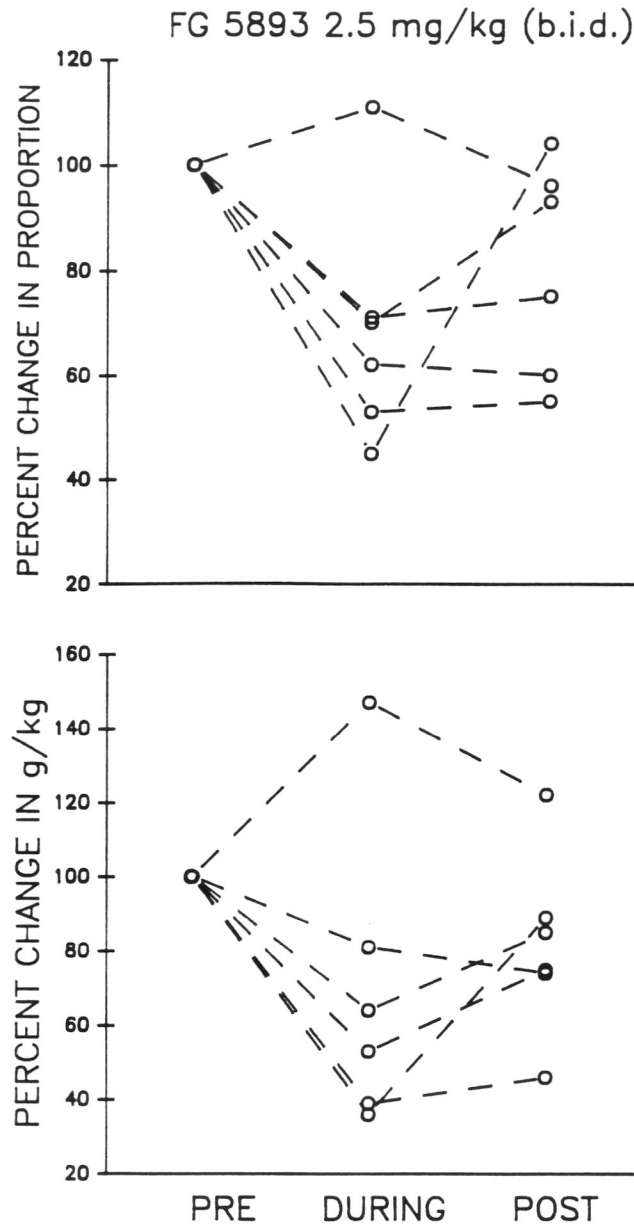graphs have the same format as fig. 3.
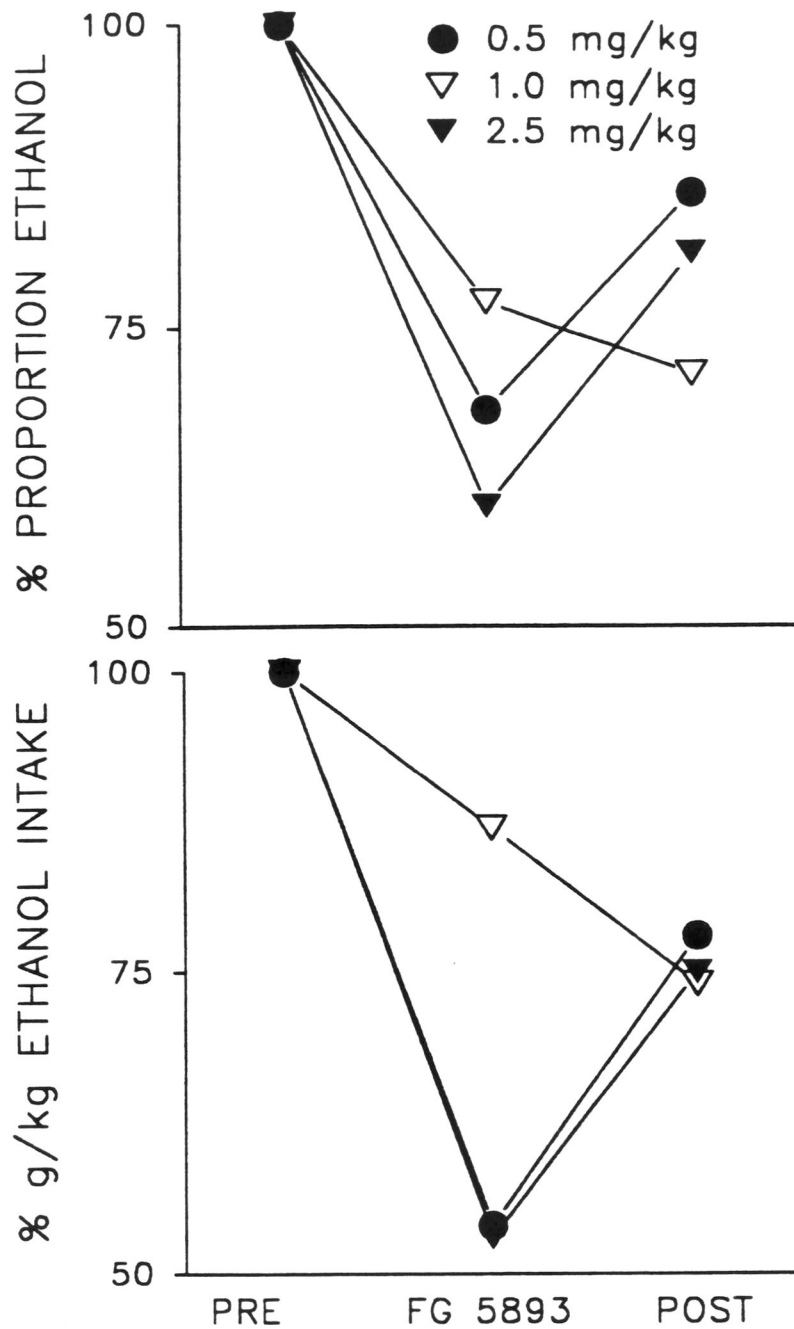
Figure 14.   Change in baseline alcohol consumption for animals that received 0.5 mg/kg, 1.0 mg/kg and 2.5 mg/kg FG 5893 (b.i.d.) as measured by percent changes in proportion and 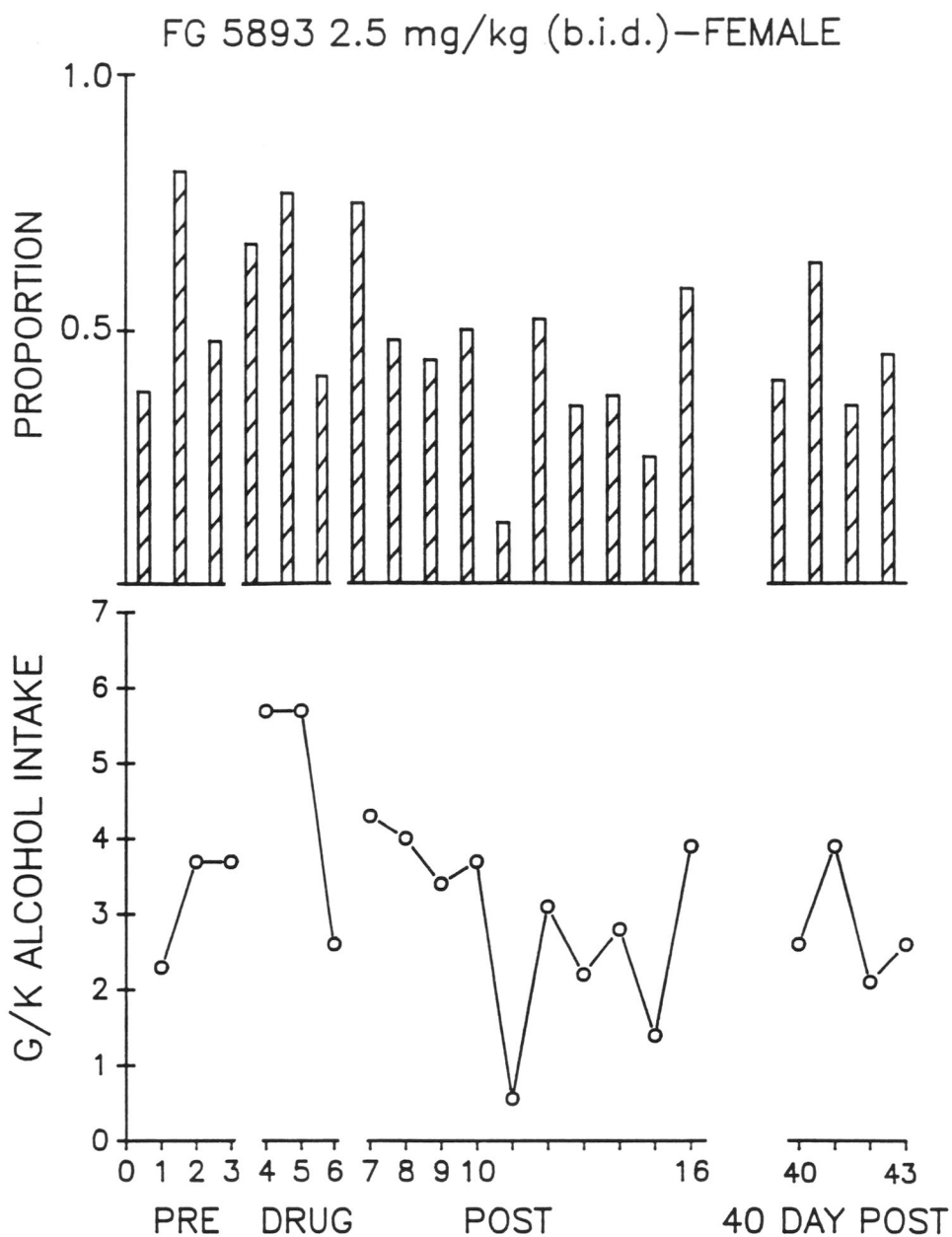g/kg -- pre for 3 days, drug for 3 days and post for 4 days.  The data represent male subjects only; N=5 for each group.
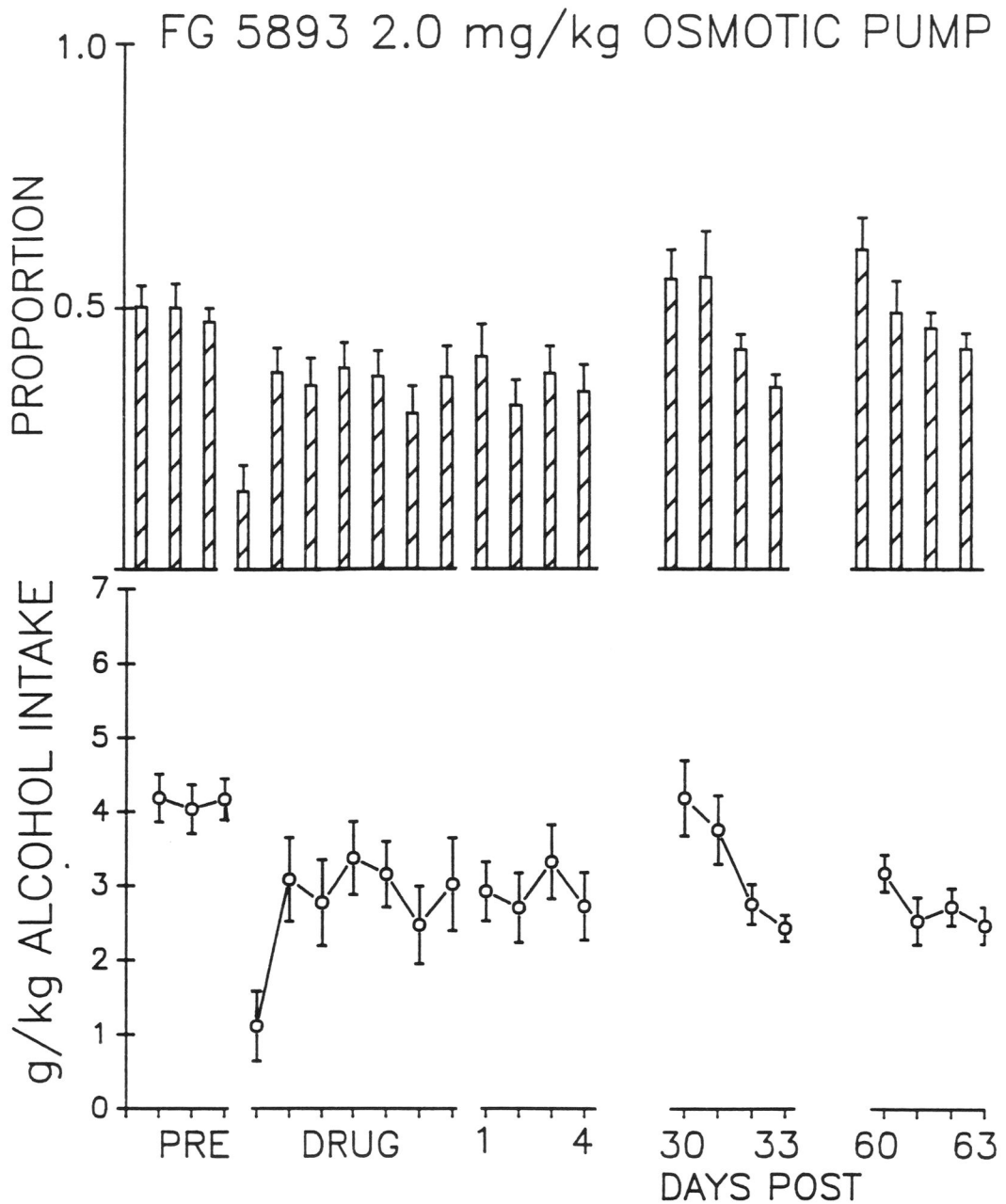
Figure 15. Mean daily alcohol consumption data for animals (N=6) implanted subcutaneously with Alzet mini-osmotic pumps delivering 2.0 mg/kg FG 5893 daily for seven days -- pre for 3 days, drug for 7 days and a 4, 30 and 60 day post period. The top and bottom graphs have the same format as fig. 3.
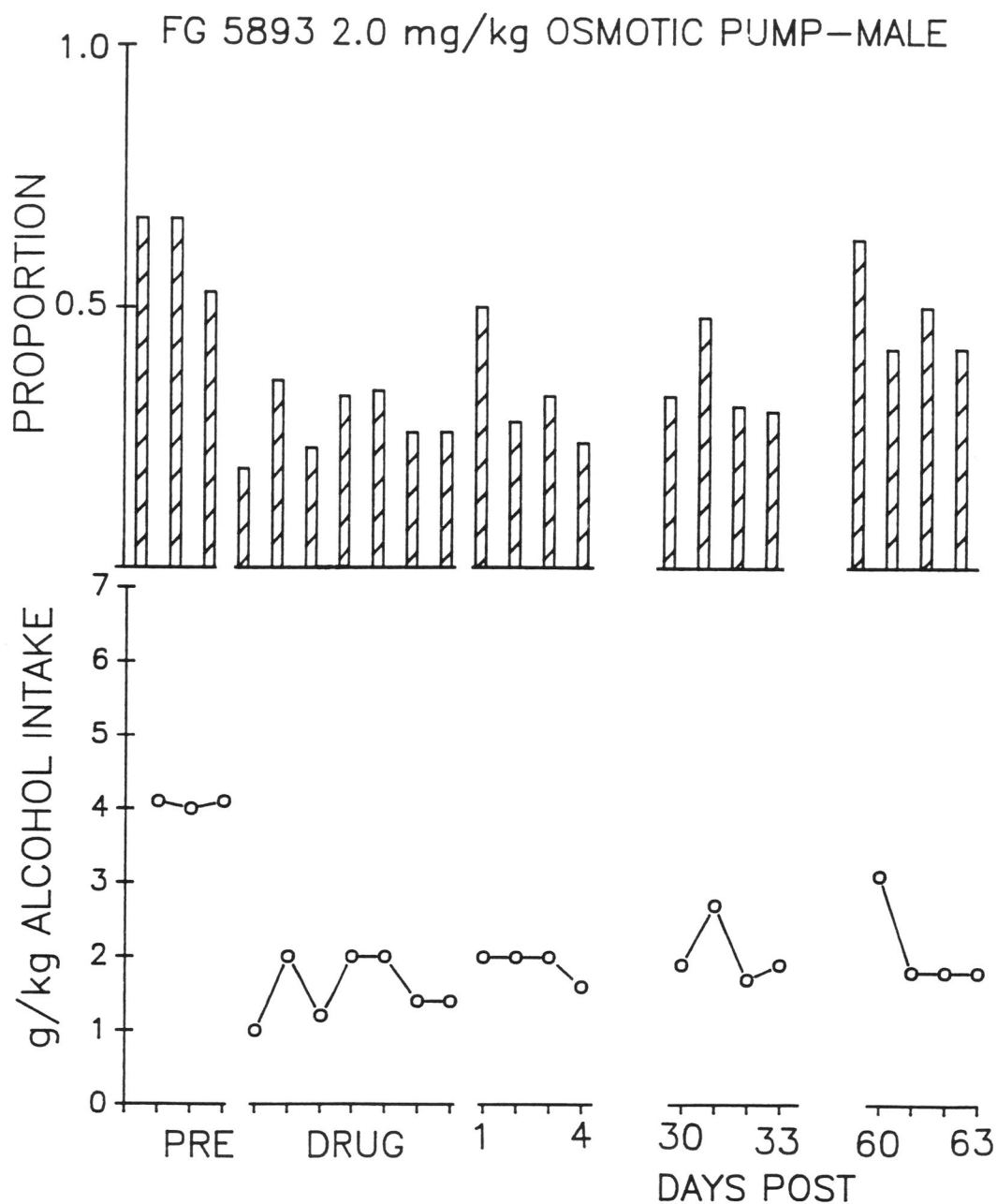
Figure 16.   Daily alcohol consumption for an individual male implanted
            subcutaneously with an Alzet mini-osmotic pump delivering
            2.0 mg/kg FG 5893 daily for seven days -- pre for 3 days,
            drug for 7 days and a 4, 30 and 60 day post period.  The top
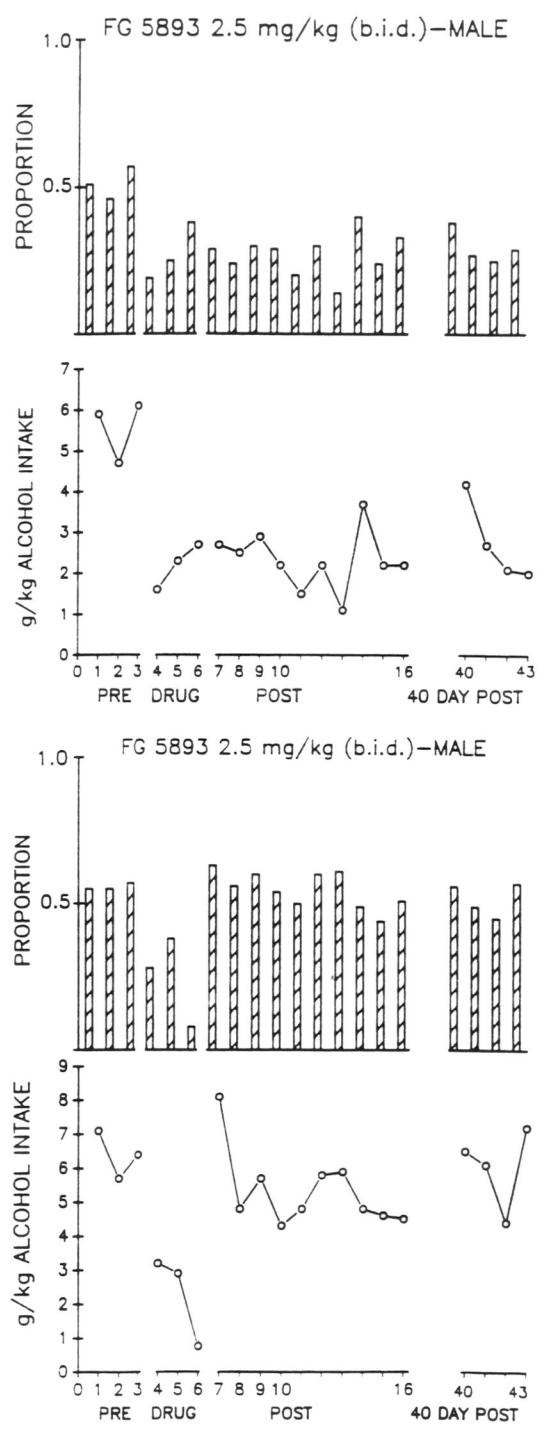            and bottom graphs have the same format as fig. 3.

Table 3

**MEAN ± S.E. EFFECTS ON FOOD, WATER, ETOH AND BODY WEIGHTS OF SIX MALE RATS IMPLANTED SUBCUTANEOUSLY WITH ALZET MINI-OSMOTIC PUMPS DELIVERING 2.0 MG/KG FG 5893 DAILY FOR 7 DAYS. PRE-CONTROL FOR 3 DAYS, DRUG FOR 7 DAYS, AND POST CONTROLS FOR 4 DAYS**

| | FOOD (g) | WATER (ml) | ETOH (ml) | WEIGHT (g) |
|---|---|---|---|---|
| **PRE** | 20.0 ± 0 | 25.4 ± 1.9 | 23.8 ± 1.3 | 605.2 ± 12.0 |
| **DRUG-FG 5893** | 20.0 ± 0 | 29.8 ± 1.4 | 16.2 ± 1.4 | 608.3 ± 8.2 |
| **4 DAY POST** | 20.0 ± 0 | 28.8 ± 1.4 | 17.5 ± 1.6 | 609.3 ± 11.7 |
| **30 DAY POST** | 20.0 ± 0 | 22.2 ± 1.5 | 20.9 ± 1.7 | 650.6 ± 13.9 |
| **60 DAY POST** | 19.8 ± 0.17 | 19.3 ± 1.6 | 18.1 ± 1.1 | 697.7 ± 16.9 |
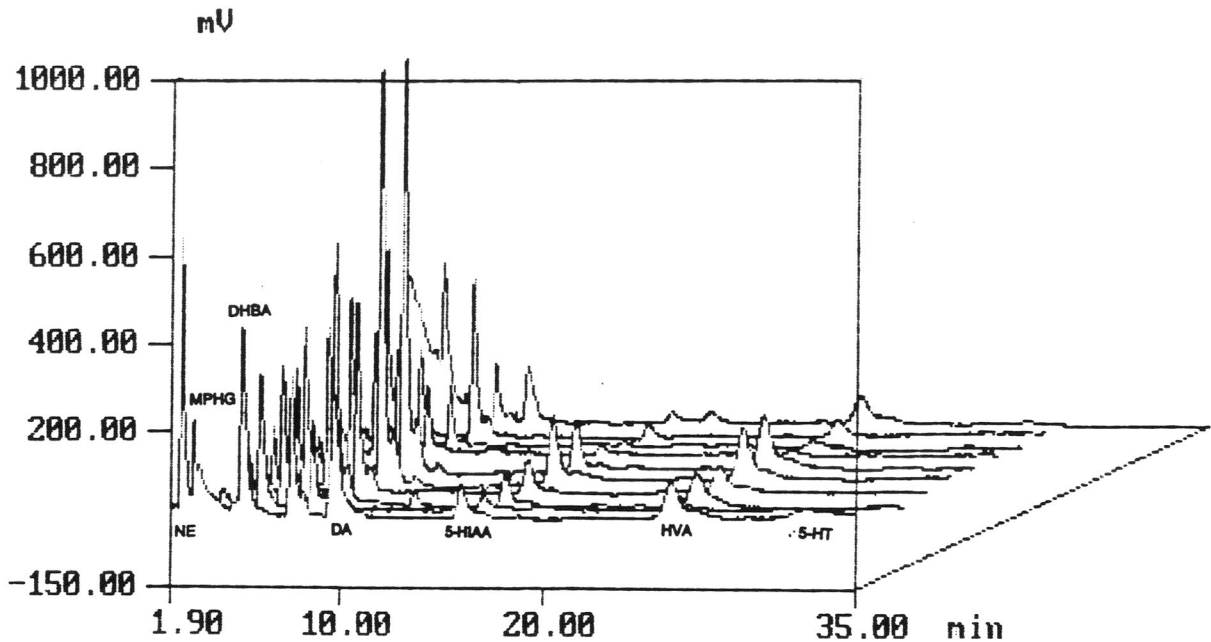
Figure 17. HPLC-CD chromatogram of a representative rat perfused in the nucleus accumbens (8 mm below dura) at a rate of 15 µl/min for 36 minutes. Control CSF perfused 3 times, followed by 3 amperozide perfusions (5 µg/ml), followed by 3 post CSF perfusions. Each perfusate was assayed for content of monoamines and their metabolites. Abbreviations are as follows: NE, norepinephrine; MHPG, methoxyhydroxy-phenylglycol; DHBA, dihydroxy-phenyl acetic acid; DA, dopamine; 5-HIAA, 5-hydroxy acetic acid; HVA, homovanillic acid; 5-HT, 5-hydroxytryptamine.

## TABLE 4

### HPLC-CD ANALYSIS OF AMPEROZIDE SAMPLES (MEANS)

|              | NE    | MHPG  | DOPAC  | DA    | 5-HIAA | HVA    | 5-HT    |
|--------------|-------|-------|--------|-------|--------|--------|---------|
| **PRE**      | 72.1  | 10.2  | 117.7  | 44.5  | 156.1  | 100.5  | 17.0    |
|              | n=22  | n=10  | n=38   | n=14  | n=26   | n=26   | n=7     |
| **AMP LOW**  | 14.8  | 7.7   | 12.2   | 1.20  | 49.7   | 17.9   | 0.0     |
|              | n=22  | n=14  | n=15   | n=5   | n=9    | n=5    | n=0     |
| **AMP LOW POST** | 5.8   | 59.2  | 22.0   | 0.46  | 228.8  | 36.6   | 7.9     |
|              | n=15  | n=2   | n=13   | n=2   | n=4    | n=3    | n=4     |
| **AMP HIGH** | 53.4  | 16.7  | 83.7   | 27.9  | 17.7   | 60.3   | 934.0   |
|              | n=27  | n=14  | n=23   | n=11  | n=16   | n=18   | n=4     |
| **AMP HIGH POST** | 8.4   | 5.97  | 102.1  | 2.72  | 10.3   | 58.1   | 1000.0  |
|              | n=17  | n=11  | n=24   | n=5   | n=15   | n=20   | n=2     |

## DISCUSSION

Since the late 1960's the indoleamine neurotransmitter, 5-HT, has been implicated and investigated extensively for its involvement in the addictive liability of alcohol. Numerous pharmacological studies of drugs which alter the synthesis, turnover or degradation of 5-HT have all affirmed its involvement; however, some conflicting results have raised further questions as to the fundamental mechanisms underlying alcohol drinking and the levels of 5-HT in the brain.

In a 1968 study by Myers and Veale, rats treated with p-chlorophenylalanine (a tryptophan hydroxylase inhibitor) exhibited both a decrease in 5-HT in the brain and a subsequent rejection in the intake of alcohol. Still, further studies in which 5-HT or 5-hydroxytryptophan (a 5-HT precursor) was administered by the intracerebroventricular (ICV) route also confirmed a reduction in alcohol drinking (Myers, Evans and Yaksh, 1972). Rats given 5,7-dihydroxytryptamine lesions in the Nucleus Accumbens showed an elevated pattern in alcohol drinking. This elevation in drinking was ameliorated by sertraline, a 5-HT reuptake inhibitor (Myers and Quarfordt, 1991). Several other pharmacological compounds which augment the levels of 5-HT in the central nervous system by affecting its synaptic reuptake, such as fluoxamine and fluoxetine, also reduce the volitional

selection of alcohol (Gill et al., 1989; Haraguchi et al., 1990). Amperozide, a 5-HT2 antagonist, also exhibits some properties of inhibiting the reuptake for 5-HT, and also decreases alcohol intake in a dose dependant manner in rats (Myers et al., 1992).

FG 5893 exhibits a high affinity for hippocampal $5-HT_{1A}$ and cortical $5-HT_2$ receptors (Pettersson et al., 1992), thereby exhibiting properties of buspirone and amperozide, which both selectively attenuate consumption of alcohol (Privette et al., 1988; Myers et al., 1992). Functional tests indicate $5-HT_1$ antagonist and $5-HT_{2A}$ agonist properties of FG 5893 (Pettersson et al., 1992). By acting on these subtypes of 5-HT receptors, FG 5893 could exert its effect on the same structures of the limbic system as aldehyde adducts such as THP or a ß-carboline, which have both been implicated in inducing the addictive drinking of alcohol (Myers, 1991).

In the present experiments, acute and chronic administration of FG 5893 exerts an ameliorative effect on alcohol consumption. Of particular significance is the fact that FG 5893 did not affect food or water intakes during and following its administration. Unlike other drugs such as naloxone, naltrexone and sertraline, which simultaneously alter alcohol consumption and caloric intake (Myers et al, 1982, Critcher et al, 1982, and Myers and Quarfordt, 1991),

FG 5893 apparently does not impair the central mechanisms controlling caloric intake. Consequently, the reduction in alcohol drinking is not a result of any side effect or secondary action of the drug to inhibit hunger and the intake of calories.

Although the three doses of FG 5893 administered twice daily caused significant reductions in both g/kg and proportion of alcohol to total fluid consumed, a dose-dependent effect of the drug in drinking was not relevant. For example, the lowest dose of 0.5 mg/kg produced the greatest initial percent decline by the 6th injection (37.2% ± 7.8%) as compared to the intermediate dose of 1.0 mg/kg and the highest dose of 2.5 mg/kg (18.5% ± 9.2% and 30.0% ± 16.8% respectively). However, during the 4 day and 40 day post FG 5893 tests, 0.5 mg/kg exhibited the greatest rebound in drinking as compared to the other two doses; i.e. 1.0 mg/kg FG 5893 produced the greatest continual temporal decline. One explanation for these results could be that the optimum excitatory or inhibitory effects of this drug on 5-HT release or selective blockade of $5-HT_2$ receptors is best seen with a low concentration FG 5893. Acute and excessive stimulation of the specific neurons in the CNS normally may be followed by neuronal fatigue and exhaustion of stores of transmitters, which might occur with the higher doses. This could explain

why (1) the 0.5 mg/kg dose was the most immediate efficacious dose, (2) the 2.5 mg/kg dose was no more effective than the 0.5 mg/kg dose, and (3) the 1.0 mg/kg dose produced greater sustained effects.

Differences in the drinking response to FG 5893 not only occurred within the group, but also between males and females receiving the same dose. For instance, Fig. 5 depicts a male and a female rat which that received the same dose of 0.5 mg/kg FG 5893. Although both animals reduced their drinking of alcohol during the days of FG 5893 treatment, the cyclic nature of the female is also seen in its drinking patterns following treatment, while the male rat exhibited a steady reduced drinking pattern after the injections of the drug. Additional examples can be seen in Figs. 9 and 13. Figure 9 depicts two males that received 1.0 mg/kg (b.i.d.), yet both reacted differently to the drug. Although a transient decrease was seen in both cases following treatment, one male was notably affected by the drug, even at the 40 day post test. Similarly, Fig. 13 represents the results of two males given 2.5 mg/kg (b.i.d.) which also were affected differentially. The difference in magnitude of responses to FG 5893 may be due in part to a genetic difference between rats in the number of serotonergic receptors, production of neurotransmitter, its release, the rate of reuptake, or other neuronal processes. Although genetic differences between

different strains of rats is well documented, (Lumeng et al, 1977, Lankford et al, 1991, and Gatto et al, 1987), rats of the same species may exhibit subtle differences which could affect their drinking behavior.

Rather than decreasing alcohol intake during treatment, some animals continued to drink the same amount, while one rat initially doubled its normal consumption, which was followed by an eventual decline. This result could be attributed to non-specific such as the "same room phenomenon" in which a vocal response to an injection of one rat could cause a "fear" response in another rat present in the same room. This could result in an increase in drinking to overcome the "fearful" state. Secondly, the trauma of the subcutaneous injection could increase the level of stress in an animal, which would subsequently augment the drinking of alcohol for its pharmacological effect.

Once alcohol is consumed for an extended period, cessation in drinking can result in severe symptoms of withdrawal (Myers and Melchior, 1977). Most animals treated with FG 5893 exhibited withdrawal-like symptoms, which was characterized by an alcohol "binging-like response". This is clearly seen in Fig. 13, representing a male rat given high dose treatment. It is believed that during periods of withdrawal, enzymes affecting 5-HT metabolism increase; as a result 5-HT metabolism significantly increases during this

period (Griffiths et al, 1974, Tabakoff et al, 1974).

Chronic administration of FG 5893 clearly reduced alcohol consumption over an extended period of time. The "binge-like" drinking observed after FG 5893 acute treatment was not seen during chronic administration, and the decline in drinking was apparent up to 60 days post FG 5893 treatment. It is possible that the chronic treatment with FG 5893 imposes a certain adaptive metabolic or functional change in the nervous system which may be opposite to that seen acutely. As a result, the perturbation caused by chronic treatment of a drug such as FG 5893 may be irreversible; however, this still remains to be investigated by more extensive, long term studies.

Overall, it is known that FG 5893 affects serotonergic neurons; however, the exact mechanism of action in the central nervous system is not yet known. It is conceivable that the $5-HT_2$ antagonist properties of the drug increase the release of 5-HT as a result of the selective blockade of these receptors. At the same time, $5-HT_{1A}$ agonist properties could result in an increased stimulation of these receptors. Future in vivo studies of FG 5893 will ultimately reveal the answers to these questions.

Whether or not treatment with FG 5893 may be applied to the problem of human alcoholism is unknown, especially with problems comparing the human condition with animal models.

Further research, therefore, is essential to examine the effect of FG 5893 on genetically bred animals predisposed to drink, rather than those induced to drink as a result in an impairment in an aldehyde dehydrogenase isozyme. Although several drugs have been tested for their action in reducing the drinking of alcohol, the development of specific treatments for the different aspects of alcoholism, nevertheless, must be considered concomitantly with those of pharmacological intervention in the human condition (Litten and Allen, 1991).

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