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# LINKS BETWEEN INTAKE OF ETHANOL AND NICOTINE AND REWARD-RELATED MECHANISMS

Mara Roisin Laura Bermingham

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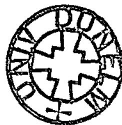
Submitted for the attainment of a Master of Science degree



University of Durham
Department of Psychology
1997

1 2 MAY 1998

# ABSTRACT

1. Examination was made of the effects of housing conditions on the preference of mice for dilute ethanol or water in a two-bottle choice. Isolating mice from groups of five after 10 days significantly increased their ethanol preference, compared to mice remaining group-housed or mice accustomed to single housing.

2. The effects of sucrose choice, followed by ethanol administration, were examined on behaviour on the plus maze, to determine whether the use of sucrose as a "comparison" solution altered behaviour. It was found that behaviour did not vary significantly with the level of mean daily voluntary sucrose consumption. Ethanol decreased anxiety-related behaviours of mice independent of their level of daily sucrose consumption.

3. Dilute nicotine was offered to mice in a two-bottle choice test. The effect of subsequent administration of ethanol was examined on behaviour on the plus maze. Ethanol exerted some behavioural effects indicative of decreased anxiety after nicotine choice, but a wider range of these behaviours were seen in control mice (water drinkers.)

4. The effects of offering dilute nicotine, dilute ethanol, or a mixture of the two, in a chronic two-bottle choice paradigm, were measured. The mean daily ethanol intake of mice with and without the addition of nicotine to the drinking solution did not significantly differ. However, the mean daily intake of nicotine alone was significantly lower than the intake of nicotine where ethanol was added to the solution.
All mice were exposed to the elevated plus-maze twice: once whilst in withdrawal and once when not in withdrawal. Plus-maze results indicated that withdrawal from both chronic ethanol and nicotine simultaneously showed a wider range of anxiety-indicative behaviours than withdrawing from chronic ethanol alone.

5. Alterations were made to the conditioned place preference paradigm but it did not prove possible to obtain conditioned preference to morphine within the time available.

## Declaration

The material contained within this thesis has not previously been submitted for a degree in this or any other university.

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Mara Bermingham
October 1997

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# INTRODUCTION

"A cigarette is the perfect type of a perfect pleasure. It is exquisite and it leaves one unsatisfied." From: The Picture of Dorian Gray by Oscar Wilde.

"If all be true that I do think,
There are five reasons we should drink;
Good wine, a friend, or being dry,
Or lest we should be by and by;
Or any other reason why."
From: A Catch by Henry Aldrich (1647-1710)

" A branch of the sin of drunkenness, which is the root of all sins."
From: A Counterblast to Tobacco by James I of England and VI of Scotland (1566-1625)

## 1.1 Origins of drug use

The production of ethanol by fermentation of various plant products is an ancient art practised by many primitive civilizations worldwide. Tobacco was introduced into Britain in the sixteenth century and since then has become a habit for a sizeable proportion of the population. All over the world, awareness of the psychoactive properties of plants such as hemp, coca, the opium poppy and tea-shrub goes back many centuries. Today, ethanol and nicotine are widely accepted in many societies, particularly in the Western world.

## 1.2 Alcoholism

Alcoholism is a major problem, particularly among the unemployed, publicans and those with jobs which involve high stress levels and/or prolonged isolation from their families. It has been defined as "a primary, chronic disease whose onset and cause is

influenced by genetic, psychosocial and environmental factors" (Madden, 1993.) The annual cost of alcoholism in the UK for as long ago as 1987 was estimated to be in the range of £60 million- 2 billion. This estimate included factors such as direct health care costs, years of working life lost, and the cost of car accidents and other crimes committed whilst under the influence of alcohol (Crofton, 1987.)

## 1.3 Nicotine use

In the early 1960s, most experts believed that smoking was a psychologically-based habit people indulged in to experience the taste and smell of the smoke and to gain oral satisfaction in the psychoanalytic sense. Since the 1970s, the detrimental health consequences of smoking cigarettes began to receive publicity. By 1980 few researchers in the field questioned the addictive nature of tobacco. However, doctors and the general public even today are slow to accept this finding, because drugs such as heroin tend to be seen as a model for all addictions (Stolerman, 1990.)

Excessive cigarette smoking does not engender the same antisocial behaviour as alcoholism although the habit itself is increasingly considered to be one that is antisocial. There is no official scale of daily nicotine consumption which can be used to diagnose the presence of an addiction to nicotine as there exists for alcohol. The major conclusion of a United States Surgeon General's Report on nicotine addiction (USDHHS, 1988) was that people smoke because they are addicted to nicotine. However, the acknowledgement that nicotine is addictive has been questioned (Robinson and Pritchard, 1992, Reynolds Tobacco Company) mainly because the drug does not produce intoxication in the same way as other classically addictive drugs. In addition, the symptoms of withdrawal from long-term tobacco use are quite different from, and less severe than, withdrawal from chronic alcohol or opiates.

## 1.4 Treatments for drug dependence

Many different treatments are available to help those who wish to stop being alcohol or nicotine-dependent. Behavioural and pharmacological therapeutic approaches are often used in combination. A minor part of the present study investigated the effects of drugs such as acamprosate and nimodipine, which can be used to treat alcohol dependence. Acamprosate has been shown clinically to reduce relapse and/or its severity

in alcoholics undergoing detoxification (Whitworth et al, 1996.) Nimodipine is a calcium-channel blocker which has been shown in animal studies to prevent the development of tolerance to ethanol (Little and Dolin, 1987), and suppress preference for ethanol (Pucilowski et al, 1992.) However, nimodipine has not yet been used clinically because of its extensive actions at calcium channels in the periphery.

## 1.5 Alcoholism, nicotine use and other compulsive activities

A major part of the present study was spent determining some of the links between voluntary intake of ethanol and nicotine, because tobacco and alcohol are frequently used together (deFiebre and Collins, 1992.) Drugs of abuse are rarely taken in isolation. Links between drug abuse and various activities such as excessive gambling have lead many psychologists to attempt to define the characteristics of a dependence-prone personality. However, retrospective personality assessment, where the individual's personality before drug use started is determined, is notoriously difficult to carry out and very unreliable (Ghodse, 1989.)

The recreational use of other psychoactive drugs such as ecstasy, other amphetamines, cocaine, cannabis, opiates and benzodiazepines, although largely illegal, is widespread across most Western societies. It is still consumption of alcohol and tobacco, though, that is perceived by any standard as being 'normal' and incorporated into every aspect of daily life (Ghodse, 1989.) The enormous revenue from these two drugs' taxation is highly conducive to governments maintaining their legality.

## 2.1 Reward

It would be naive to assume that the alcohol produced by ancient societies was drunk purely as a dietary supplement or only because the quality of the drinking-water was dubious. Similarly, it would be absurd to suggest that thousands of people taking ecstasy at a weekend rave were doing so simply because of the thrill of purchasing power they experienced when buying tablets. Reward is a common factor linking all psychoactive drugs throughout history and is a critical theme running through this investigation.

Drugs such as ethanol and nicotine could serve as "rewards." This term means that humans and other animals can learn or become "conditioned" to find these drugs

rewarding, and so continue to seek them out (Stolerman, 1990.) Reward can be attained on administration of a psychoactive drug itself; as triggered by environmental cues normally associated with the drug or even in the absence of either, simply by thinking about drug-associated events (Robinson & Berridge, 1993.) Many neural pathways have been suggested as substrates of drug reward, particularly the mesolimbic dopamine system (Nestler, 1992.)

### 3.1 The present study

Mice were used as models to attempt to measure the reward associated with intake of ethanol, nicotine, and the two drugs in combination. Variables such as the number of mice housed together in a cage, the concentration of the drug offered or administered, and the independent effect of sucrose (often added to sweeten drug solutions) were examined as to their bearing on the measured reward.

The broad aims of the present study were to investigate the effects of nicotine and ethanol on mouse behaviour; in particular, behaviour indicative of reward. A clearer understanding of the reward attributable to each drug might lead to an explanation as to why these two drugs are often simultaneously used (and abused) by humans.

### 4.1 Experimental methods

Because of the nature of reward, it can only be measured indirectly and inferentially. In this investigation, two methods were used which can give some measure of reward. These were the two-bottle choice paradigm and conditioned place preference test. A further method used was withdrawal anxiety testing. It is necessary to describe further the rationale for using these procedures and to illustrate more fully how they work.

### 4.2 The two-bottle choice test

The two-bottle choice test is useful in that it provides a good model for human behaviour, because intake of the drug is voluntary, with a constant supply of water and food available at all times. Particularly for ethanol, the oral route of administration of the drug is of course within the normal experience of all typical laboratory subjects, and the

normal route of ethanol administration in humans. A salient feature of voluntary oral intake of drugs is the palatability of the solution. This factor was particularly important in planning nicotine-drinking experiments, necessitating a preliminary investigation into the effects of sweeteners themselves before adding them to disguise the taste of drug solutions.

In using the two-bottle choice paradigm, it is preferable to house mice in single cages. However, it is known that isolating mice constitutes a stress (Brain, 1975.) Pertinently, the stress caused by simply weighing the bottles and animals and cleaning-out cages has been shown to have significant effects on factors such as ethanol preference (Smith et al, 1994) so the first experiment carried out was intended to investigate the effects of housing and the normal maintenance routine on the drinking patterns of mice. The effects on drinking patterns incurred by isolating TO mice from their groups was monitored. The results from this experiment provided a reference point for the rest of the project.

### 4.3 Conditioned Place Preference

Investigating the existence of a conditioned place preference is another way to test reward in animals. It uses a Pavlovian conditioning procedure. A general version of the test is where animals experience two distinct neutral environments subsequently paired spatially and temporally with distinct drug states. Later, in a drug-free state, the animal is given free run of both environments. The duration of time spent in either environment is seen as an index of the reinforcing value of the drug. Evidence of a positive reinforcement experience from a drug is assumed if the animal spends more time on the side previously paired with that drug. In this case the previously neutral stimuli becomes a secondary positive reinforcer. Conversely, if the subject spends less time in the environment paired with the drug, the drug is assumed to have an aversive effect on the animal, and the previously neutral environment becomes a secondary negative reinforcer.

An early demonstration of conditioned place preference was carried out by Olds and Milner (1954.) Rats stimulated in one particular environment with an intracranial electrode returned to that environment when allowed free run of both that and another neutral environment.

## 4.4 Withdrawal anxiety

Withdrawal from many drugs of abuse causes symptoms typically described as a mixture of anxiety, dysphoria and drug craving (Naranjo and Sellers, 1986.) Observations of animals confirm the anxiogenic effects of drug withdrawal (Emmett-Oglesby et al, 1983.) That withdrawal anxiety exists at all has been demonstrated widely for ethanol (Rezazadeh et al, 1990, among others) but only partially for nicotine (Emmett-Oglesby et al, 1990.) This induced anxiety may be critical in the genesis and maintenance of alcoholism (Emmett-Oglesby et al, 1990) and may have some role in other addictions too (Markou et al, 1994.) The elevated plus-maze was used to obtain a behavioural measures of anxiety in the mice used. In this apparatus, the combined qualities of the elevation of the maze platform and the exposure of two 'open arms' of the maze produce a potentially aversive environment which only mice with lower levels of anxiety will be inclined to explore.

One symptom common to withdrawal syndromes, and largely irrespective of the class of drug of abuse, is a negative motivational/affective state. Intracranial self-stimulation reward thresholds can provide a quantitative measure of this state. For example, rats were made dependent on ethanol by exposure to ethanol vapour for two weeks. The blood alcohol level was measured for ten days preceding and following cessation of the drug. On cessation of the ethanol, the magnitude and duration of the reward thresholds measured over time increased in direct proportion to the decrease in blood alcohol level measured. The experiment was repeated for cocaine and morphine withdrawal with similar findings. (Markou et al, 1994.)

A fall in blood drug levels is a physiological marker which follows withdrawal of a drug. An increase in reward thresholds is purported, above, to be another such marker. My idea was that yet another effect of drug withdrawal- anxiety- could be directly related to the pre-withdrawal rewarding effects of the drug. It occurred to me that the more rewarding a drug, the higher the level of anxiety that might be engendered on removal of the same. The anxiety which followed withdrawal of ethanol and nicotine, and predicted change in anxiety when the drugs were reintroduced, were postulated as giving some index of the drug's associated reward.

It would be incorrect to suggest that the two-bottle choice test, conditioned place preference paradigm and withdrawal anxiety test are equivalent in scale or quality of reward measurement. Whether they are directly comparable or not is neither here nor

there; the tests just provide three useful and distinct behavioural tools for the study of a subjective effect.

## 5.1 Limitations

As with all studies of the effect of psychoactive drugs on living organisms, one must constantly bear in mind the extraordinary plasticity of the interface between drugs and behaviour. This is not intended as a disclaimer for the work described in the following pages; in fact all behavioural psychopharmacological research must develop within these boundaries and my research builds on mainly well-established methods and principles. Awareness of the limits of the applications for these results is as important as the awareness of potential research directions generated from them.

# METHODS

## I ETHANOL PREFERENCE EXPERIMENT, GROUPS/SINGLY HOUSED

### 1.1 Aims

This first experiment was intended as a 'control' for the planned future drinking experiments. The results of this experiment were intended to show the expected drinking patterns and preferences for ethanol of male TO mice when housed in groups and when isolated from the same groups. Isolating mice would be necessary in future experiments in order to be able to determine the precise drug intake per animal, so the effect the act of isolation itself had on mice's preference for ethanol was important to investigate. The effect that routine procedures (such as cage-cleaning and weighing of mice) had on ethanol drinking was also monitored.

### 1.2 The two-bottle choice paradigm

This test was chosen in order to give an index of the effect ethanol had on the mice tested, because ethanol drinking and preference for ethanol over other fluids have long been regarded as possible indices of ethanol's pharmacological effects (Myers and Veale, 1972.) Two-bottle choice experiments involve offering the subject continuous access to a second, test solution, in this experiment 8% (v/v) ethanol, in addition to the bottle of drinking-water, and measuring the amount drunk from each bottle every day. A measure of 'preference' can be calculated by finding the ratio of test solution drunk to the total fluid drunk (test solution plus drinking-water.) In this experiment, the position of the two bottles was swapped each day after weighing to avoid the possibility that the mouse might just be favouring a particular bottle position rather than the solution inside the bottle. The mice used were accustomed to drinking tap-water, so this was used in all two-bottle choice experiments as drinking-water and as a solvent.

## 1.3 Rationale behind experiment design

Male TO mice were chosen for this experiment. This choice was made because the same strain were being bred in-house, thus ensuring their availability for future experiments. Mice are cheaper to run in experiments because they have relatively lower ongoing maintenance costs and, because of their smaller size compared to rats, require and consume lower quantities of experimental drugs. Literature abounds for both rats and mice of many strains which have undergone various two-bottle choice experiments, so points of reference were available whatever the species chosen. The effect of housing conditions and isolation on ethanol preference in mice has been investigated and discussed before, notably by Smith et al (1994) and Brain (1975.) The size of the groups of mice, as opposed to the singly-housed mice, was chosen to be 5 individuals. Admittedly, this number was based on other researchers''standard group' sizes (Young and Bristow, 1995;Wolffgramm and Heyne, 1995) although their reasoning behind this choice never appeared to be discussed in these references.

8% (v/v) was chosen as the ethanol concentration used in the two-bottle choice test. Several strains of mice will drink higher concentrations than 8% in two-bottle choice experiments (Phillips et al, 1994; Belknap et al, 1993) but in general, the lower the concentration of the ethanol solution, the more likely it is that the mouse will drink it. Smith et al (1994) used 8% (v/v) ethanol in their two-bottle choice studies of the effect that handling and cage cleaning had on ethanol preference in mice. Using the same concentration in the present experiment allowed some useful comparisons to be made between the two. (A later experiment of mine examined TO mice preferences for a range of ethanol concentrations- see Methods section 4.1.)

## 1.4 Method

The subjects used were 140 male three-month old TO mice. There were four main treatment groups. All groups were given a two-bottle choice test; the second bottle containing tap water. Standard laboratory chow was available at all times. Test solutions for the four groups were as follows:-

a) 20 singly-housed controls (tap water)

b) 20 singly-housed ethanol (ethanol 8% v/v)

c) 10 control groups of mice housed in groups of 5 (tap water)

d) 10 ethanol groups of mice housed in groups of 5 (8% v/v ethanol)

The consumption of both solutions was measured at 1400h each day for the entire duration of the experiment. When cleaning-out of cages and weighing of mice were carried out, both were done on the same day to minimise the stress caused to the animals.

Table summarising method

| Time course | Treatment/housing groups | | | |
|---|---|---|---|---|
| | n=20 single ethanol | n=20 single control | 10 groups x5 ethanol | 10 groups x5 control |
| 1st 10 days | No change | No change | No change | No change |
| Days 10-16 | +n=10 newly single from 2 of ethanol group-housed | +n=10 newly single from 2 of control group- housed | 8 groups x5 ethanol | 8 groups x5 control |
| Days 17-28 | +n=20 always single, newly ethanol from control singles | Same mice minus the original n=20 single controls | + 6 groups from control group-housed | 2 groups x5 control |
| Day 28 | +n=10 newly single, newly ethanol from 2 control groups | no change | no change | none |

# II SUCROSE DRINKING EXPERIMENT

## 2.1 Aims

Alcohol is normally made more palatable to humans by being sweetened and flavoured, rather than being served in a straight ethanol/water mix. Sweeteners can also be used in drinking experiments in order to mask the aversive taste of high-concentration ethanol or other bitter drugs which might not otherwise be ingested voluntarily (Ksir and Mellor, 1992; Wolffgramm and Heyne, 1995; Schulteis et al, 1996.) It was planned later to carry out drinking experiments with nicotine, a bitter-tasting substance in solution, so the use of sucrose or another sweetener, in a 'fading' procedure (Samson, 1986) was anticipated. (Sucrose 'fading' is a way of weaning an animal onto an aversive-tasting, psychoactive drug by gradually decreasing the concentration of sucrose 'mask' until the animal learns the association between the drug and its effects.) However, first it was considered important to determine what behavioural effects, if any, sucrose exerts, both alone and in conjunction with ethanol. This fundamental question was the main aim of this experiment.

The effect that nimodipine (a calcium-channel blocker) had on voluntary sucrose consumption was also investigated. Administration of calcium-channel blockers has been shown to prevent the development of tolerance to ethanol (Little and Dolin, 1987), and suppress preference for ethanol (Pucilowski et al, 1992) and sucrose solutions (Pucilowski et al, 1994.) The results from this experiment could indicate the extent of the underlying influence of calcium channels on sucrose consumption.

## 2.2 Rationale behind experiment design

Male, singly-housed TO mice were used. Singly-housing the subjects allowed precise day-to-day monitoring of every individual's sucrose consumption. As in the first experiment, a two-bottle choice test was used, where a 10% (w/v) sucrose solution was offered alongside tap water. This concentration was chosen because it is a typical starting point in sucrose-fading procedures (Samson, 1986; Petry and Heyman, 1995.)

The two doses of ethanol chosen to administer to mice prior to exposure to the elevated plus-maze were 1 g/kg and 1.75 g/kg. At a dose of 1.5 mg/kg (i.p.) ethanol has been shown to produce both anxiolytic and slight motor activity-depressing effects on the elevated plus-maze in mice (Melchior and Ritzmann, 1994) so two doses above and below this value were chosen.

The doses of nimodipine chosen were 5 mg/kg and 50 mg/kg. The latter dose has been shown to decrease (Dolin and Little, 1989) tolerance to ethanol in rats, depending on the time of drug administration, and it was assumed to be pharmacologically active for mice at this dose too.

## 2.2 The elevated plus-maze

The elevated plus-maze has been used as an animal model of anxiety. In designing animal analogues of anxiety, animals are usually exposed to stimuli which can be interpreted as capable of causing anxiety in humans. The stimuli come under two broad categories: exteroceptive stimuli, (such as unavoidable electric shocks) which originate outside the body, and interoceptive stimuli, (such as administering an anxiogenic drug) which originate inside the body. Animals can be observed for responses or behavioural deficits resulting from those stimuli in order to provide an index of anxiety (Lal and Emmett-Oglesby, 1983.)

The elevated plus-maze was developed from the work of K.C.Montgomery in the 1950s and is based on the observation of spontaneous activity of rodents placed an aversive environment produced by height and open spaces (Reiband and Bohme, 1993.) Montgomery used a Y-shaped elevated maze composed of open and closed arms. Rats were found to explore the enclosed arms significantly more frequently than the open arms. He reasoned that while both the open and closed arms would evoke the same exploratory drive, the open arms would evoke more fear than the closed arms, resulting in less exploration therein (Montgomery, 1958.) An elevated plus-maze was used in this experiment to obtain a measure of the behaviour exhibited in mice after chronic exposure to sucrose and the effect of ethanol on this behaviour. The type of maze used in this experiment consisted of a cross-shaped apparatus made of two open arms facing each other and two closed arms (which had walls made from clear perspex) disposed at right angles to the open arms. The maze was elevated forty-five centimetres above the floor by a single central support. The mouse to be

tested was placed in the centre of the apparatus and its exploratory behaviour was recorded over several minutes using a video camera linked to a VCR and monitor in an adjacent laboratory. Variations in light intensity modify the basal state of "anxiety" of the animals and therefore change the sensitivity of the test to drug effects. When exploring the open arms of the maze, rodents are particularly sensitive to sound disturbances, thus remote video surveillance was the preferred way of monitoring events (Reiband and Bohme, 1993.)

The test period for observation of each mouse's behaviour on the elevated plus-maze was five minutes. This time was chosen because Montgomery (1958) demonstrated that avoidance behaviour was particularly marked over this time but began to decrease towards the end of a 10-minute period. After five minutes the mouse was replaced in its cage. The events of the test session were classified later in terms of the behaviour exhibited. The computer programme used to aid this continuous assessment was Hindsight 1.4. A list of the behavioural parameters scored off the videotape follows:

1) Total number of entries made into open arm
2) Total number of entries made into closed arm
3) Total number of entries made into either arm
4) Percent of total entries into both arms made onto the open arm
5) Percent of total entries into both arms made into the closed arm
6) Percent of total time spent in the closed arm
7) Percent of total time spent on the open arm
8) Non-exploratory behaviour: The combined duration(s) of immobility and grooming.
9) Closed arm return,frequency of: exiting a closed arm with only two paws and returning (doubling back) into the same arm (after Moser, 1989.)
10) Head dip: an exploratory forward head/shoulder movement over the side of the maze and down towards the floor. This behaviour was differentiated as 'protected' (occurring on or from the relative security of the closed arms or central platform) or 'unprotected' (occurring on the open arms)(Cole and Rodgers, 1994.)
11) Stretch attend posture, mean net duration of: an exploratory body posture where the mouse stretches forward and retracts to its original position without actually moving from its pre-stretch location (Pollard and Howard, 1988.) This behaviour was also categorised as 'protected' or 'unprotected' under the same criteria as for head-dips, above.

## 2.4 Method

The subjects used were 70 male TO mice, placed in single housing with food and water and left to habituate for 14 days. During the 15 days following the habituation period, the mice were given the choice of drinking from an additional bottle on the right hand side (not the normal water bottle position) containing 10% sucrose (100 g diluted in 1litre tap water.) Both bottles were weighed each day. Standard laboratory chow was available at all times. Cleaning-out of cages was done on the same day as weighing the mice, at least once a week. The mice were assigned to various treatment groups based on their mean sucrose consumption per day during the post-habituation period. The groups were:-

| Sucrose consumption range, g/kg/day | Number in group | Treatment(s) (all injections i.p.) |
|---|---|---|
| 0-20 | 6 | all saline 0.9% |
| 20.5-30 | 25 | n=9: 1 g/kg ethanol; n=9: 1.75 g/kg ethanol; n=7: saline 0.9% |
| 30.5-40 | 18 | n=9: 1.75 g/kg ethanol; n=9: saline 0.9% |
| 40.5-50 | 14 | n=7: 1.75 g/kg ethanol; n=7: saline 0.9% |
| 50.5-70 | 7 | all saline 0.9% |

Mice were tested on the plus-maze fifteen days after the first introduction to sucrose. (By this time the individual variance in mean daily sucrose consumption was low.) Lights came on in the holding-rooms at 08:00h. The mice were transferred to the behavioural laboratory (lit by two 60W red lights) at exactly 09:00h. (Red light is invisible to mice so conducting experiments by this light would help to reduce the anxiety due to factors other than the plus-maze itself.) At 09:40h. the first injection was administered, and this mouse was put on the plus-maze at 10:00h. (i.e. a contact time of 20 minutes.) Whilst the mouse was on the maze (five minutes,) the bottles were weighed and replaced. Straight after testing, the mouse was returned to its cage.

The activity of the mice on the maze was recorded on a video and analysed using the Hindsight 1.4 programme. Post-test monitoring of sucrose and water consumption was carried out for a week. After this week, those mice which had had ethanol injections and those in group L underwent euthanasia , leaving 30 mice which had so far received only saline injections. These mice were split into 3 groups:

1) n=5 received nimodipine 5 mg/kg, n=5 received tween 80 (0.05%)
2) n=5 received nimodipine 50 mg/kg, n=5 received tween 80 (0.05%)
3) n=10 received tween 80 (0.05%)

The subjects were matched as closely as possible between groups for their average recent sucrose consumption (g/kg.) Injections (all i.p.)were given at 19:00h, (one hour before lights off) on four consecutive days. The side of the injection was altered on a daily basis to reduce peritoneal irritation. Sucrose consumption was monitored for four days after the last injection.

## III ETHANOL AND NICOTINE DRINKING EXPERIMENTS

### i) NICOTINE DRINKING

#### 3.1 Aims

This experiment was intended as a preparation for a planned long term nicotine/ethanol drinking study, in which the voluntary intake of both drugs would be examined. In the present experiment, it was necessary to investigate firstly whether TO mice would drink an unadultered nicotine solution. This was because results from the sucrose drinking experiment, carried out immediately prior to this experiment, did not indicate that sucrose was merely an inactive vehicle, whose effects on the mouse could be disregarded when used as a mask for unpalatable drugs. Therefore, it would be far preferable if mice would voluntarily drink nicotine without the use of sucrose fading. One aim of the

experiment was to achieve just this, but equally to ensure that the resulting mean daily nicotine intake was not so low as to be negligible. A further aim was to investigate the behavioural effects of nicotine on mice, both alone and when in conjunction with ethanol.

### 3.2 Rationale behind experiment design

The two-bottle choice test was chosen, where mice were given continuous access to a bottle containing nicotine solution and another containing tap water. The dose of nicotine chosen was 1 g/l (v/v). It was hoped that this concentration of nicotine was weak enough not to be too aversive in taste to the mice. However, if this was found not to be the case, it was intended to lower the concentration of nicotine in a second pilot study. Of the very few two-bottle choice experiments for mice using unsweetened nicotine, Meliska et al (1995) used a considerably lower concentration range of 1.0-40.0 μg/ml nicotine, compared with the equivalent 1 mg/ml nicotine used in this experiment. It was calculated that by drinking 2 ml of a 1 g/l nicotine solution per day, a 35 g mouse would be consuming nearly 60 mg/kg/day nicotine. This would be a far from negligible intake; a typical injected dose of nicotine would be, for mice, between 0.2 mg/kg (Johnson et al, 1995) and 4.0 mg/kg/h (Collins et al, 1993.) In studies where nicotine is administered by intubation, Leblebicioglubekcioglu et al (1995) used a dose of 12 mg/kg three times daily.

After twenty-nine days of nicotine exposure, all mice were tested on the elevated plus-maze. (Problems with leaky bottles necessitated a wait of 29 days until individual daily nicotine consumption levels could be measured accurately and found to have a low day-to-day variance.) The effect that an injection of 1.75 mg/kg ethanol or saline had on the anxiety of the nicotine-drinking mice was measured using the plus-maze. This dose of ethanol was the higher of the two doses used in the preceding sucrose/ethanol experiment. It was chosen again because the results of the preceding experiment showed that mice injected with this dose were found to exhibit significant changes in several behavioural measures on the elevated plus-maze, compared to mice receiving saline injections.

The mice were exposed to the plus-maze a second time one week after the first exposure so that the results of the two test days could be compared, and to measure the effect of both plus-maze exposures on subsequent daily nicotine consumption.

## 3.3 Method

Thirty-two male TO mice were used. They were presented with a choice of two bottles in their single cages. Half the mice had two water bottles, whilst the other half had one water bottle on the left hand side plus an additional bottle containing 1 g/l (v/v) nicotine solution. Standard laboratory chow was available at all times. Bottles were weighed at 14:00h daily and the mice were weighedevery three days.

After fourteen days of two-bottle choice, the mice in the nicotine group were given only one bottle which contained freshly made-up nicotine solution. (The control group were given one, rather than two, bottles of water.) This regime lasted for five days. Return to two-bottle choice for all mice was prompted by a weight drop in the nicotine-drinking mice. After 29 days of exposure to nicotine or water, all 32 mice were exposed to the elevated plus-maze. For both the nicotine/water and the water/water choice groups, the treatments were as follows:-

n=8 were injected with 1.75 g/kg ethanol i.p.
n=8 were injected with 0.9% saline i.p.

Lights came on in the holding-rooms at 08:00h. The mice were transferred to the behavioural laboratory (lit by two 60W red lights) at 09:00h. At 09:40h the first injection was done, and this animal was put on the plus-maze at 10:00h (i.e. a contact time of 20 minutes.) Whilst the mouse was on the maze, the bottles were weighed and replaced. Straight after testing, the mouse was returned to its cage. The activity of the mice on the maze was recorded on a video and analysed using the Hindsight 1.4 programme. The nicotine/water consumption was measured daily in the week following this first plus-maze exposure.

After one week, the plus-maze was carried out exactly as before (the same mice received the same injections.) The daily nicotine/water consumption continued to be measured for one week following the final plus maze experiment.

## ii) ETHANOL DRINKING

### 4.1 Aims

Like the nicotine drinking experiment described above, this experiment was intended as another preparation for a long term nicotine/ethanol drinking study. The main aim here was to find the optimum concentration of ethanol to use for TO male mice in this future experiment. The 'optimum' ethanol concentration would be decided on several bases; firstly, whether this concentration was palatable enough to TO mice to be consumed voluntarily and without a sucrose sweetener. (As explained in the 'Aims' section of the nicotine experiment above, it would be preferable to avoid the use of a sweetener entirely.) Secondly, the 'optimum' ethanol concentration would be sufficiently high so that the actual dose of ethanol self-administered on drinking it would be appreciable and consistent for all mice in that treatment group. (Clearly, the lower the concentration of ethanol in solution, the greater the quantity of that solution which must be drunk in order to achieve the same effect.) 8% ethanol (v/v) was used in the first experiment but it would have been invalid to assume that 8% was the optimal choice without investigating a range of other concentrations too. A further aim of this experiment was to compare the behavioural effects on mice of withdrawing them from the different concentrations of ethanol. This knowledge would be useful in planning the following long-term ethanol/nicotine experiment (section 5.1) in which withdrawal from ethanol was to be a crucial part.

### 4.2 Rationale behind experiment design

The two-bottle choice test was chosen, where mice were given continuous access to a bottle containing ethanol solution and another containing tap water. The nine doses of ethanol chosen ranged from 0 (tap water) to 20.0% (v/v) in increments of 2.5%. The elevated plus-maze was used to test for the behavioural effects of withdrawal from these solutions after three weeks, by which time day-to-day variance in individual ethanol consumption was low.

## 4.3 Method

The subjects used were 72 male TO mice, which were randomly assigned to one of nine treatment groups: either 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 or 20.0% (v/v) ethanol solutions (8 mice per group.) The mice were housed singly and did not undergo an habituation period prior to introduction of their respective test solutions, which were always placed on the left hand side of the cage. All ethanol solutions (v/v) were made with tap-water. All the bottles used were of the same type (rubber-bunged) and were weighed at 14:00h each day. Standard laboratory chow was available at all times. Cleaning-out and weighing of mice were carried out on the same day each week to limit the possible stress incurred by these procedures. Three weeks later all mice were tested on the elevated plus-maze. Testing had to be conducted over two days because of the large number of mice involved. The procedure on these two days was as follows:

Lights came on in the animal holding rooms at 08:00h, and the mice to be tested that day were transferred to the behavioural laboratory at 09:00h. Their ethanol bottle was removed at the same time (or the left hand water bottle for the water/water group.) The first mouse was tested on the maze for 5 minutes at 11:20h. The mouse was weighed and its ethanol bottle was replaced straight after removal from the maze. In effect, the length of withdrawal from ethanol prior to testing ranged between 2h 20 minutes and 5h. Post-test monitoring of ethanol consumption was carried out for three days after the last plus-maze testing session to measure the effect of the plus-maze procedure on subsequent daily ethanol consumption.

# iii) ETHANOL/NICOTINE DRINKING

## 5.1 Aims

The aim of this experiment was to investigate the links between voluntary nicotine and ethanol drinking on TO mice. Information gained from the preceding two experiments was combined to plan the present long-term investigation A further aim of this experiment

was to compare the behavioural effects (as measured on the plus-maze) exhibited by mice in withdrawal from ethanol and/or nicotine, after long-term exposure to these drugs.

## 5.2 Rationale behind experiment design

The two-bottle choice test was chosen, where mice were given continuous access to a bottle containing drug solution and another containing tap water. 10% (v/v) was chosen as the ethanol solution concentration following the results of the ethanol drinking experiment, above. These results showed that although 2.5% ethanol was the highest-preferred concentration in terms of volume of solution drunk per day, the most consistent and high daily mg/kg ethanol consumption was by those mice drinking the 10% ethanol solution. 1g/l (v/v) nicotine was chosen as the nicotine solution concentration, following the results of the nicotine drinking experiment, above. The results demonstrated that T0 mice would voluntarily drink an unsweetened 1 g/l nicotine solution and by so doing, their mean nicotine consumption was 35 mg/kg per day. After fourteen weeks of nicotine and/or ethanol exposure, mice were tested twice on the elevated plus-maze, once when, and once when not undergoing withdrawal from their test drug(s).

## 5.3 Method

The subjects used were 70 male TO mice which were randomly assigned to various treatment groups and administered with drug solutions in a two-bottle choice set-up (drug/ tap water choice.) Standard laboratory chow was available at all times. The mice were all housed singly and the experiment commenced without an habituation period prior to the start of drug treatment. The treatment groups, ten mice per group, were as follows (contents of 'drug' bottle):

Group 1 : tap water
Group 2 : ethanol (10%, v/v)
Group 3, 5, 6 and 7 : Mixture of ethanol (10%, v/v) and nicotine (1 g/l, v/v)
Group 4 : nicotine (1 g/l, v/v)

Both bottles were weighed at 14:00h each day. Cleaning-out of cages and weighing of were carried out on the same day each week to minimise any stress incurred. The mice were maintained on their test solutions for fourteen weeks.

First plus-maze test

After fourteen weeks of two-bottle choice all mice were tested once on the plus-maze following a 4h-withdrawal period. (Again, owing to the large size of the group, plus-maze testing had to be conducted over two days.) The withdrawal procedure for each group was as follows. The left-hand water bottles remained in all cages at all times.

Group 1: fresh water was substituted for the right-hand water bottle
Group 2: fresh water was substituted for the ethanol solution
Group 3: fresh water was substituted for the ethanol/nicotine mixture
Group 4: fresh water was substituted for the nicotine solution
Group 5: 1 g/l nicotine was substituted for the ethanol/nicotine mixture
Group 6: 10% ethanol was substituted for the ethanol/nicotine mixture
Group 7: the ethanol/nicotine mixture was not withdrawn at any point.

Lights came in in the holding-rooms at 08:00h. The mice to be tested that day were transferred to the behavioural laboratory just prior to 08:00h. The behavioural laboratory was lit by two red 60W bulbs positioned near the elevated plus-maze. Withdrawal of solutions from two cages commenced at 08:00h and continued with two further cages having solutions withdrawn every ten minutes thereafter. At 12:00h the mouse which had by then undergone a 4h drug withdrawal was placed on the plus-maze for 5 minutes. The original drug solutions were replaced immediately after exposure to the plus-maze. The video recordings of both days' plus-maze tests were analysed using Hindsight 1.4.

Second plus-maze test

One week later the elevated plus-maze testing was repeated. The procedure this time was exactly the same as the first test, except this time none of the test solutions were withdrawn or changed in the 4h prior to testing on the plus-maze. Again, the video recordings of both days' plus-maze tests were analysed using Hindsight 1.4.

# IIII CONDITIONED PLACE PREFERENCE EXPERIMENTS

## i) MORPHINE/ ACAMPROSATE

### 6.1 Aims

Morphine has been shown to produce a place preference effect in mice (Funada et al, 1993) and rats (Suzuki and Misawa, 1995; Higgins et al, 1992.) The aim of this experiment was firstly, to determine whether morphine produces a place preference effect in TO mice, and secondly, to investigate whether acamprosate alters morphine's observed effect. Acamprosate is a drug which has been shown in clinical trials to reduce relapse and/or its severity in alcoholics undergoing detoxification.

### 6.2 Conditioned Place Preference

Conditioned place preference is one of the ways to measure reward in animals, and was the paradigm chosen for the next few experiments of this investigation. It uses a Pavlovian conditioning procedure. A general version of the test is where animals experience two distinct neutral environments subsequently paired spatially and temporally with distinct drug states. Later, the animal is given free run of both environments. The duration of time spent in either environment is seen as an index of the reinforcing value of the drug (Schechter and Calcagnetti, 1993.)

The first procedure in testing for the existence of a conditioned place preference is to allow animals free run of both test environments, to observe the animals' preference for either place, if any such preference exists. Half the subjects are then restricted to one of the environments under drug conditions and the other half are paired with the other environment under placebo conditions. This 'conditioning' phase may be repeated a number of times over a few days. After this training, the subjects are tested for their preference. In the test, the animals again have free run of both environments in a drug-free state. When the animal is found to spend less time in the environment paired with the drug, the drug is assumed to have an aversive effect on the animal , and the previously neutral environment can be assumed to have become a secondary negative reinforcer (Treit, 1985.)

There are two main experimental designs for testing conditioned place preference. The first is the biased design, in which subjects are first tested for their baseline preference, then the environments are 'levelled out,' e.g. by putting (aversive) vinegar in the preferred, darker environment. The drug is paired initially with the non-preferred environment, and simultaneously saline or the drug vehicle is paired with the preferred environment. The subjects are then tested in a drug-free state with the run of both environments, as before. The second type of design is known as unbiased. In this set-up, manipulation of the two environments is made such that there is no apparent initial preference. The drug is subsequently paired with either environment, the control substance being paired with the other environment. The design used in this investigation was not strictly unbiased or biased, because no manipulation of the environments was made after measuring initial drug-free preference. The drug was simply paired with the non-preferred side of box. This conditioned place preference design had not been used in our laboratory before, so the first task was to set it up and check that it worked. Obtention of a place preference effect for a rewarding drug such as morphine is one way of checking the reliability of the apparatus, so this test was attempted first.

The same conditioned place preference setup was used in all experiments, unless stated otherwise. All six conditioned place preference boxes (60cmx15cm wide, 20cm deep) were of a two-section design, where one end was enclosed with clear, and the other with black perspex. On the floor of the clear section was placed a metal wire mesh (12mm$^2$ holes) which was included as an additional differentiation cue. Dividers (black and clear perspex) were used on conditioning days to separate the two environments. Each environment had

exactly the same dimensions. Two 60W red anglepoise lamps were directed against the wall above the CPP boxes to provide a diffuse light over the apparatus. (These lamps provided the only light source for the laboratory when the experiments were being run.) Black perspex squares were placed between the white wall and the clear ends of the CPP boxes. The six boxes were positioned so that alternate black/clear ends were against the backing wall.

The mouse to be tested was placed in the centre of the apparatus and its behaviour was recorded over the next 30 minutes using a video camera linked to a VCR and monitor in an adjacent laboratory. After the 30-minute exposure to the boxes, the mice were placed back in their respective cages and the conditioned place preference boxes were cleaned. The procedure for cleaning was wiping out the interior with a damp towel, then wiping it with a separate, dry towel.

## 6.3 Rationale behind experiment design

A pilot study was carried out first. Twelve male, group-housed TO mice underwent a drug-free preference test by allowing them free run of the conditioned place preference boxes for 30 minutes. The purpose of this pilot test was to gain some idea of the preference score to be expected, but also so that the procedure could be run through once, checking that the apparatus was in working order and video recording went to plan.

Following the initial pilot study was the conditioned place preference procedure. This involved one drug-free, baseline preference testing day, followed by ten conditioning days (pairings of the drug and one side of the conditioned place preference apparatus,) followed by a final, drug-free test day. The dose of morphine used was 10 mg/kg and the dose of acamprosate used was 400 mg/kg. The morphine dose was chosen for two reasons; firstly, because Cunningham et al (1992a) obtained a conditioned place preference in two strains of mice after repeated pairings with 10 mg/kg morphine. Secondly, the same group tested other, lower doses of morphine which also caused a place preference effect but 10 mg/kg gave the highest mean activity count in the mice during the conditioning trials. Schechter et al (1995) produced evidence to support the idea that it is the locomotor stimulating effects of drugs that can correlate with the strength of their reinforcing effect upon behaviour.

## 6.4 Method- initial pilot study

Twelve male TO mice (housed in groups of 6) were moved from their holding-room to the behavioural laboratory. They were habituated to this laboratory for one hour, prior to a 30-minute exposure to the conditioned place preference apparatus, which was set up in the same laboratory. No injections were administered to the mice prior to placing them in the centre of the conditioned place preference boxes. The duration of time spent by each mouse in either of the two sides during the next 30 minutes was monitored using a video camera, and scored later using the Hindsight 1.4 package, where an "active" rating was equivalent to an entry into the clear section.

The results from this initial pilot study showed that mice spent an average 67% of the total exposure time (30 minutes) in the clear side of the conditioned place preference box. If the results of the baseline preference testing in the main study matched this pilot study, and assuming that this result showed that the mice 'preferred' the clear side to the black side, the morphine and acamprosate-treated mice would be paired with the black, non-preferred side of the box in the main experiment.

## 6.5 Method- main experiment

Thirty male TO mice were used, housed in groups of 6, with weight ranges of 35-50g, similar to those used in the pilot study. Exactly the same setup was used as for the pilot study when conducting the initial drug-free baseline preference test. The procedure followed was also identical to that used in the pilot test. The results showed that the mice spent an average 60% of the total exposure time (30 minutes) in the clear side of the conditioned place preference box. This clear-side 'preference' was certainly not significantly different from what could be expected to occur by chance. However, the morphine and acamprosate-treated mice were paired with the black, 'non-preferred' side of the box in the conditioning phase, as was planned in the pilot study, above.

24h after the baseline preference test was the first of ten consecutive conditioning days. All mice were habituated to the behavioural laboratory (with the red lights on) for at least one hour before treatment. The habituation period varied because only twelve animals

could be placed in the apparatus at any one time. Starting with the first two cages, mice were injected and tail marked, and placed back into their cages. Their drug treatments were as follows, all injections being administered i.p:-

n=10 mice: saline (0.9%) every day

n=10 mice: saline (0.9%) / morphine (10 mg/kg) on alternate days, such that each mouse received 5 saline pairings and 5 morphine pairings.

n=10 mice: saline (0.9%) / [morphine (10 mg/kg) plus acamprosate (400mg/kg)] on alternate days, such that each mouse received 5 saline pairings and 5 [morphine plus acamprosate] pairings.

The contact time for the drugs was 10 minutes (i.e. the interval between the time of injection and the time of placing the treated mouse in the conditioned place preference box.) After 30 minutes the mice were removed from the boxes and returned to their cages in the holding-room. The preference for either side of the conditioned place preference box, in the absence of any drugs, was tested again 24h after the final conditioning trial. The procedure for this test day was exactly the same as for the baseline testing day, videoing and scoring the events as before.

ii) ETHANOL/NICOTINE

7.1 Aims

The aim of this experiment was to determine; firstly, whether ethanol produced a place preference effect in TO mice in the conditions used in our laboratory, and secondly, whether nicotine produced the same effect. The third aim was to determine the effect produced when the two drugs were combined.

7.2 Rationale behind experiment design

This was the second experiment carried out using the conditioned place preference paradigm, and the procedure differed from the first experiment only in the drugs used in the conditioning trials. The dose of ethanol chosen to be administered was 2.5 g/kg. Several factors influenced this choice; one was because Ali et al (1995) found 2.5 g/kg ethanol produced a conditioned place preference in the BKW mouse, whereas lower doses of the drug failed to produce this effect. Considering ethanol's interaction with nicotine, Johnson et al (1995) found that a challenge with ethanol (2.5 g/kg, i.p.) induced locomotor stimulation in mice subchronically treated with nicotine. A higher dose was not used, in spite of Cunningham et al (1992a) finding that 3 and 4 g/kg ethanol were the lowest in a range of doses to induce a place preference in DBA/2J mice. This was because Williams et al (1993) found that repeatedly injecting SAF mice with 3 g/kg ethanol i.p. produced marked hypothermia, although tolerance to this effect was rapid.

The dose of nicotine chosen to be administered was 0.4 mg/kg. Risinger and Oakes (1995) carried out studies of place conditioning in mice for a range of doses of nicotine. They found that 2.0 mg/kg nicotine produced locomotor depression and conditioned place aversion. No conditioning was produced by 0.25 and 1.0 mg/kg nicotine but enhanced locomotor activity and conditioned place preference was produced by 0.5 mg/kg nicotine. It was important to consider nicotine's interaction with ethanol, however. Lapin et al (1995) showed that ethanol (0.125- 2.0 g/kg i.p.) enhanced the locomotor stimulation induced by 0.4 mg/kg s.c. nicotine in rats, so this slightly lower dose was favoured.

The nicotine and ethanol were to be paired with the non-preferred side of the conditioned place preference box, because Schechter et al (1995) demonstrated a conditioned place preference in mice for 0.75 mg/kg s.c. nicotine after they paired the drug with the less-preferred side of the apparatus.

7.3 Method

Thirty-six male TO mice were used, housed in groups of six. As before, the procedure involved one drug-free baseline day, followed 24h later by ten conditioning days

(pairings of the drug(s) or saline with one side of the conditioned place preference box,) followed 24h after the final conditioning day by a drug-free test day.

On the baseline day, mice in their cages were habituated to the behavioural laboratory for one hour, prior to a 30-minute exposure to the conditioned place preference apparatus. The results of this drug-free baseline preference test showed that the mice spent an average 69% of the total exposure time in the clear side of the conditioned place preference box. Therefore, the ethanol and nicotine-treated mice were to be paired with the black, non-preferred side of the box in the conditioning phase.

On each of the ten conditioning days, all mice were habituated to the behavioural laboratory for at least one hour before treatment. Once all the mice from one cage had received their injections they were placed immediately into their respective sides of the conditioned place preference boxes (in other words the contact time for the both drugs was minimal.) The injections received each day were followed according to the following schedule:-

n=9 mice: saline (0.9% i.p.) every day

n=9 mice: saline (0.9% i.p.) / ethanol (2.5 g/kg i.p.) on alternate days, such that each mouse received 5 saline pairings and 5 ethanol pairings.

n=9 mice: saline (0.9% i.p.) / nicotine (0.4 mg/kg s.c.) on alternate days, such that each mouse received 5 saline pairings and 5 nicotine pairings.

n=9 mice: saline (0.9% i.p.) / [ethanol (2.5 g/kg i.p.) plus nicotine (0.4 mg/kg s.c.)]on alternate days, such that each mouse received 5 saline pairings and 5 [ethanol plus nicotine] pairings.

(As stated before, mice receiving ethanol and nicotine injections were always paired with the black side of the conditioned place preference box.)

The preference for either side of the conditioned place preference box, in the absence of any drugs, was tested again 24h after the final conditioning trial. The procedure for this test day was exactly the same as for the baseline testing day, videoing and scoring the events as before.

## iii) ETHANOL/NICOTINE: AUTOMATED EXPERIMENT

### 8.1 Aims

This experiment was intended as a modified repeat of the preceding ethanol/nicotine conditioned place preference experiment. The main modification was to automate the whole procedure. An extra cue in the conditioned place preference box was also added. As before, the overall aim of this experiment was to determine; firstly, whether ethanol produced a place preference effect in TO mice in the conditions used in our laboratory; secondly, whether nicotine produced the same effect; and thirdly, the effect produced when the two drugs were combined.

### 8.2 Rationale behind experiment design

i)The first objective was to set up and test the automated apparatus. Most research groups using the conditioned place preference paradigm use such apparatus, as it saves the time spent traditionally analysing videos, and removes some of the human error inherent otherwise. Infrared light sources and photodetectors (two sets) were mounted opposite each other at the boundary of the black/clear section of each of the conditioned place preference boxes. The sources were placed 5 cm apart and 2cm from the floor on one side of the box. Occlusion of the infrared light beams enabled detection of the animal's position (left versus right side) within the box. Data were recorded every second by an Elonex PC, programme "mouselog."

ii)To enable results using the automated apparatus to be compared directly with results of the conditioned place preference experiments just completed, the new method had first to be tested against the video analysis method. Only if the two methods were acceptably comparable could the main experiment be undertaken. After setting up the automated conditioned place preference procedure, a pilot study was conducted to compare the results of the old and new methods after monitoring the same events. An extra differentiation cue was added to the dark side of the conditioned place preference apparatus- a smear of

chocolate on the floor. It has been shown that preferences for novelty shown by mice in an exploration box are suppressed by bulbectomy and olfactory lesions (Misslin and Ropartz, 1981) so it was expected that association and differentiation processes in the mice in the present study would also rely to a great extent on olfactory cues. The odour of chocolate has been found to be generally aversive to mice, as has odours such as those from cat fur clippings and untreated sheep wool (Garbe et al, 1993.) Since the purpose in the present study was simply to provide an effective additional cue, (whether aversive or not), to aid the mice in differentiating between the two sides of the apparatus, the most easily obtainable substance- chocolate- was used.

iii) Once the comparability of the automated apparatus had been verified, the next stage was to test the apparatus with a rewarding drug which normally produces a conditioned place preference effect in mice. Morphine was chosen for this phase.

iv) Once a conditioned place effect had been demonstrated successfully for morphine, the next stage was to test mice in apparatus with a non-rewarding drug, such as haloperidol. An absence of a conditioned place preference effect with haloperidol would suggest that the procedure was sensitive to the effects of both rewarding and non-rewarding drugs.

v)Having ascertained this fact, the last stage would be to actually repeat the previous ethanol-nicotine experiment using the automated apparatus.

<u>Method i) Setting up the automated conditioned place preference apparatus</u>

The computer programme was loaded and one mouse was given free run of one of the six conditioned place preference boxes. The computer monitor and mouse were observed for several minutes. This was to ensure that the computer was registering information for the correct box, that each beam-crossing was registering correctly, and that the side of the box in which the mouse was at any point in time was recorded correctly. Another five mice were exposed to the other five conditioned place preference boxes, and

the computer monitor and mice were again observed to ensure that the mice's movements were correctly registered.

Method ii) Comparing results of the automated apparatus with those from video analysis

The automated apparatus was compared with a video recording carried out simultaneously, using six further male TO mice. The conditioned place preference boxes used were the same design as before, with a metal grille on the floor of the clear section. A piece of milk chocolate (Wispa) was smeared in a 1-inch strip on the far-end floor of each box (dark sections only.) The main room lights were kept on throughout.

Mice in their cages were habituated to the behavioural laboratory for one hour, prior to a 30-minute exposure to the conditioned place preference apparatus. After the 30-minute exposure to the boxes, the mice were placed back in their cage and the boxes were cleaned. First, the interior was washed down with water, then wiped with a dry towel. Care was taken during washing to ensure that the water ran into the dark end (where the chocolate was smeared) rather than in the other direction, so that the chocolate smell would remain localised.

The preference for either of the two sides during the 30 minutes was monitored both by using a video camera and simultaneously by the computer. The dark, chocolate-paired side was found to be the non-preferred side (45.1% of the total time was spent on the dark side.) It was found that the video results differed from the automated results by about 5%. This was considered an acceptable difference.

Method iii) Testing with morphine

Twelve further TO male mice were used to test for a conditioned place preference effect with morphine. As before, the procedure involved one drug-free baseline day, followed 24h later by ten conditioning days, followed 24h after the final conditioning trial by a final test day (drug-free.) The initial baseline test was analysed using both the automated and video methods. Both methods showed that the clear side of the conditioned place preference box was the preferred side, so the rewarding drug was to be paired with the non-preferred, dark side of the boxes. On each of the ten conditioning days, all mice were

habituated to the behavioural laboratory for an hour before treatment. The treatment was as follows:-

n=6 mice: saline (0.9% i.p.) every day
n=6 mice: saline (0.9% i.p.) / morphine (10 mg/kg i.p.) on alternate days, such that each mouse received 5 saline pairings and 5 morphine pairings.

The mice receiving morphine were injected 10 minutes before being placed in the dark, chocolate-smeared side of the conditioned place preference box for 30 minutes. The mice receiving saline were paired with the clear side of the box. The final test day was conducted and analysed in the same way as the initial baseline day.

The results did not show a significant conditioned place preference effect with this rewarding drug, so the experiment was repeated with one modification. Wet sawdust was placed underneath the metal grille on the clear side, to provide an additional differentiating cue. Method iii) was repeated including this modification, still pairing the morphine with the dark, chocolate-paired side.

Method iv) Testing with haloperidol (Planned)

(Owing to a lack of time, this experiment had to remain in the planning stage.)

Once a place preference effect for morphine had been achieved, the experiment would be re-run using a non-rewarding drug such as haloperidol. A dose of 0.025 mg/kg haloperidol would be used, as this dose has been shown to produce behavioural modifications in mice (Cole and Rodgers, 1994) without the locomotor activity suppression seen in the conditioned place preference setting with higher doses (Cunningham et al, 1992b.)

Method v) Testing with ethanol/nicotine (Planned)

(Owing to a lack of time, this experiment had to remain in the planning stage.)

When the non-rewarding haloperidol had been shown to produce no significant place-preference effect, the experiment would be repeated using ethanol and nicotine. The

procedure used would be a repetition of the earlier attempt, but using an automated apparatus. The dose of ethanol used would be reduced this time from 2.5 g/kg to 1.0 g/kg due to the motor suppressing effects observed at the higher dose in the first ethanol/nicotine conditioned place preference experiment. The dose of nicotine used would remain at 0.4 mg/kg.

# RESULTS

## I ETHANOL PREFERENCE EXPERIMENT, GROUPS/SINGLY HOUSED

### 1.1 Introduction

This first experiment was intended to give a profile of drinking patterns and preference for 8% (v/v) ethanol in male TO mice when housed in groups and when isolated from the same groups. In addition, data generated was expected to indicate the effects of routine procedures (such as cage-cleaning and weighing of mice) on ethanol preference. For both single and group-housed animals, the position of the drinking-bottles containing water and 8% ethanol were swapped daily.

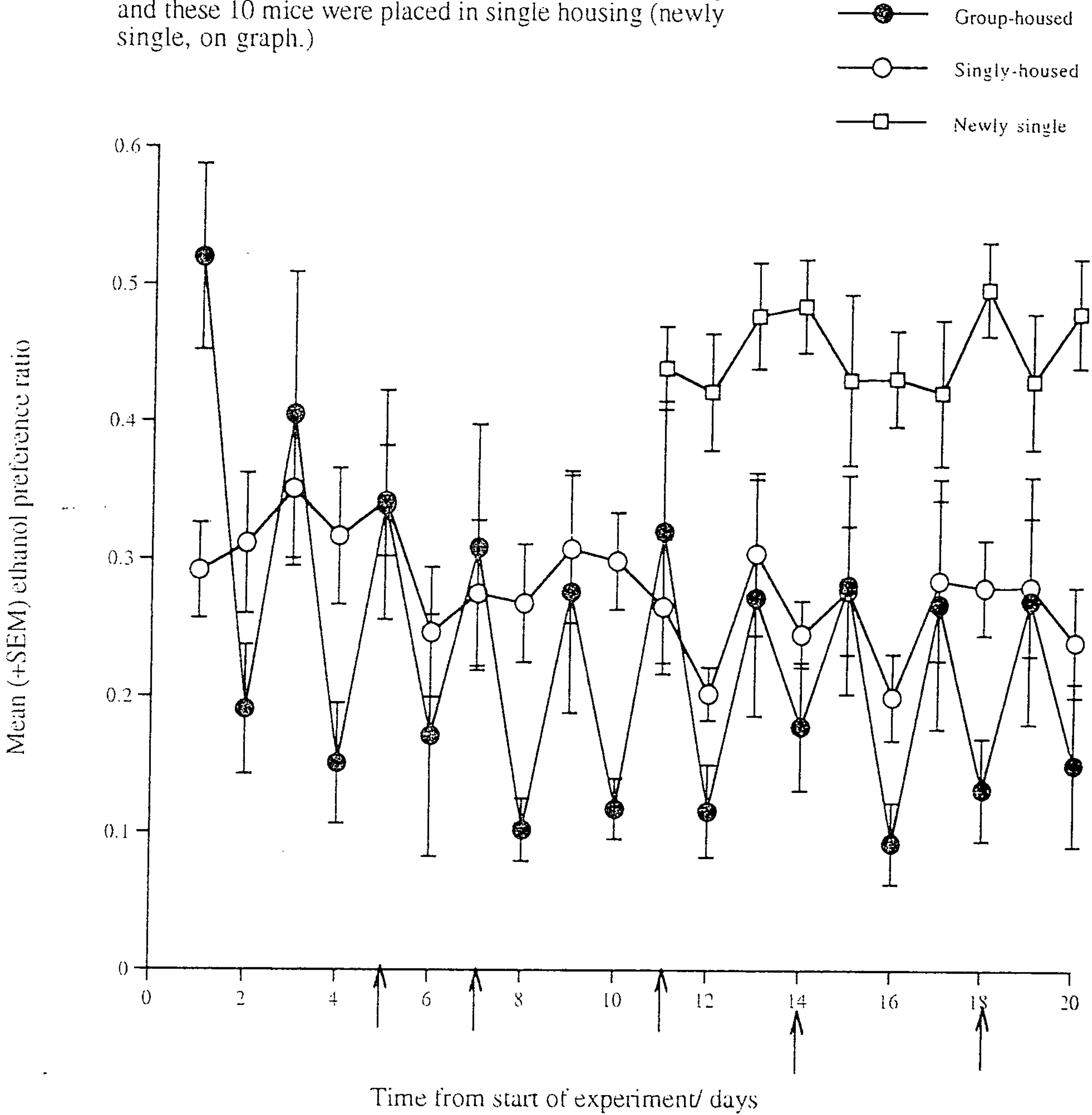
### 1.2 Overall Ethanol Preference Patterns, Figs. 1.0 and 1.1

The most striking feature of both graphs of mean preference for group-housed mice was their daily fluctuation (Figs. 1.0, 1.1.) This preference pattern was noticeable only for singly-housed mice in Fig. 1.1. Ethanol preference of group-housed mice was generally highest on days when the bottle containing ethanol was placed on the left side of the cage. An apparent decrease in preference for ethanol over time among the group-housed mice when the ethanol was placed on this side was not significant.

### 1.3 Effect of Isolation, Fig. 1.0

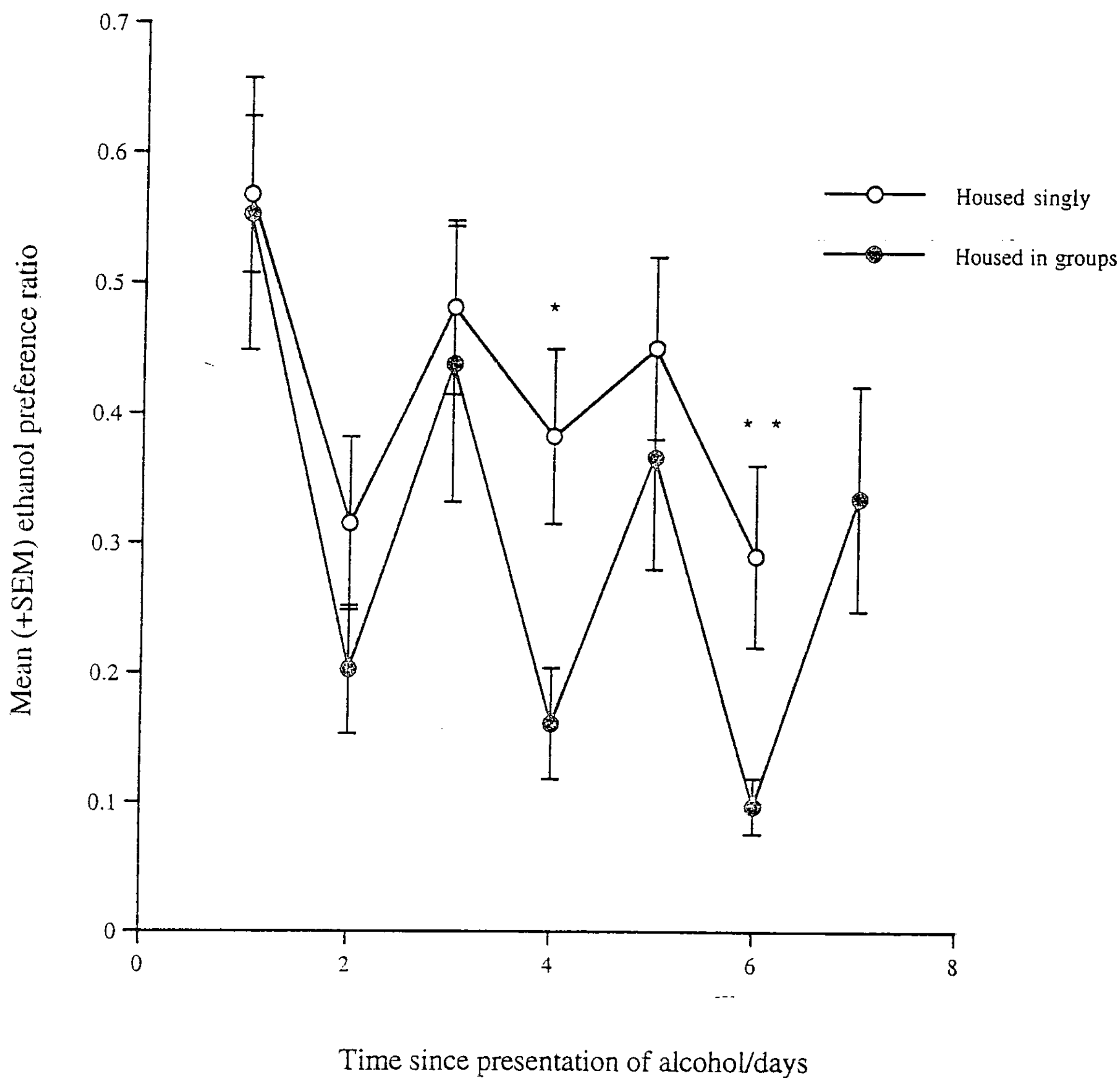
The increase in ethanol preference after moving mice from groups of five into single housing on day 10 was significant. Considering days 11-20 inclusive, the mean daily ethanol preference for group-housed mice was significantly lower ($p<0.0001$, Mann-Whitney Rank Sum test) compared to the mean daily ethanol preference for newly-single mice over the same time period. The mean daily ethanol preference among singly-housed

Fig 1.0 Effect of housing conditions on ethanol 8%(v/v) preference ratio in TO mice. All mice were alcohol naive prior to day 1. Mice were housed in ten groups of n=5, or singly (n=20.) On day 10, two of the groups were split and these 10 mice were placed in single housing (newly single, on graph.)




Arrows: indicate days cages were cleaned out

Fig.1.1 A comparison of control, group-housed TO mice with controls housed in single cages. Both groups were alcohol naive prior to testing. Water/ ethanol 8% (v/v) drinking bottle positions were swapped daily. (*P<0.01, c.f. group ratio,day 4. **P<0.01, c.f. group ratio,day 6)




Time since presentation of alcohol/days

mice from day 11-20 inclusive was also significantly lower ($p<0.0001$, Student's t-test) than the mean daily ethanol preference for newly-single mice over the same time period.

### 1.4 Effect of Introduction of Ethanol, Fig. 1.1

This figure shows the preference for ethanol of mice habituated to either single or group housing for 17 days but newly-exposed to 8% ethanol. Singly-housed mice (n=20) had a significantly higher preference than those housed in groups (n=6 groups) on the fourth and sixth day after first presentation of ethanol (both days $p<0.01$, Mann-Whitney Rank Sum test.)

### 1.5 Effect of Cleaning-Out of Cages, Fig. 1.0

Cleaning-out of cages did not have a significant effect on preference for 8% ethanol the following day, on any of the five occasions, in any of the three groups of mice (singly-, group- or newly-singly-housed.)

## II SUCROSE DRINKING EXPERIMENT

### 2.1 Introduction

Because of later plans to use sucrose to mask aversive-tasting drugs, the aim of this experiment was to determine what behavioural effects, if any, sucrose exerted, both alone and in conjunction with ethanol. All mice were continually exposed to 10% (w/v) sucrose for four weeks in a two-bottle choice test. Mice were found to vary in their individual daily sucrose consumption levels and any effects that their level of sucrose consumption had on their behaviour on the elevated plus-maze was measured. The effect that injections of nimodipine had on voluntary sucrose consumption was also investigated. In retrospect, the

inclusion of an additional, water-drinking experimental group of mice would have been a useful control; this option is considered in more detail in the Discussion section, later.

2.2 Plus-maze testing- effect of ethanol. Figs. 2.0-2.3 inclusive

Mice were injected with ethanol (1.75 g/kg or 1.0 g/kg) or saline prior to testing on the elevated plus-maze. First of all, the effect of ethanol on the mice was considered without taking into account the specific sucrose consumption group to which each mouse belonged. For detailed descriptions of every behavioural parameter measured, e.g. an exact definition of a 'closed arm return,' please refer back to the Methods, section 2.2.

Fig. 2.0 shows the effect of ethanol on the mean percent of total time spent in the closed arm of the elevated plus-maze. Administering1.75 g/kg ethanol significantly decreased this mean percentage compared to saline-treated mice ($P<0.05$, Dunnett's test.) The lower dose of 1.0 g/kg ethanol did not produce a significant change in the mean percentage compared to saline-treated mice.

Fig. 2.1 shows the effect of ethanol on the mean percent of total time spent in the open arm of the elevated plus-maze. Administering 1.75 g/kg ethanol significantly increased this mean percentage compared to saline-treated mice ($P<0.005$, Dunnett's test.) However, 1.0 g/kg ethanol produced no significant change in the mean percentage compared to saline-treated mice.

Fig. 2.2 shows the effect of ethanol on the mean net duration of stretch attend postures exhibited on the elevated plus-maze. Administering 1.75 g/kg ethanol significantly decreased this mean net duration compared to saline-treated mice ($P<0.0001$, Dunnett's test.) Again, 1.0 g/kg ethanol produced no significant change in the mean net duration compared to saline-treated mice.

Fig. 2.3 shows the effect of ethanol on the mean total number of entries into either arm of the elevated plus-maze. Administering 1.75 g/kg ethanol significantly increased this mean

Fig. 2.0 Effect of ethanol i.p. injection dose on mean percent of total time spent in closed arm of plus-maze. All subjects (TO mice) had uninterrupted access to 10% w/v sucrose solution for 4 weeks prior to plus-maze testing. (*$P<0.05$, c.f. saline treatment group)

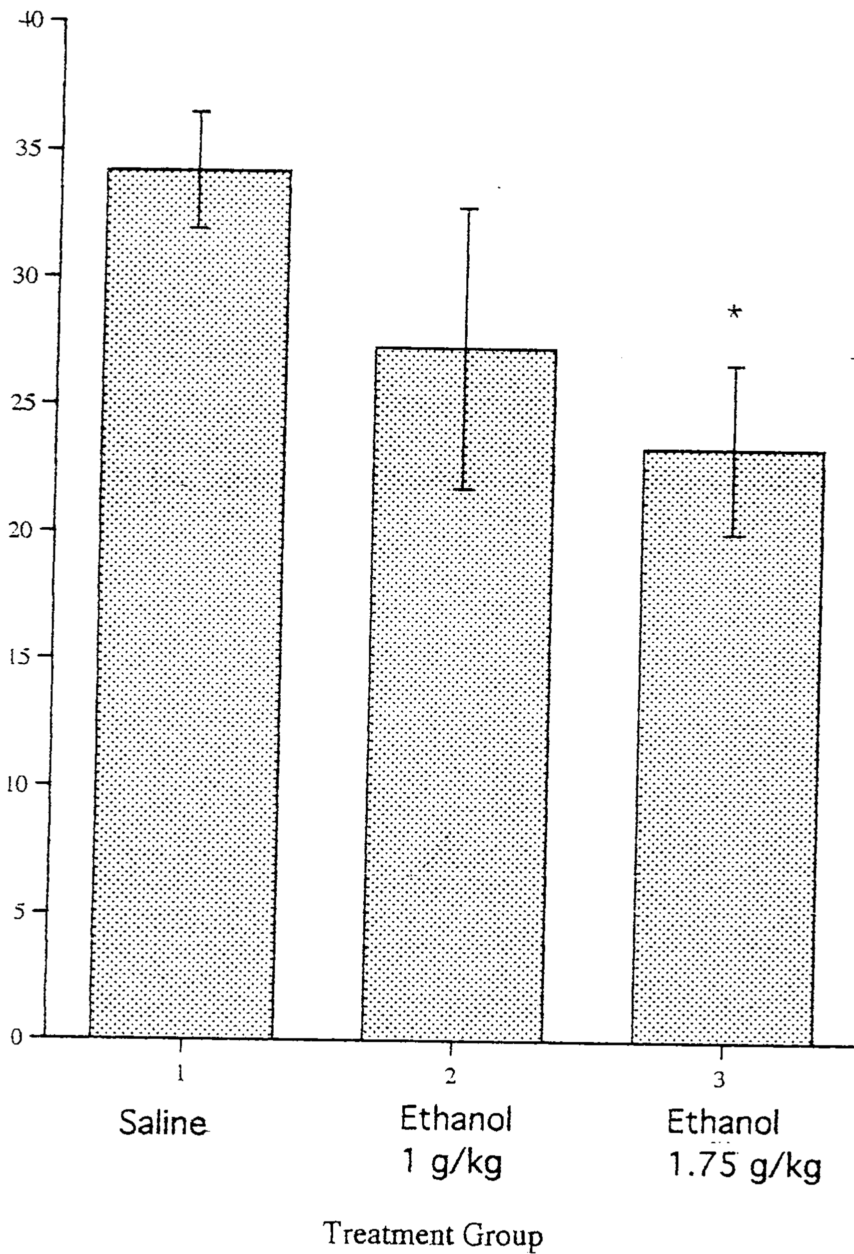


Fig. 2.1 Effect of ethanol i.p. injection dose on mean percent of total time spent on open arm of plus-maze. All subjects (TO mice) had uninterrupted access to 10% w/v sucrose solution for 4 weeks prior to plus-maze testing. (*P<0.005, c.f. saline treatment group)

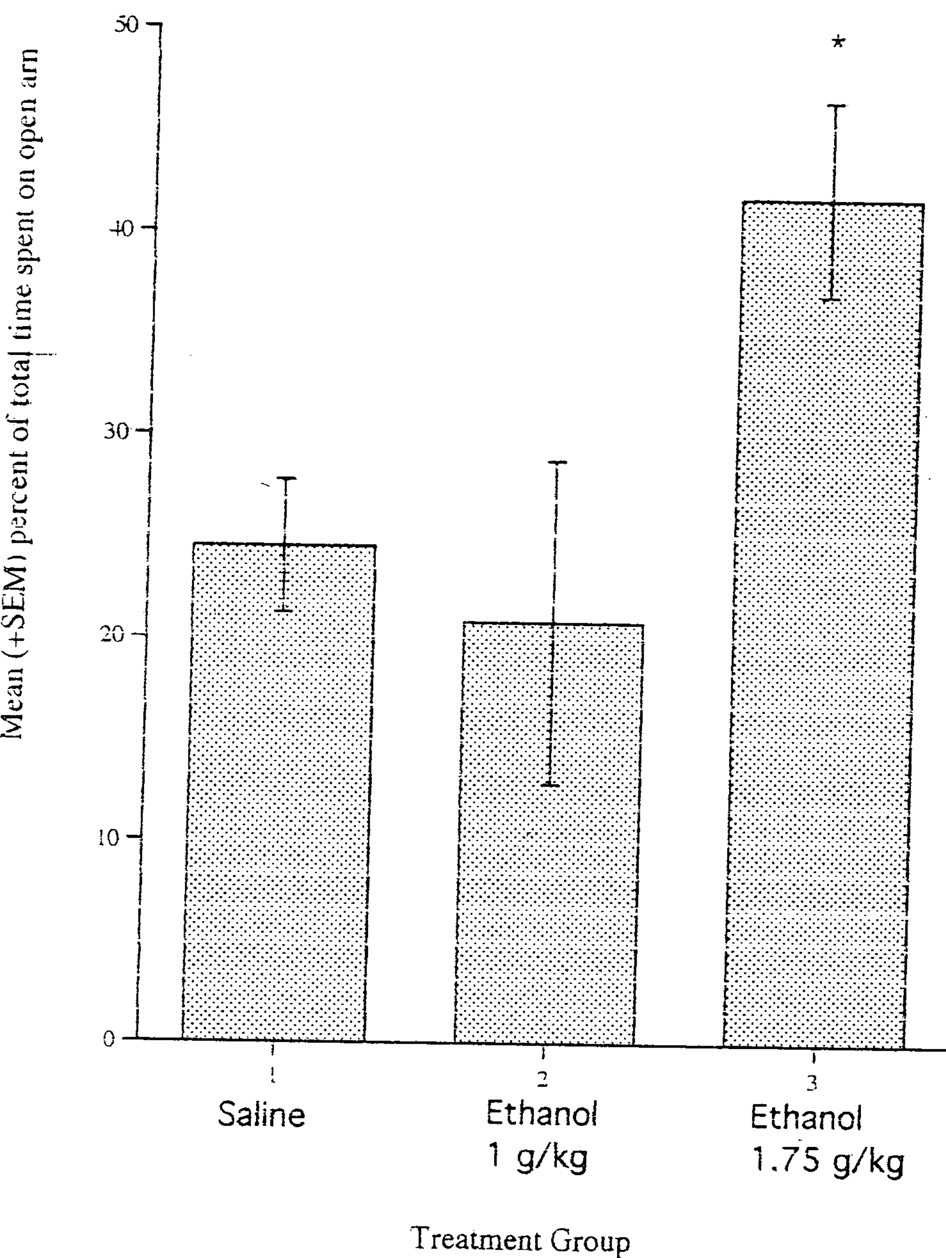


Fig. 2.2 Effect of ethanol i.p. injection dose on mean net duration of stretched attend postures exhibited on the plus-maze. All subjects (TO mice) had uninterrupted access to 10% w/v sucrose solution for 4 weeks prior to plus-maze testing. (*P<0.0001, c.f. saline treatment group)

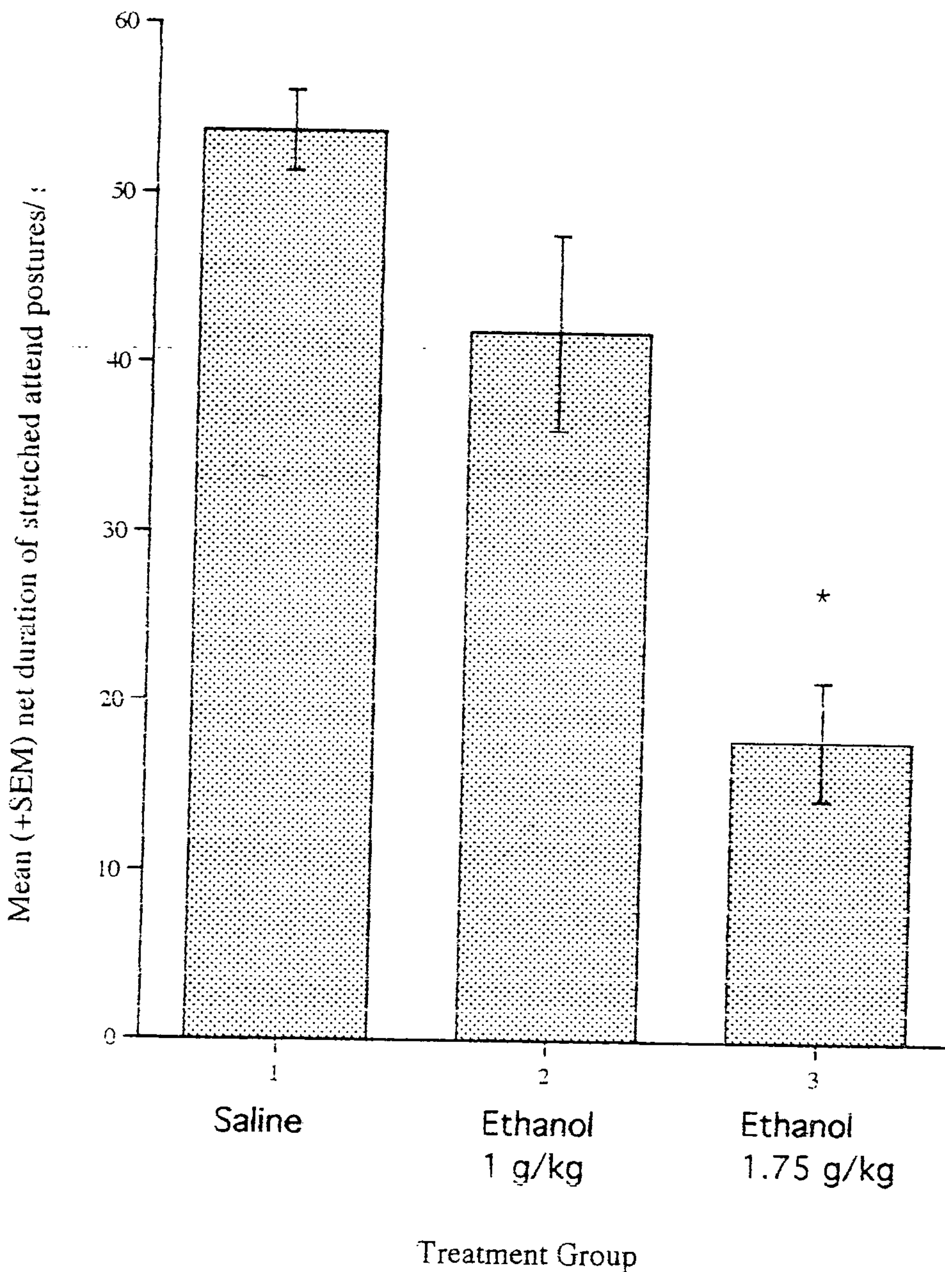
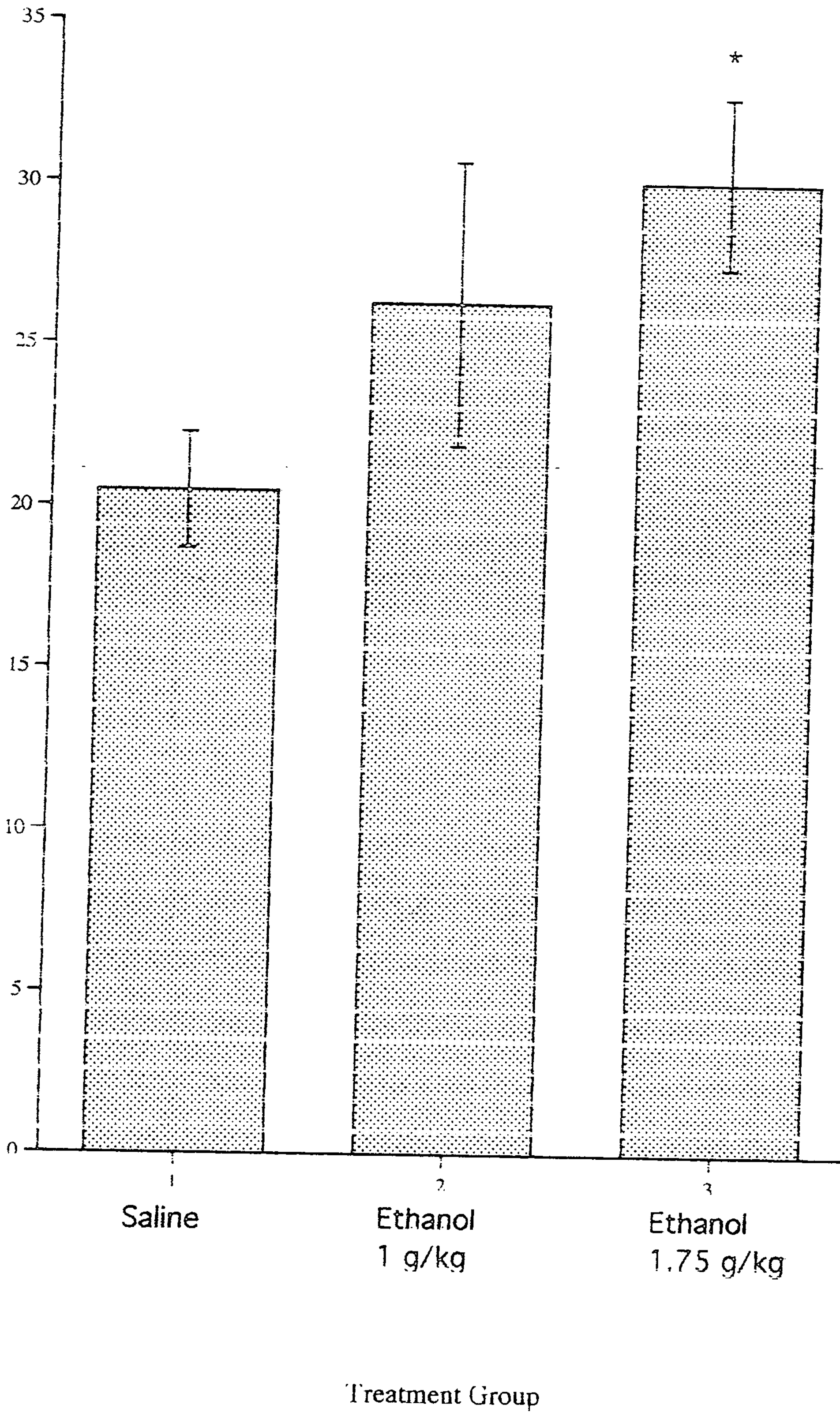


Fig. 2.3 Effect of ethanol i.p. injection dose on mean total number of entries into either arm of the plus-maze. All subjects (TO mice) had uninterrupted access to 10% w/v sucrose solution for 4 weeks prior to plus-maze testing. (*P<0.05, c.f. saline treatment group)



total number compared to saline-treated mice ($P<0.05$, Dunnett's test) whereas 1.0 g/kg ethanol produced no significant change in the mean total number compared to saline-treated mice.

Table 1 The following table gives a summary of all significant effects produced by either dose of ethanol on mice exposed to the elevated plus-maze. The statistical test used in all cases was Dunnett's test.

| Plus-maze parameter tested | Figure number of graph, if applicable | Which ethanol dose (g/kg) caused significant difference from saline group | Direction of difference from saline- increase or decrease (Probability value) |
|---|---|---|---|
| % of total time spent in closed arm | 2.0 | 1.75 | Decrease ($P<0.05$) |
| % of total time spent on open arm | 2.1 | 1.75 | Increase ($P<0.005$) |
| Mean net duration of stretch attend postures | 2.2 | 1.75 | Decrease ($P<5.0 \times 10^{-11}$) |
| Total number of entries into either arm | 2.3 | 1.75 | Increase ($P<0.05$) |
| Number of head dips | | 1.75 and 1.00 | Both Increase ($P<5.0 \times 10^{-8}$) |
| Number of closed-arm returns | | 1.75 | Decrease ($P<0.01$) |
| % of head dips which are unprotected | | 1.75 | Increase ($P<0.0005$) |
| Number of open arm entries | | 1.75 | Increase ($P<0.005$) |

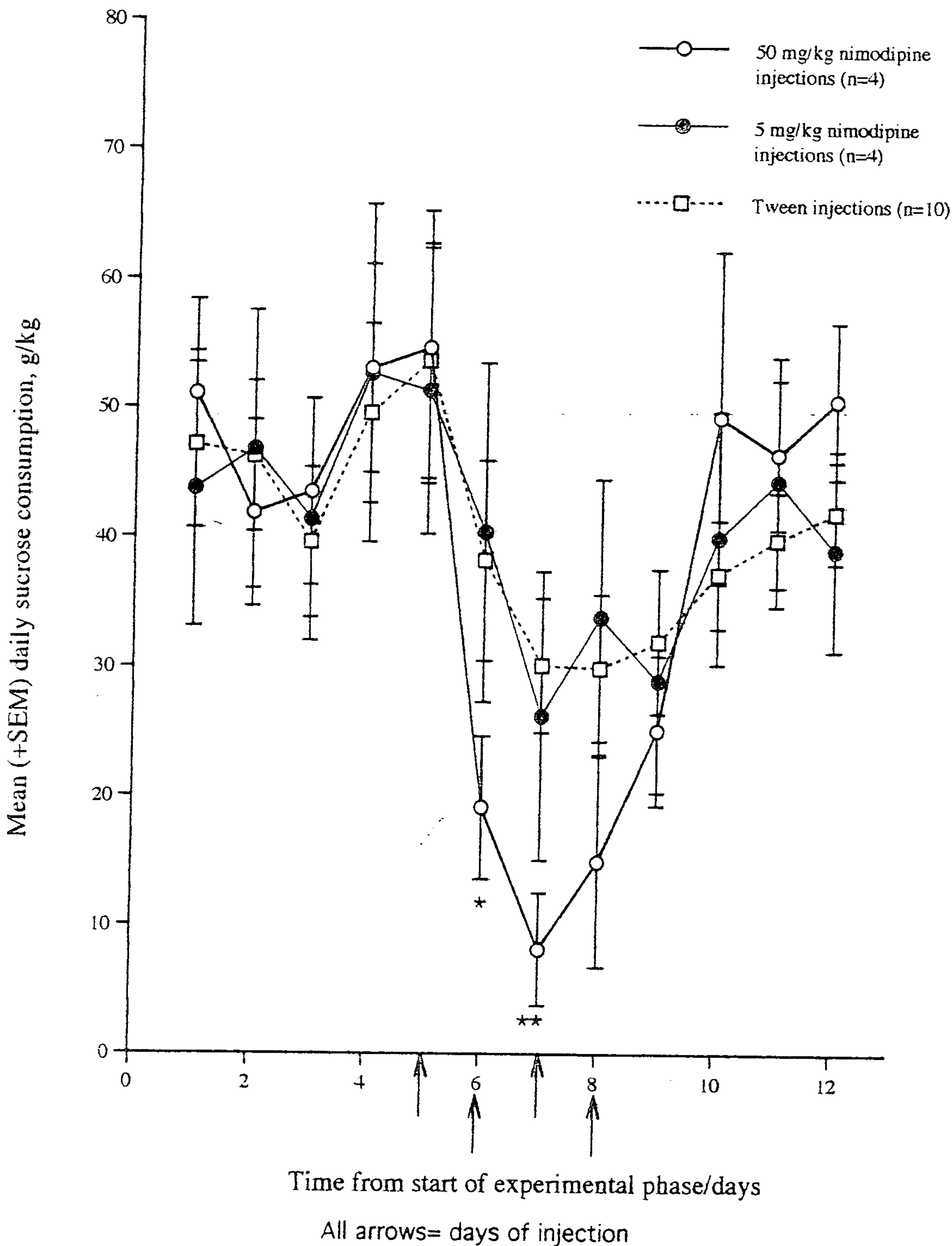
2.3 Effect of nimodipine on sucrose consumption, Fig. 2.5

One week after the plus-maze testing, a course of daily nimodipine injections were administered to those mice which had had saline injections only and had a mean daily sucrose consumption range of between 20.5 and 70 g/kg/24h. The aim was to determine whether the doses of nimodipine chosen altered mean daily sucrose consumption. Mice whose daily sucrose consumption range was lower than 20 g/kg/24h were not used. This was because some mice in this low-drinking range only drank a negligible quantity of sucrose each day, so the additional effects of nimodipine would also be negligible. The three treatment group were as follows:

1) n=5 received nimodipine 5 mg/kg, n=5 received tween 80 (0.05%)
2) n=5 received nimodipine 50 mg/kg, n=5 received tween 80 (0.05%)
3) n=10 received tween 80 (0.05%)

Fig. 2.4 shows the effect of the injections on sucrose consumption. The mean sucrose intake of mice in the 50 mg/kg nimodipine dose group decreased significantly below that of the Tween treatment group on day 6 (the first day after the initial injection)($P<0.05$, Student's t-test) and day 7 ($P<0.01$, Student's t-test.) It must be stressed that the number of mice in each of the nimodipine treatment groups was only 4 throughout the 12-day experimental phase, due to bottle leakage. The mean sucrose intake of the lower-dose (5 mg/kg) nimodipine group did not differ significantly from that of the Tween group throughout the 12-day experimental phase.

Fig. 2.5 Effect of daily injections of nimodipine on sucrose consumption in TO mice, compared with daily Tween injections. All mice had had continuous access to 10% (w/v) sucrose solution for 4 weeks prior to 'day 5' and were matched into groups based on mean daily sucrose consumption prior to injections. (*P<0.05, **P<0.01: compared to Tween-injected mice, same day.)
N.B. n=4 for both nimodipine-treated groups, throughout days 1-12.



# III ETHANOL AND NICOTINE DRINKING EXPERIMENTS

## i) NICOTINE

### 3.1 Introduction

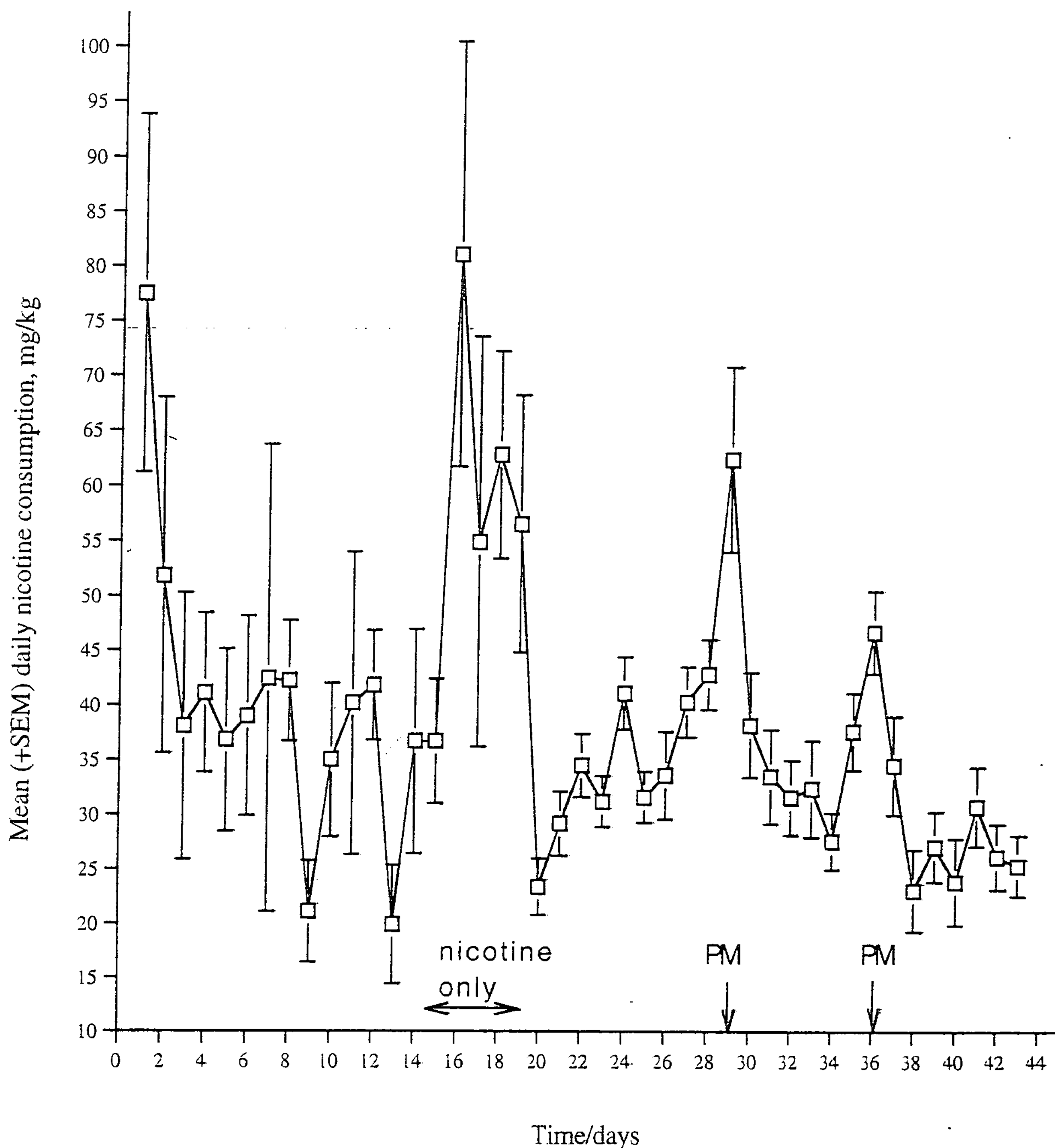
This experiment was intended as one preparation for the long-term nicotine/ethanol drinking study. The main aim was to find a concentration of nicotine which the TO mice would drink voluntarily without the need for an added sweetener. To this end, mice were exposed to 1 g/l nicotine in a two-bottle choice test for a total of six weeks. However, from days 15-19 inclusive, nicotine was given as the sole fluid to all mice in the nicotine-drinking group. The purpose of this was to determine whether a period of forced nicotine intake would raise voluntary nicotine intake on the resumption of a two-bottle choice paradigm.

A further aim was to investigate the behavioural effects of nicotine on mice, both alone and when in conjunction with ethanol. The elevated plus-maze was employed for this purpose and behaviour of mice on the maze was observed after ethanol or saline administration. The plus-maze test was repeated after one week, which allowed the effect of this procedure itself on daily nicotine drinking to be monitored.

### 3.2 Daily consumption of nicotine up to day 15 inclusive, Fig. 3.0

Fig. 3.0 shows the daily nicotine intake of the mice in a two-bottle choice paradigm. The large error bars were mainly due to the low n value (4 for days 1-19), as data for the other twelve nicotine-drinking mice had to be discounted due to the leakiness of the bottles. After day 20 a less leaky bottle type became available. The mean nicotine intake for the four mice from days 2-15 inclusive was 37 mg/kg/day, s.e.m.2.3 mg/kg/24h.

Fig.3.0 Daily consumption of 1 g/l nicotine solution by TO mice. Two-bottle choice throughout except for days 15-19 inclusive when water bottle was removed. Less leaky bottle bungs became available after day 20. Large variance in ethanol consumption on day of plus-mazing partly due to leakage when transferring cages.n=4 until day 16, then n=16.




Arrows: PM= day of plus-maze

3.3 Effect of removing water choice on daily nicotine consumption, Fig. 3.0

From days 15-19 inclusive, two-bottle choice was denied and nicotine (1 g/l) was the sole fluid source. There appeared to be some increase in the daily nicotine consumption during this period but again it must be borne in mind that the number of subjects at this point was a mere 4. The resumption of a two-bottle choice was prompted by a sharp weight reduction in the nicotine-drinking group mice. The mean daily intake of nicotine for days 20 to 28 was 34 mg/kg/day, s.e.m. 2.2 mg/kg/day (n=16). The mean daily nicotine intake from days 20 to 28 was not significantly different from the mean intake before the period of forced nicotine intake.

3.4 Effect of plus-maze exposure on daily nicotine consumption, Fig. 3.0

The mean measured nicotine consumption value on day 29 (the day of the first plus-maze) should be discounted. It was artificially high due to bottle leakage when transferring the cages to the behavioural laboratory. The same problem did not occur on the day of the second plus-maze as this time the bottles were removed before the transit (and replaced immediately afterwards.) The mean intake for days 30-35 inclusive (the period between the two plus-maze tests) was 33 mg/kg/day, s.e.m.1.8 mg/kg/day. Following the second plus-maze the mean nicotine intake (for days 37-43 inclusive) was 27 mg/kg/day, s.e.m.1.7 mg/kg/day), which was not significantly different from mean daily nicotine intake for days 30-35 inclusive.

3.5 Plus-maze testing- first time, day 29.

After 29 days of exposure to nicotine or water, all 32 mice were tested on the elevated plus-maze. For both the nicotine/water and the water/water choice groups, the treatments were as follows:-

n=8 were injected with 1.75 g/kg ethanol i.p.

n=8 were injected with 0.9% saline i.p.

Raw data for this plus-maze is shown in Table 3.1, below. None of the behavioural parameters observed varied significantly between subjects which had had a water/water or nicotine/water choice prior to this plus-maze test. There were not any significant differences between subjects injected with saline or ethanol, from either drinking group, for any of the behavioural parameters measured.

Table 3.1 averaged raw plus-maze data values for each treatment group on the first plus-maze day, presented as mean values (with standard errors.)

| Plus-maze parameter tested | Group 1 ± SEM | Group 2 ± SEM | Group 3 ± SEM | Group 4 ± SEM |
|---|---|---|---|---|
| Total number of entries made into closed arm | 17.0±2.9 | 15.5±1.4 | 18.3±2.6 | 14.8±2.6 |
| Total number of entries made into either arm | 34.6±4.6 | 39.8±4.0 | 41.9±5.2 | 34.9±5.2 |
| % of total time spent in the central section | 23.9±3.3 | 17.6±1.0 | 28.0±6.4 | 20.1±2.1 |
| % of head dips which are unprotected | 99.0±1.0 | 100±0.0 | 99.5±0.5 | 99.5±0.5 |
| % of stretched attend postures which are unprotected | 50.0±16.7 | 50.0±18.9 | 28.6±18.4 | 50.0±18.9 |
| Number of head-dips | 23.3±6.6 | 36.1±7.3 | 38.4±5.3 | 28.0±6.5 |
| % of total time spent in closed arm | 33.5±5.0 | 29.9±2.7 | 27.5±3.9 | 27.8±3.4 |
| % of total time spent in open arm | 41.8±6.7 | 52.3±2.6 | 44.5±5.4 | 50.4±4.0 |
| % of total arm entries made onto open arm | 50.4±5.3 | 60.0±2.6 | 56.5±3.0 | 57.9±3.1 |
| Number of closed arm returns | 0.22±0.2 | 0.13±0.1 | 0.0±0.0 | 0.75±0.3 |
| Number of open arm entries | 17.6±2.9 | 24.3±3.0 | 23.6±3.1 | 20.1±3.2 |
| Number of rears | 5.89±1.0 | 8.63±1.6 | 5.14±2.0 | 11.6±2.7 |
| Duration of non-exploratory behaviour | 2.66±1.3 | 5.21±1.4 | 6.46±1.7 | 0.82±0.6 |
| Mean net duration of stretched attend postures | 1.33±0.5 | 1.63±1.0 | 1.63±0.30 | 0.625±0.18 |

In figs. 3.1 to 3.4 inclusive, the shaded bar closest to the ordinate always represents the 'control' group value, i.e. the water-drinking mice which received saline injections.

Fig. 3.1 shows the mean percent of total time spent in the closed arm of the plus-maze after administration of saline or ethanol. The administration of ethanol significantly decreased the total time spent in the closed arm of both drinking-groups compared to the control group (both $P<0.05$, Dunnett's test.)

Fig. 3.2 shows the mean percent of total time spent on the open arm of the plus-maze after administration of saline or ethanol. The administration of.ethanol significantly increased the total time spent on the open arm of both drinking-groups compared to the control group (both $P<0.05$, Dunnett's test.)

Fig. 3.3 shows the mean percent of the total number of entries made onto the open arm of the plus-maze after administration of saline or ethanol. The administration of ethanol significantly increased the percent of open-arm entries of the water-drinking group only compared to the control group ($P<0.05$, Dunnett's test.)

Fig. 3.4 shows the mean number of total entries made into either arm of the elevated plus-maze after treatment with ethanol or saline. Treatment with ethanol did not cause the number of total arm entries to differ significantly from the control value, and neither did the nicotine-drinking, saline-injected group differ significantly from the control group in this respect. This figure was included as an example of a 'non-significant result' (although in one sense this is a contradiction in terms!- see discussion.)

3.6 Plus-maze testing- second time, day 36, Figs. 3.1-3.4 inclusive.

The second and final plus-maze test was an exact repeat of the first test: the same drugs were administered to the same mice. This time however, mice exhibited some significant behavioural differences between drinking groups and drug treatment groups

(ethanol or saline injections) as observed on the plus-maze. Raw data is shown in Table 3.2 below. Again, there were four groups to compare, n=8 in each group:

Group 1: water-drinkers which had received a saline injection ('Control' group)

Group 2: nicotine-drinkers which had received a saline injection

Group 3: water-drinkers which had received a 1.75 g/kg ethanol injection

Group 4: nicotine-drinkers which had received a 1.75 g/kg ethanol injection

Table 3.2 gives the averaged raw plus-maze data values for each treatment group on the second plus-maze day, presented as mean values (with standard errors.)

| Plus-maze parameter tested | Group 1 ± SEM | Group 2 ± SEM | Group 3 ± SEM | Group 4 ± SEM |
|---|---|---|---|---|
| Total number of entries made into closed arm | 15.1±2.5 | 17.4±2.2 | 12.3±4.3 | 15.6±3.6 |
| Total number of entries made into either arm | 31.0±4.8 | 34.5±2.5 | 45.3±5.8 | 43.5±7.0 |
| % of total time spent in the central section | 31.7±5.6 | 27.4±3.5 | 23.5±6.8 | 31.2±4.7 |
| % of head dips which are unprotected | 98.3±1.1 | 82.8±12.5 | 100±0.0 | 83.8±12.5 |
| % of stretched attend postures unprotected | 63.5±16.2 | 67.2±15.4 | 42.9±20.2 | 58.3±25.8 |
| Number of head-dips | 8.11±2.5 | 7.75±2.5 | 14.29±6.4 | 19.38±8.5 |
| % of total time spent in closed arm | 38.7±6.6 | 38.3±3.5 | 17.0±6.0 | 19.6±4.4 |
| % of total time spent in open arm | 28.7±5.3 | 33.8±4.2 | 59.4±9.7 | 48.9±5.4 |
| % of total arm entries made onto open arm | 50.2±3.4 | 49.0±6.9 | 74.9±8.0 | 66.6±6.2 |
| Number of closed arm returns | 1.33±0.6 | 0.38±0.2 | 0.0±0.0 | 0.25±0.3 |
| Number of open arm entries | 15.9±2.7 | 17.1±2.4 | 33.1±4.7 | 27.9±4.3 |
| Number of rears | 15.89±2.9 | 24.0±6.9 | 2.0±1.3 | 4.63±2.2 |
| Duration of non-exploratory behaviour | 9.89±4.5 | 5.77±2.1 | 14.99±4.4 | 9.95±3.8 |
| Mean net duration of stretched attend postures | 2.667±0.8 | 5.250±3.7 | 0.429±0.2 | 1.125±0.5 |

Fig. 3.1 Nicotine or Water- Drinking TO mice (n=16 per group) Mean percent of total time spent in closed arm of plus-maze after saline (shaded bars) or 1.75 g/kg ethanol (white bars) i.p. injection. Second Plus-Maze Day. (*P<0.05, c.f. water-drinking, saline-injected group.)

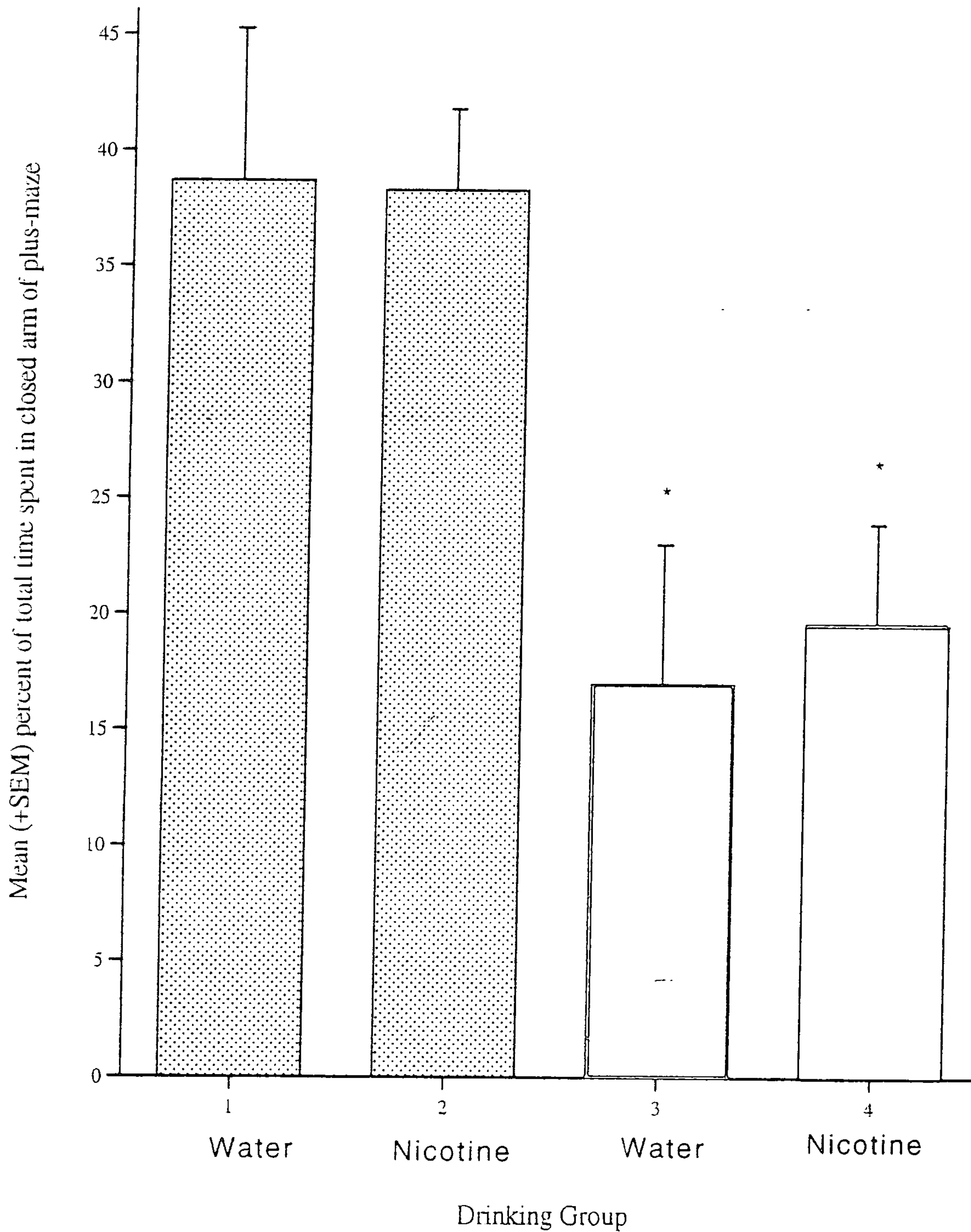


Fig. 3.2 Nicotine or Water-Drinking TO mice (n=16 per group). Mean percent of total time spent on open arm of plus-maze after saline (shaded bars) or 1.75 g/kg ethanol (white bars) i.p. injection. Second Plus-Maze Day.(*P<0.05, c.f. water-drinking, saline-injected group.)

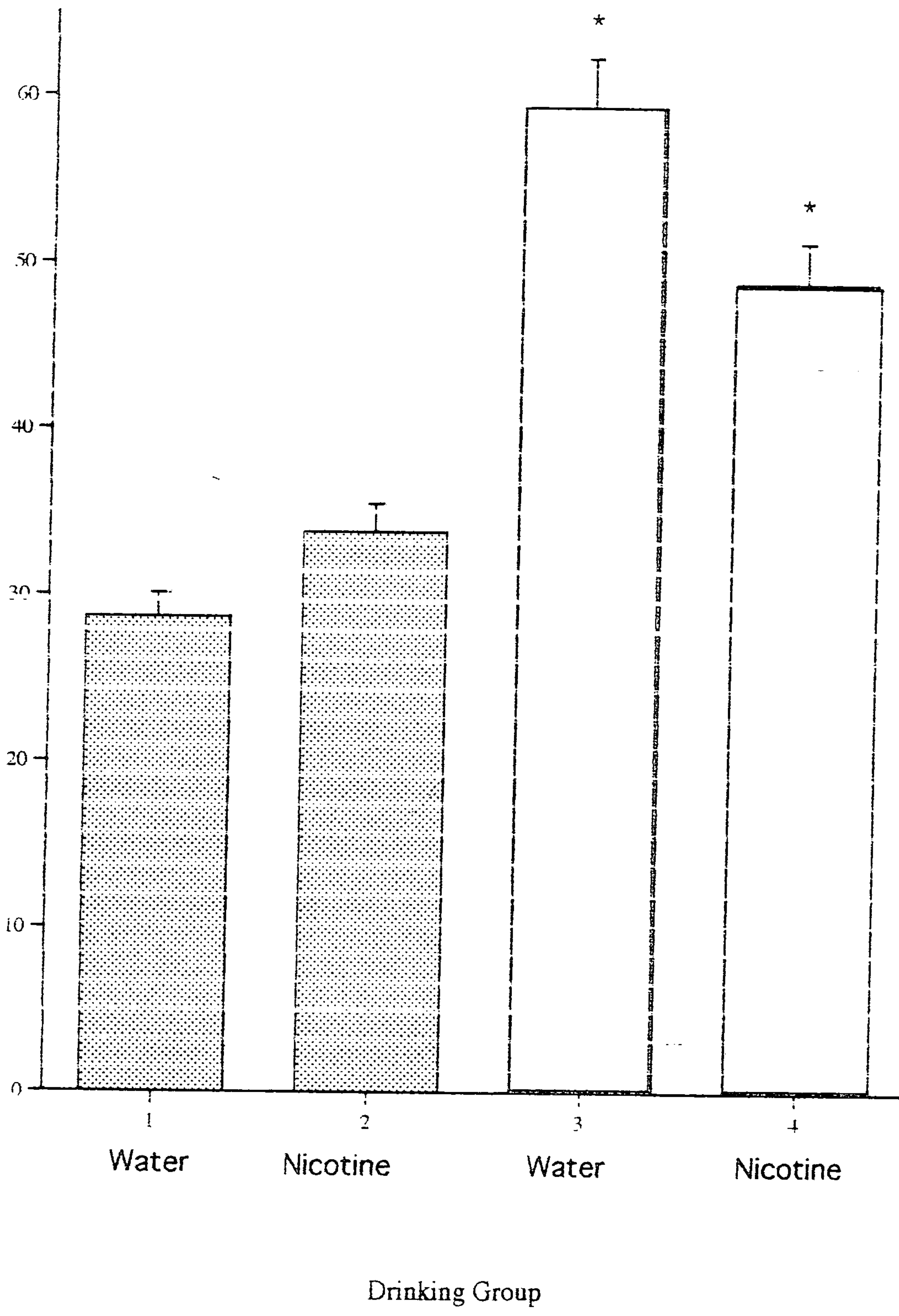


Fig. 3.3 Nicotine or Water-Drinking TO mice (n=16 per group) Mean percent of total number of entries made into open arm after saline (shaded bars) or 1.75 g/kg ethanol (white bars) i.p. injection. Second Plus-Maze Day. (*$p<0.05$,c.f.water-drinking, saline-injected group)

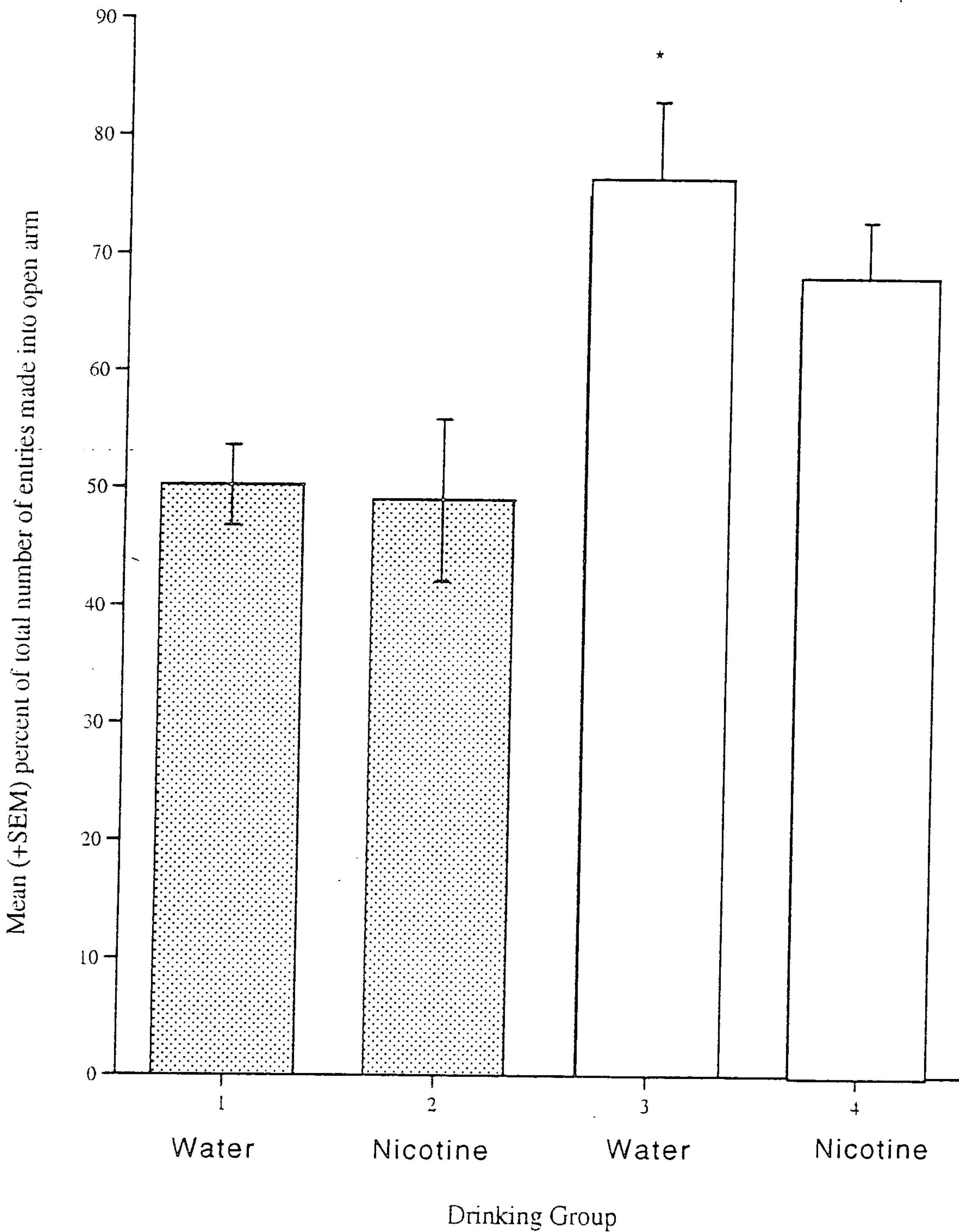
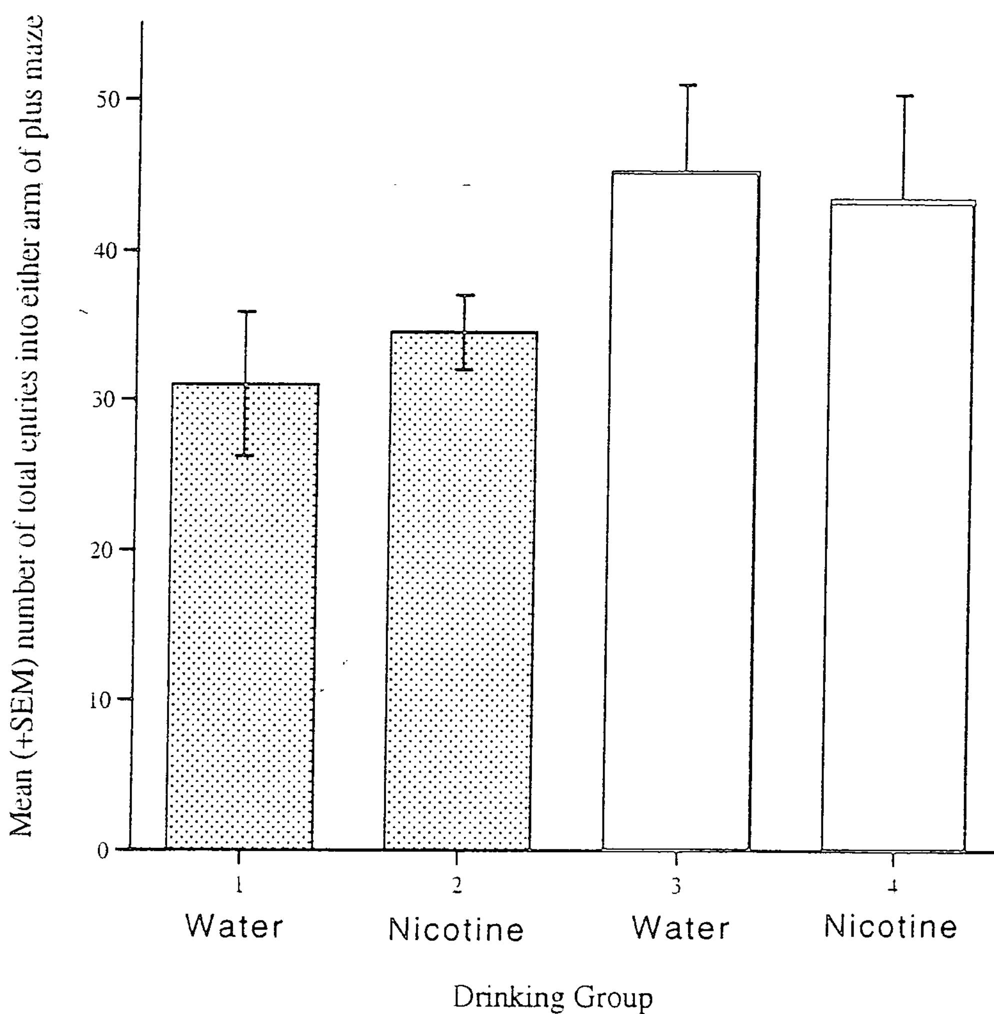


Fig 3.4 Nicotine or Water- Drinking TO mice (n=16 per group)
Mean number of total entries into either arm of plus-maze after saline (shaded bars) or 1.75 g/kg ethanol (white bars) i.p. injection. Second Plus-Maze Day.



The following table summarises all significant results of the second plus-maze on day 36.

Table 4 Summary of all significant results from the second plus-maze testing session. The 'control' group referred to is always the water-drinking, saline-injected group.

| Plus-maze parameter tested | Figure number of graph, if applicable | Which treatment produced significant difference from 'control' group | Direction of difference from control group: increase or decrease (Probability value, statistical test.) |
|---|---|---|---|
| % of total time spent in closed arm | 3.1 | Ethanol injections, both drinking groups | Both decrease (both P<0.05, Dunnett's test.) |
| % of total time spent in open arm | 3.2 | Ethanol injections, both drinking groups | Both increase (both P<0.05, Dunnett's test.) |
| % of total arm entries made onto open arm | 3.3 | Water-drinkers with ethanol injections only | Increase (P<0.05, Dunn's test.) |
| Number of closed arm returns | | Water-drinkers with ethanol injections only | Decrease (P<0.05, Dunn's test.) |
| Number of open arm entries | | Ethanol injections, both drinking groups | Both increase (both P<0.005, Dunnett's test.) |
| Number of rears | | Water-drinkers with ethanol injections only | Decrease (P<0.005, Dunn's test.) |

## ii) ETHANOL

### 4.1 Introduction

This experiment was intended as another preparation for the long-term, voluntary nicotine/ethanol drinking study. The main aim here was to find the optimum concentration of ethanol to use in this future experiment. A range of nine ethanol concentrations (0-20% v/v) were presented to singly-housed TO mice for three weeks in a two-bottle choice

paradigm. After three weeks all mice were tested once on the elevated plus-maze whilst undergoing withdrawal from ethanol. The behaviour exhibited by the mice thereon was compared between the nine ethanol concentration groups.

4.2 Daily intake of ethanol across concentration groups, Fig. 4.0

Fig. 4.0 shows the mean daily intake of ethanol (g/kg) for the different ethanol concentration groups. ('Daily' intake meant total intake over 24h.) The group which drank 12.5% (v/v) ethanol in a two-bottle choice had the highest mean daily intake of ethanol. The lowest mean daily intake was among the mice in the 2.5% (v/v) ethanol group.

4.3 Plus-maze testing, Fig. 4.1

After three weeks of continuous access to ethanol and water, all mice were tested once on the elevated plus-maze. All mice underwent withdrawal from ethanol prior to exposure to the maze. The length of this withdrawal period varied between a minimum of 2h20minutes and maximum of 5h, because although all ethanol bottles were removed at 09:00h, they were only replaced after that mouse had been exposed to the maze (last mouse was tested at 14:00h.)

Only one behavioural parameter measured on the plus-maze varied significantly between the subjects from one of the eight ethanol concentration groups compared with those in the water-only group. Fig. 4.1 shows this significant difference in the mean number of total entries made into both arms of the plus-maze. Mice in the 20% ethanol group exhibited a significantly lower mean total number of arm entries compared to mice in the water group ($P<0.05$, Dunnett's test.)

Table 5 **next page**: Raw plus-maze data for mice in the nine ethanol concentration groups, presented as mean values (with standard errors.)

| Plus-maze parameter tested | Ethanol concentration group during two-bottle choice/ % (v/v) ± SEM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2.5 | 5.0 | 7.5 | 10.0 | 12.5 | 15.0 | 17.5 | 20.0 |
| Total number of entries made into closed arm | 16.5± 2.7 | 14.5± 1.5 | 12.25 ±1.44 | 12.13 ±0.92 | 14.63 ±1.63 | 14.0± 1.0 | 16.5± 1.9 | 15.25 ±2.04 | 9.88± 0.85 |
| Total number of entries made into either arm | 29.3± 3.7 | 24.4± 2.5 | 22.8± 2.4 | 23.0± 2.6 | 26.4± 2.4 | 24.8± 1.4 | 29.6± 2.8 | 30.4± 2.8 | 19.3± 1.5 |
| % total time spent in central section | 42.1± 4.2 | 47.8± 3.8 | 42.8± 5.7 | 50.7± 6.9 | 43.5± 5.5 | 52.6± 4.4 | 47.7± 2.9 | 40.7± 3.1 | 46.7± 5.0 |
| % of head dips unprotected | 96.4± 2.6 | 99.0± 1.0 | 99.6± 0.4 | 98.6± 0.7 | 100.0 ±0.0 | 98.2± 1.8 | 100± 0.0 | 98.8± 1.3 | 100± 0.0 |
| % of stretched attend postures unprotected | 81.3± 13.4 | 70.8± 16.0 | 53.9± 16.4 | 81.3± 12.3 | 97.5± 2.5 | 71.9± 16.0 | 68.8± 16.2 | 83.2± 12.2 | 100± 0.0 |
| Number of head-dips | 9.5± 1.7 | 9.88± 1.29 | 17.5± 3.86 | 13.25 ±4.06 | 8.75± 2.02 | 10.0± 1.7 | 15.38 ±1.55 | 19.0± 2.2 | 9.75± 2.19 |
| % total time spent in closed arm | 27.8± 4.1 | 28.4± 2.1 | 21.9± 2.7 | 21.4± 2.7 | 22.3± 1.8 | 16.8± 1.5 | 21.3± 1.2 | 21.4± 2.1 | 21.1± 3.7 |
| % of total time spent in open arm | 29.5± 6.0 | 23.7± 3.1 | 34.3± 6.9 | 27.7± 8.0 | 33.7± 5.4 | 30.6± 4.9 | 30.9± 2.8 | 37.5± 3.0 | 32.0± 3.5 |
| % of total arm entries made onto open arm | 44.8± 4.9 | 39.9± 4.0 | 44.3± 5.6 | 42.8± 7.0 | 44.6± 3.9 | 43.0± 3.2 | 43.9± 3.6 | 50.8± 3.3 | 46.9± 4.7 |
| Number of closed arm returns | 17.4± 3.49 | 9.88± 1.95 | 12.25 ±2.66 | 10.13 ±1.04 | 15.0± 2.75 | 13.0± 1.38 | 15.38 ±2.47 | 13.75 ±2.27 | 10.25± 1.82 |
| Number of open arm entries | 12.8± 1.78 | 9.88± 1.46 | 10.5± 2.0 | 10.88 ±2.53 | 11.75 ±1.51 | 10.75 ±1.11 | 13.13 ±1.76 | 15.13 ±1.27 | 9.38± 1.61 |
| Number of rears | 0.25± 0.16 | 1.13± 0.55 | 1.00± 0.63 | 0.88± 0.44 | 0.38± 0.18 | 2.00± 1.09 | 1.13± 0.48 | 1.13± 0.67 | 0.88± 0.61 |
| Duration of non-exploratory behaviour | 2.04± 0.65 | 3.64± 0.57 | 2.05± 0.66 | 6.14± 2.37 | 4.61± 2.25 | 4.19± 1.28 | 3.51± 1.54 | 2.99± 0.85 | 5.02± 1.14 |
| Mean net duration of stretched attend postures | 3.63± 1.02 | 2.13± 0.79 | 3.38± 0.98 | 3.63± 1.12 | 3.25± 0.65 | 1.38± 0.46 | 1.38± 0.38 | 2.50± 0.78 | 2.88± 0.55 |

Fig.4.0 Mean daily intake (over three-week exposure period) of ethanol in two-bottle choice experiment, TO mice (n=8 per group).

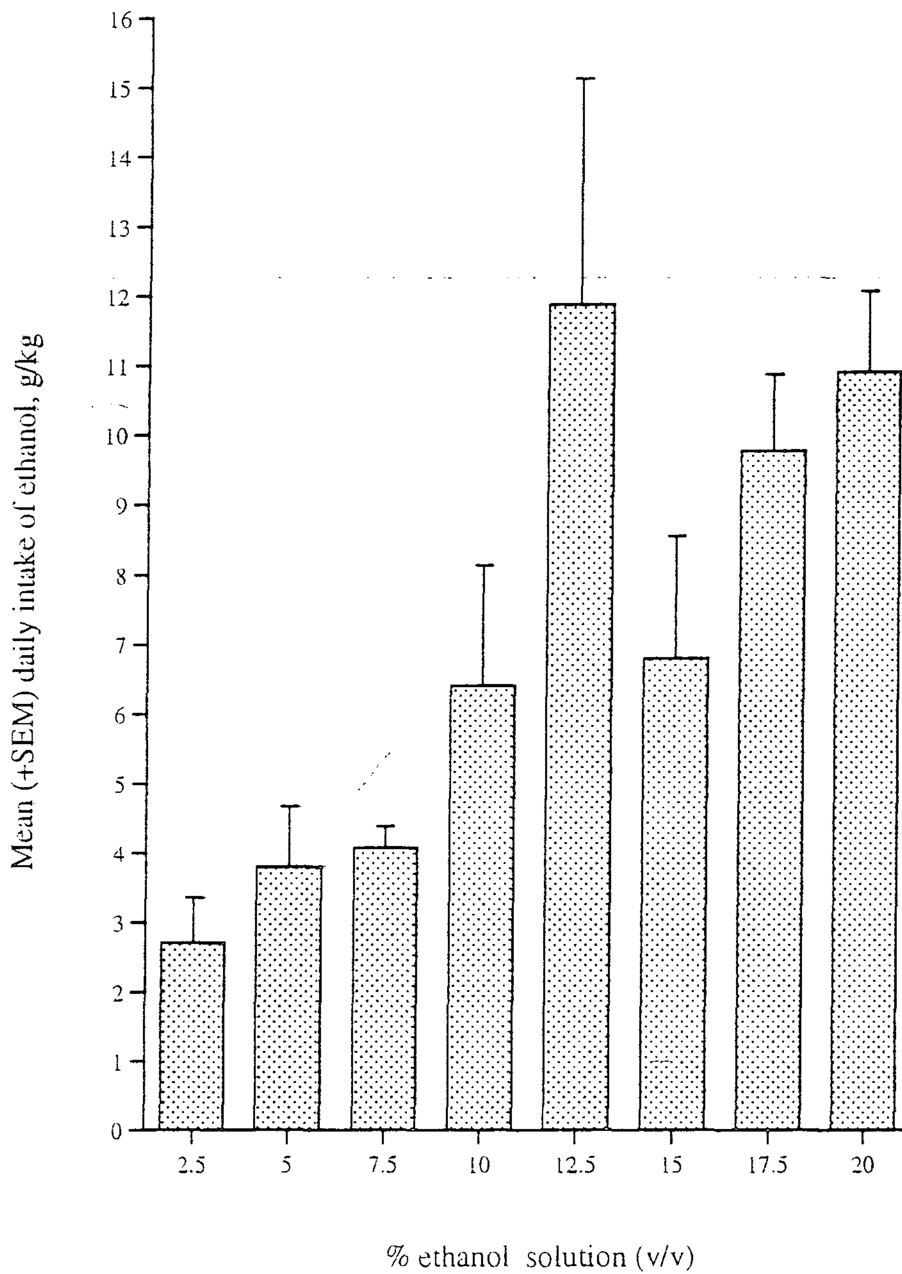
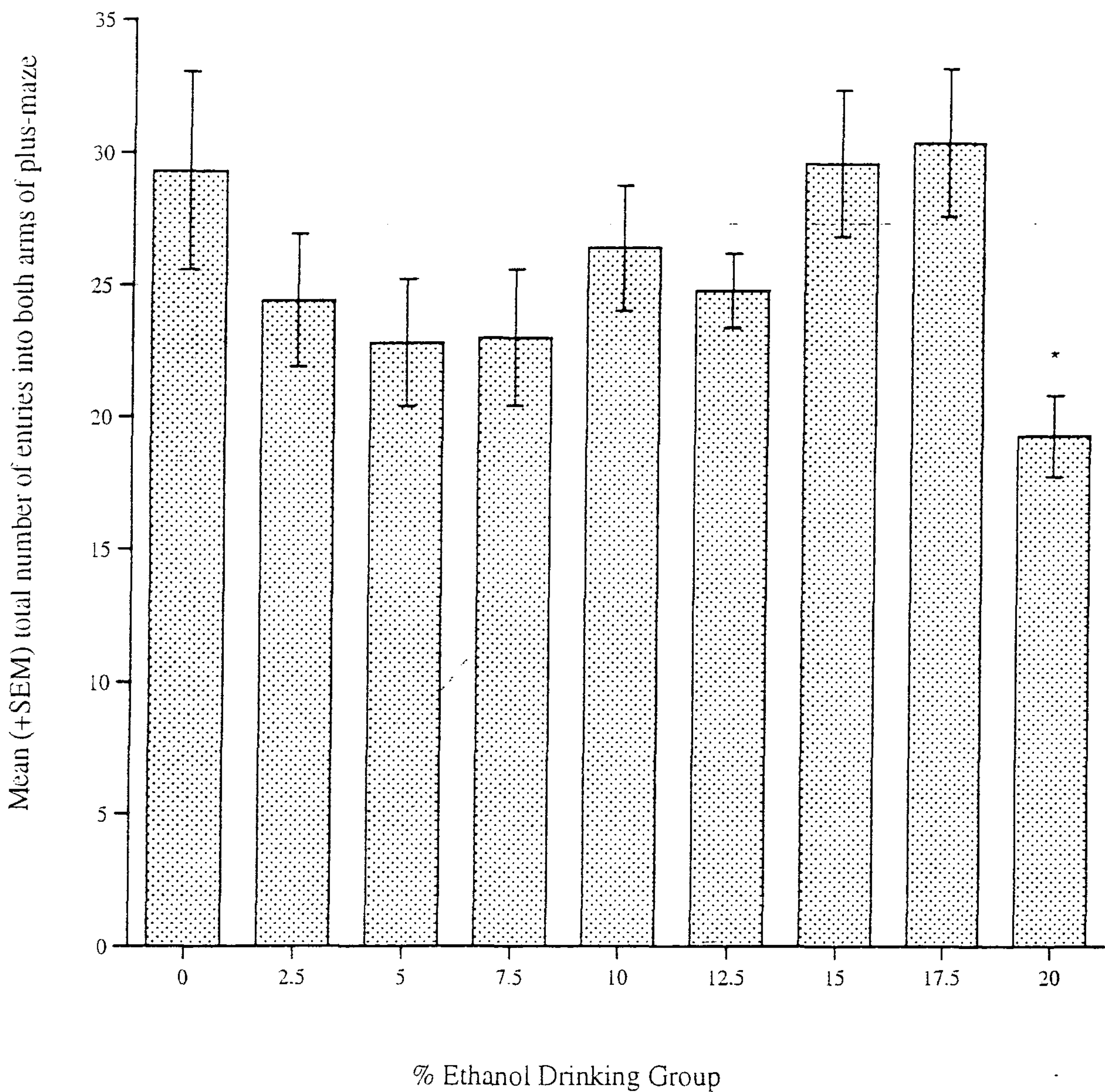


Fig. 4.1 Mean number of total entries into both arms of plus-maze. Prior to testing mice had been exposed to one of a range of ethanol solutions (v/v) in a three-week, two-bottle choice preference study. Withdrawal time before testing was 2h20 minutes minimum, 5h maximum.($P<0.05$, c.f. 0% ethanol group)



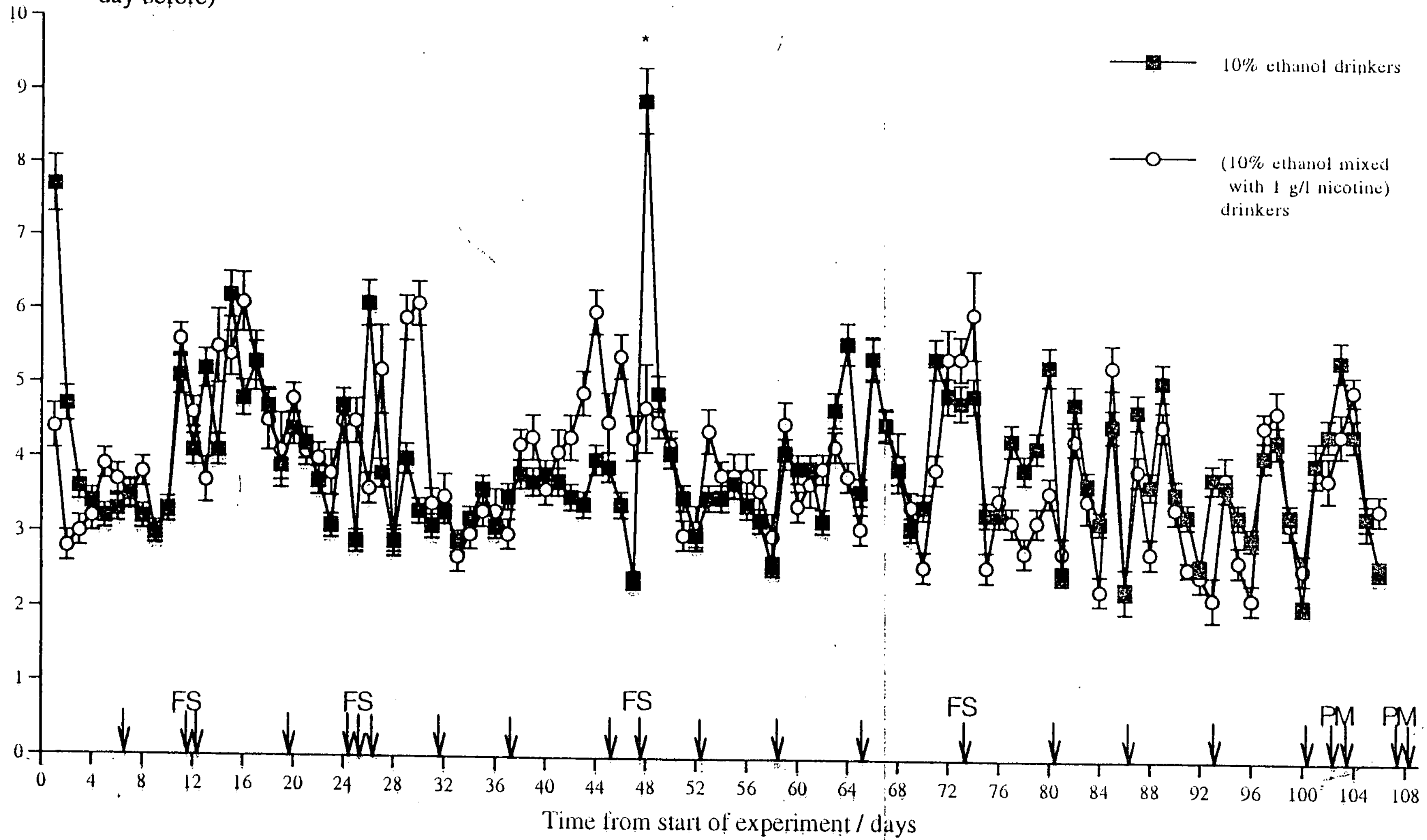



Fig. 5.0 Daily ethanol consumption of TO mice in long-term two-bottle-choice experiment. Second drinking-bottle contains either 10% (v/v) ethanol or same dose ethanol with added 1 g/l nicotine. n=10 (ethanol 10% only); n=40 (mixture of both drugs.) (*$P<0.005$, c.f. mean intake of 10% ethanol the day before)

iii) ETHANOL/NICOTINE

5.1 Introduction

The first aim of this experiment was to examine preferences for ethanol or nicotine when offered alone or in combination. (The doses of both drugs used were chosen according to the results of the preceding two experiments.) Mice were exposed to 1 g/l (v/v) nicotine, or 10% (v/v) ethanol, or a mixture of the two, for fourteeen weeks in a two-bottle choice paradigm. After fourteen weeks, all mice were exposed to the elevated plus-maze after undergoing exactly 4h withdrawal from their respective drug solutions. Five days later, all mice were re-exposed to the plus-maze but this time none had their drug solutions withdrawn prior to testing. The aim of these two sets of plus-maze tests was to compare the behaviour seen after withdrawal from ethanol and nicotine with that seen when the same mice were not undergoing withdrawal from the two drugs.

5.2 Ethanol intake over fourteen weeks, Fig. 5.0

Fig.5.0 shows the voluntary mean ethanol intake (g/kg/day, where 'day'= 24h) of mice drinking either a 10% ethanol solution or a mixture of 10% ethanol and 1 g/l nicotine. The ethanol intake of these two groups did not differ significantly throughout the duration of the experiment. Similarly, cage-cleaning was not found to produce a significant change in ethanol intake for either group on the day after this procedure, throughout the fourteen weeks. (It should be stressed that in all experiments where the effect of cage-cleaning on drug intake was measured, precautions were taken in order to minimise factors other than mouse drinking which could cause loss of fluid from the bottles. These precautions included stabilising the cage-rack (to prevent jarring of cages, leading to leakage) and always up-ending feeding bottles when cleaning-out cages, then replacing them gently.)

The day after the stale drug solutions were replaced by fresher solutions, the ethanol intake of those mice in the solely 10% ethanol solution group always increased compared with their mean intake the day before. This increase was significant only on day 48 ($P<0.005$, Student's t-test.) Giving fresh solutions to mice in the drug mixture group did not significantly

alter their mean intake of ethanol the following day, nor was the direction of this intake change consistently an increase compared to the day before.

5.3 Nicotine intake over fourteen weeks, Fig. 5.1

Fig. 5.1 shows the voluntary mean nicotine intake (mg/kg/24h) of mice drinking either a 1 g/l nicotine solution or a mixture of 1 g/l nicotine and 10% ethanol. (Note that the intake graph for the group drinking the drug mixture in figs. 5.0 and 5.1 follows the same pattern.) Considering the whole fourteen weeks, the mean daily nicotine intake of mice offered the plain nicotine solution was significantly lower than the mean daily nicotine intake of mice with the choice of the drug mixture solution ($P<0.0001$, Mann-Whitney Rank Sum Test.)

Again, cage-cleaning did not have a significant effect on daily nicotine intake among the mice in either nicotine-drinking group. Replacing stale drug solutions with fresher solutions did not significantly alter the mean intake of nicotine the following day for either nicotine-drinking group, compared with intake the day before.

5.4 First Plus-maze, days 102/103, mice in withdrawal

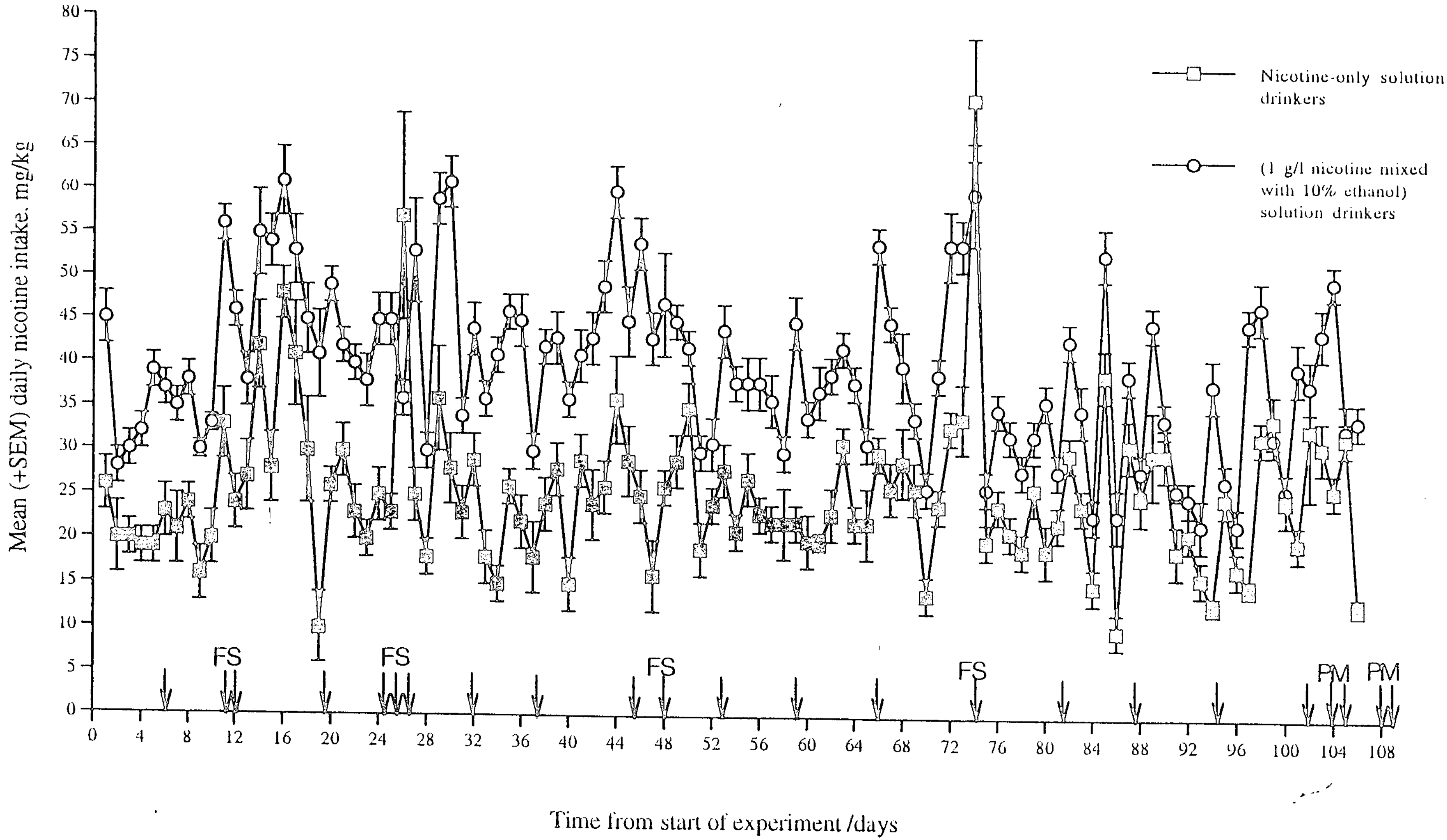
After fourteen weeks of two-bottle choice all mice except those in group 7 underwent a 4h-withdrawal period. The group descriptions and their respective withdrawal procedures follows:-

Group 1: water/water drinkers: one bottle withdrawn
Group 2: ethanol/water drinkers: ethanol withdrawn
Group 3: (ethanol+nicotine)/water drinkers: mixture withdrawn
Group 4: nicotine/water drinkers: nicotine withdrawn
Group 5: (ethanol+nicotine)/water drinkers: only ethanol withdrawn
Group 6: (ethanol+nicotine)/water drinkers: only nicotine withdrawn
Group 7: (ethanol+nicotine)/water drinkers: mixture not withdrawn.

Table 6 Raw data from the first plus-maze for mice in the seven treatment groups, presented as mean values (± standard errors.)

| Plus-maze parameter tested | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|---|---|---|---|---|---|---|---|
| Total number of entries made into closed arm | 9.1± 1.0 | 9.9± 1.1 | 11.6± 1.5 | 10.0± 0.8 | 9.3± 1.4 | 10.6± 1.3 | 12.1± 1.6 |
| Total number of entries made into either arm | 21.0± 1.3 | 18.0± 1.4 | 19.6± 1.9 | 19.9± 1.9 | 13.3± 1.8 | 15.5± 1.8 | 17.1± 2.6 |
| % of total time spent in the central section | 19.26± 2.0 | 23.21± 3.3 | 19.53± 2.7 | 17.22± 1.9 | 14.23± 2.1 | 21.63± 2.3 | 17.47± 1.9 |
| % of head dips which are unprotected | 92.0± 2.3 | 86.7± 10.0 | 79.5± 13.3 | 95.8± 2.5 | 87.7± 10.0 | 95.0± 5.0 | 99.1± 0.95 |
| % of stretched attend postures which are unprotected | 55.67± 13.8 | 45.00± 15.7 | 55.14± 12.7 | 79.78± 5.2 | 50.43± 9.6 | 67.66± 8.6 | 53.75± 16.8 |
| Number of head-dips | 18.0± 3.4 | 11.2± 2.3 | 13.6± 3.2 | 14.3± 2.5 | 5.1± 1.7 | 4.5± 1.1 | 7.625± 2.9 |
| % of total time spent in closed arm | 39.27± 3.2 | 46.27± 4.6 | 47.4± 6.3 | 56.78± 4.8 | 73.67± 3.6 | 62.04± 5.0 | 64.71± 4.2 |
| % of total time spent in open arm | 38.99± 5.2 | 29.74± 5.0 | 31.69± 6.7 | 25.27± 3.6 | 10.06± 2.3 | 16.09± 3.6 | 15.02± 4.0 |
| % of total arm entries made onto open arm | 57.58± 3.6 | 45.22± 4.3 | 38.47± 7.4 | 48.15± 3.1 | 30.49± 5.5 | 30.6± 3.6 | 26.23± 4.9 |
| Number of closed arm returns | 1.40± 0.9 | 1.90± 1.0 | 2.80± 1.2 | 2.70± 1.1 | 5.70± 1.6 | 3.10± 1.1 | 2.125± 0.9 |
| Number of open arm entries | 11.9± 0.7 | 8.1± 1.0 | 8.0± 1.8 | 9.9± 1.4 | 4.0± 0.9 | 4.9± 0.9 | 5.0± 1.3 |
| Number of rears | 14.0± 1.0 | 13.9± 1.8 | 14.3± 2.5 | 17.1± 1.9 | 19.0± 1.3 | 21.7± 2.5 | 17.3± 2.8 |
| Duration of non-exploratory behaviour | 5.954± 1.9 | 5.308± 2.0 | 4.009± 2.1 | 6.819± 2.5 | 8.557± 2.0 | 5.817± 1.4 | 7.572± 1.0 |
| Mean net duration of stretched attend postures | 4.20± 1.2 | 1.70± 0.5 | 4.30± 1.1 | 5.40± 0.7 | 4.50± 0.7 | 4.70± 0.9 | 3.50± 0.6 |

Fig. 5.1 Daily nicotine consumption of TO mice in long-term two-bottle-choice experiment. Second drinking-bottle contains either 1 g/l nicotine solution or same dose nicotine with added 10% (v/v) ethanol solution. n=10 (nicotine 1 g/l only); n=40 (mixture of both drugs.)




Arrows: FS=fresh solutions; PM= plus maze testing; other arrows ...

After exactly 4h all mice were exposed to the plus-maze. The behaviour on the maze of the mice from different treatment groups was compared with that of group 1 (the water-drinking group.) The data for this group is represented on all graphs in this set as the bar closest to the ordinate. Raw data from the first plus-maze session is shown in Table 6, previous page.

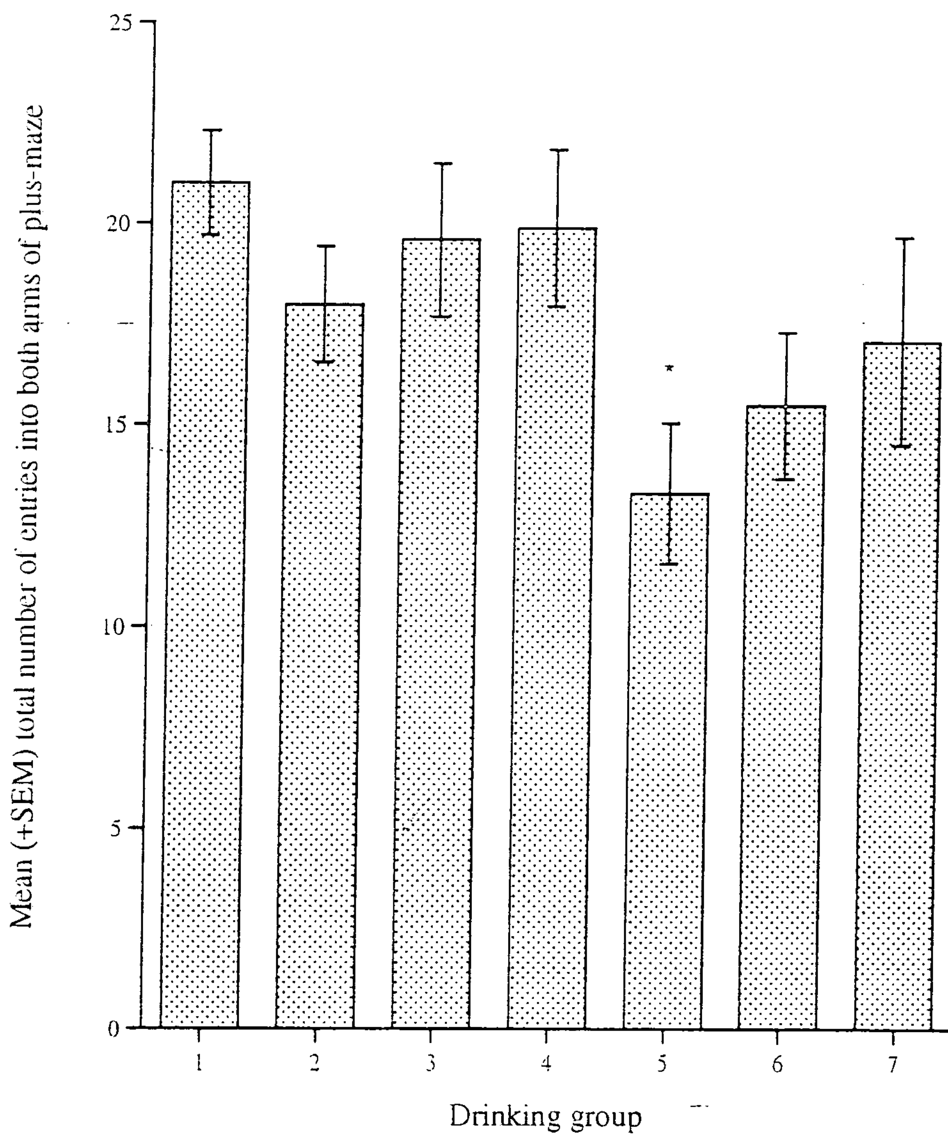
Fig. 5.2 shows the effect of the different withdrawal procedures on the mean number of total entries into either arm of the plus-maze. Compared to group 1, all treatments appeared to decrease the number of entries but only for group 5 was this decrease significant ($P<0.05$, Dunnett's test.) Mice in group 5 had had access to an ethanol/nicotine mixture for 14 weeks but at the time of testing were undergoing nicotine withdrawal.

Fig. 5.3 shows the effect of the different withdrawal procedures on the percentage of total time spent in the closed arm of the plus-maze. Compared to group 1, all treatments appeared to increase the percentage but only for groups 4-7 inclusive was this increase significant (all $P<0.00005$, Dunnett's test.) Mice in group 4 were undergoing nicotine withdrawal. Those in groups 6 and 7 had had access to an ethanol/nicotine mixture for 14 weeks, but at the time of testing group 6 were undergoing ethanol withdrawal whereas those in group 7 never underwent withdrawal.

Fig. 5.4 shows the effect of the different withdrawal procedures on the percentage of total entries made onto the open arm of the plus-maze. Compared to group 1, all treatments appeared to decrease this percentage but only for groups 3, 5, 6 and 7 was this decrease significant (all $P<0.0005$, Dunnett's test.) Mice in group 3 had had access to an ethanol/nicotine mixture for 14 weeks, but at the time of testing were undergoing withdrawal from both drugs.

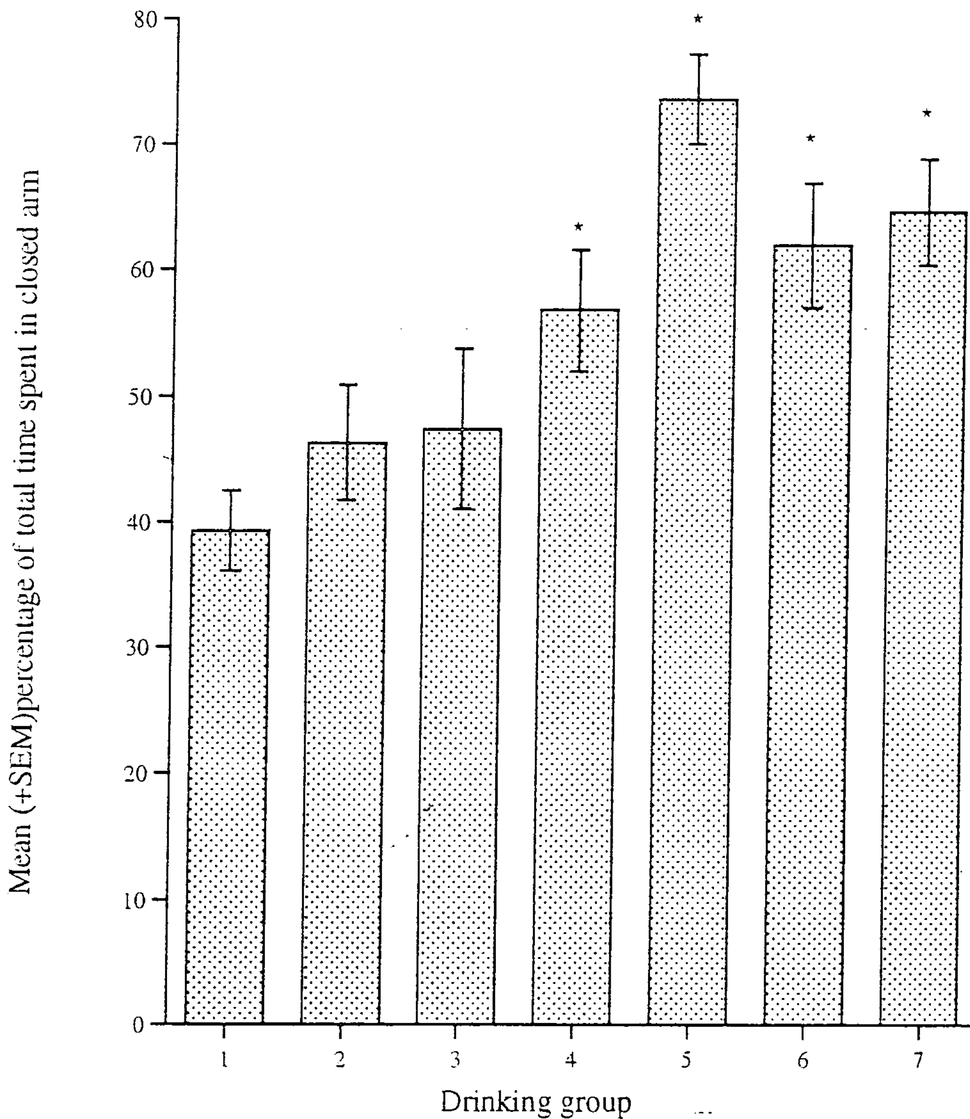
Fig. 5.5 shows the effect of the different withdrawal procedures on the mean percentage of total time spent on the open arm of the plus-maze. Compared to group 1, all treatments appeared to decrease this percentage but only for groups 5-7 inclusive was this decrease significant (all $P<0.0005$, Dunnett's test.)

Fig. 5.2 Mean number of total entries into both arms of plus-maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine- drinking TO mice. First plus-maze, some subjects in withdrawal. ($P<0.05$, c.f. drinking group 1)



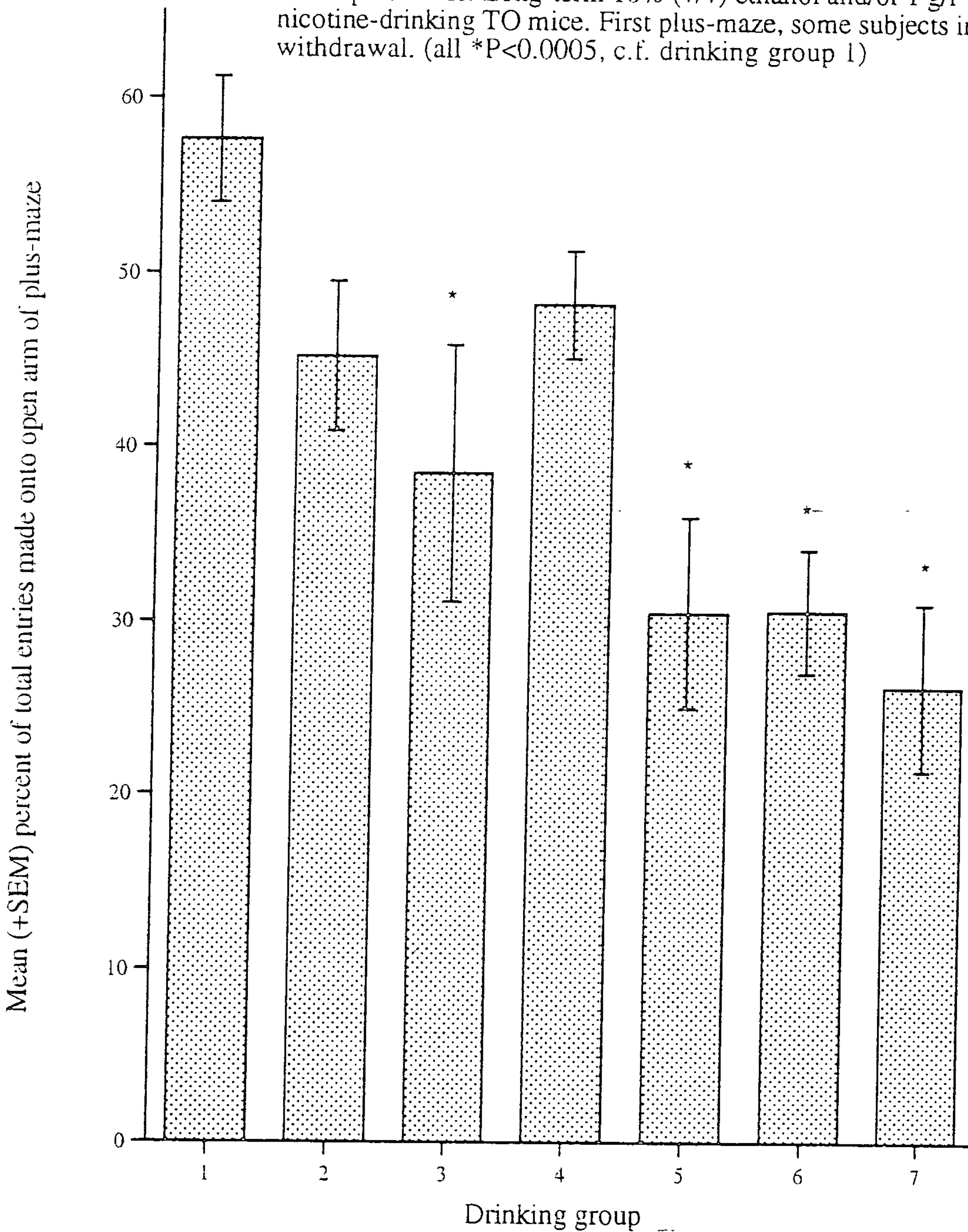

1=water
2=ethanol (withdrawn)
3=ethanol/nicotine, both withdrawn
4=nicotine (withdrawn)
5=ethanol/nicotine, withdrawing nicotine only
6=ethanol/nicotine,withdrawing ethanol only
7=ethanol/nicotine, neither withdrawn

Fig. 5.3 Mean percentage of total time spent in closed arm of plus maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine-drinking TO mice. First plus-maze, some subjects in withdrawal. (all *P<0.00005, c.f. drinking group 1)



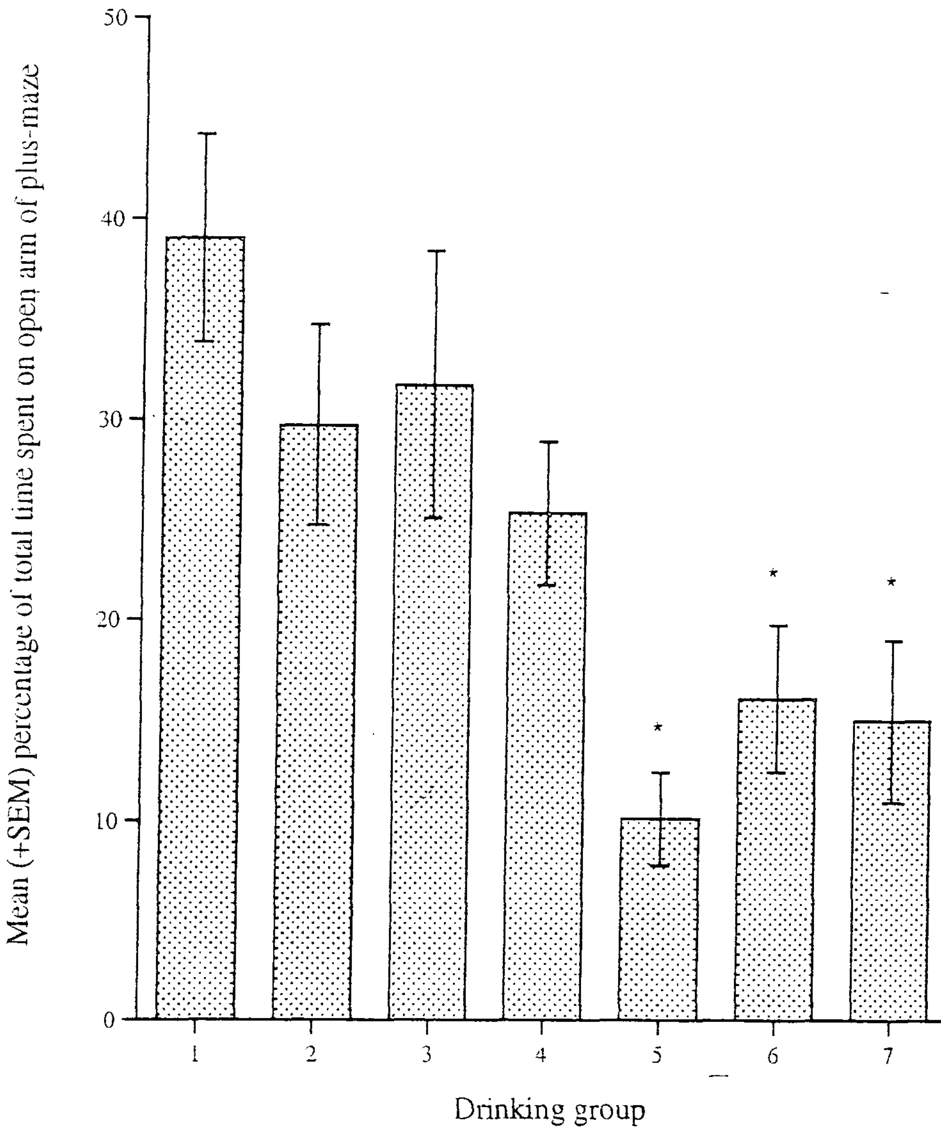

1=water
2=ethanol (withdrawn)
3=ethanol/nicotine, both withdrawn
4=nicotine (withdrawn)
5=ethanol/nicotine, withdrawing nicotine only
6=ethanol/nicotine,withdrawing ethanol only
7=ethanol/nicotine, neither withdrawn

Fig.5.4 Mean percent of total entries made onto the open arm of the plus-maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine-drinking TO mice. First plus-maze, some subjects in withdrawal. (all *P<0.0005, c.f. drinking group 1)




1=water
2=ethanol (withdrawn)
3=ethanol/nicotine, both withdrawn
4=nicotine (withdrawn)
5=ethanol/nicotine, withdrawing nicotine only
6=ethanol/nicotine,withdrawing ethanol only
7=ethanol/nicotine, neither withdrawn

Fig. 5.5 Mean percentage of total time spent on open arm of plus maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine-drinking TO mice.First plus-maze, some subjects in withdrawal. (all *P<0.0005, c.f. drinking group 1)




1=water
2=ethanol (withdrawn)
3=ethanol/nicotine, both withdrawn
4=nicotine (withdrawn)
5=ethanol/nicotine, withdrawing nicotine only
6=ethanol/nicotine,withdrawing ethanol only
7=ethanol/nicotine, neither withdrawn

Table 7 summarises the results above and all other significant results of the first plus-maze testing session. 'Group 1' was the water-drinking group.

Table 7 Summary of all significant results from the first plus-maze testing session, days 102-103.

| Plus-maze parameter tested | Graph figure, if applicable | Treatment groups differing significantly from group 1 | Direction of difference (probability value, statistical test.) |
|---|---|---|---|
| Total number of entries made into either arm | 5.2 | 5 | Decrease ($P<0.05$, Dunnett's.) |
| % of total time spent in closed arm | 5.3 | 4,5,6,7 | All increased (all $P<0.00005$, Dunnett's.) |
| % of total arm entries made onto open arm | 5.4 | 3,5,6,7 | All decreased (all $P<0.0005$, Dunnett's.) |
| % of total time spent on open arm | 5.5 | 5,6,7 | All decreased (all $P<0.0005$, Dunnett's.) |
| Number of open arm entries | | 5,6,7 | All decreased (all $P<0.00005$, Dunnett's.) |
| Number of head-dips | | 5,6 | Both decreased (both $P<0.005$, Dunn's.) |

5.5 Second plus-maze data, days 107/108, no drug withdrawal

One week after the first plus-maze test, the whole procedure was repeated using the same mice. This time, instead of following a withdrawal procedure 4h prior to testing, none of the solutions were withdrawn or changed at any time, i.e. all mice had continuous access to their respective drugs except when they were placed on the maze. A reminder of the drinking group descriptions follows:-

Group 1: water/water drinkers

Group 2: ethanol/water drinkers

Group 3: (ethanol+nicotine)/water drinkers

Group 4: nicotine/water drinkers

Group 5: (ethanol+nicotine)/water drinkers

Group 6: (ethanol+nicotine)/water drinkers

Group 7: (ethanol+nicotine)/water drinkers

The behaviour exhibited on the maze of the mice from the different treatment groups was again compared with that of group 1 (the water-drinking group, represented on all graphs as the bar closest to the ordinate.) Table 8 shows the raw data from the second plus-maze session, shown on the next page.

Fig. 5.6 shows the effect of drinking group on the median ranked percentage of total time spent in the closed arm of the plus-maze. Compared to group 1, all treatments appeared to increase this median percentage but only for groups 3-6 inclusive was this increase significant (all $P<0.05$, Dunnett's test.)

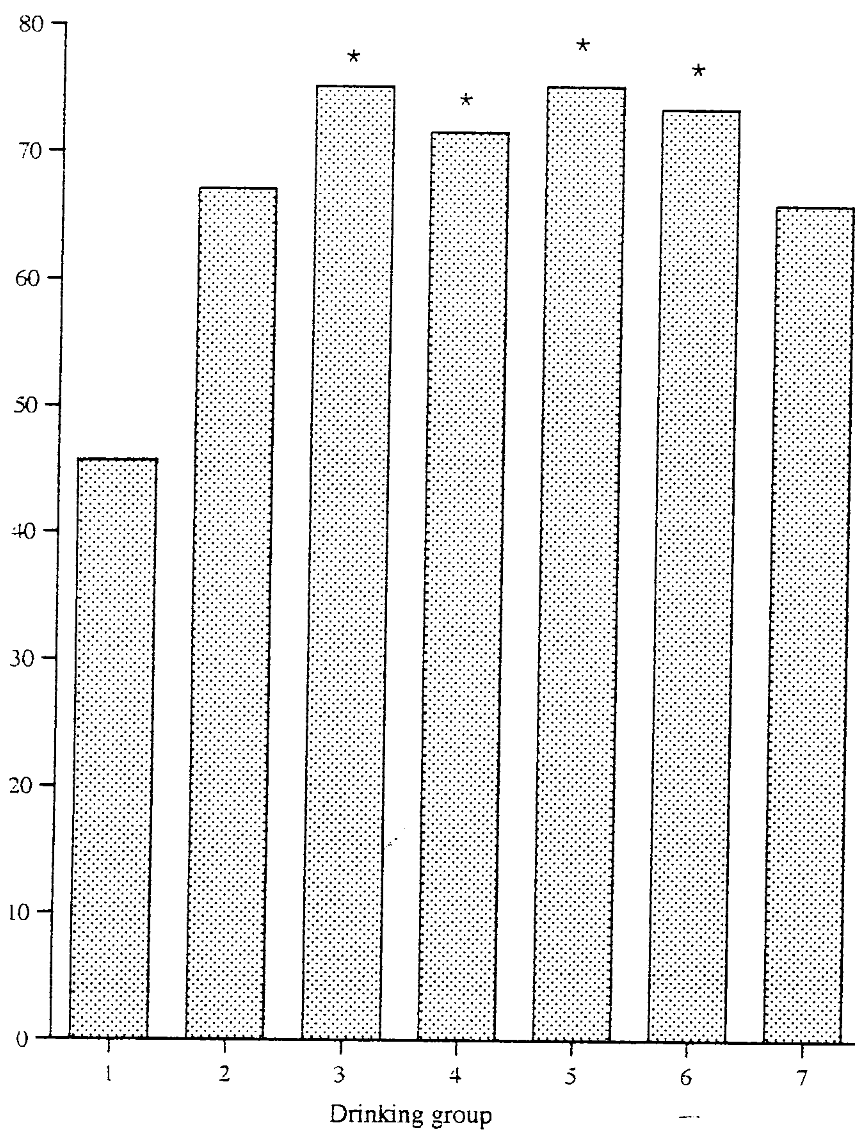
Fig. 5.7 shows the effect of drinking group on the mean number of total entries made into either arm of the plus-maze. Compared to group 1, mice in groups 4-6 inclusive made significantly fewer entries into either arm (all $P<0.05$, Dunnett's test.)

Fig. 5.8 shows the effect of drinking group on the mean percentage of total entries made into the open arm of the plus-maze. Compared to group 1, all treatments appeared to decrease this percentage but only for groups 3, 5 and 6 was this decrease significant (all $P<0.05$, Dunnett's test.)

Table 8  Raw data from the second plus-maze for mice in the seven treatment groups, presented as mean values (± standard errors.)

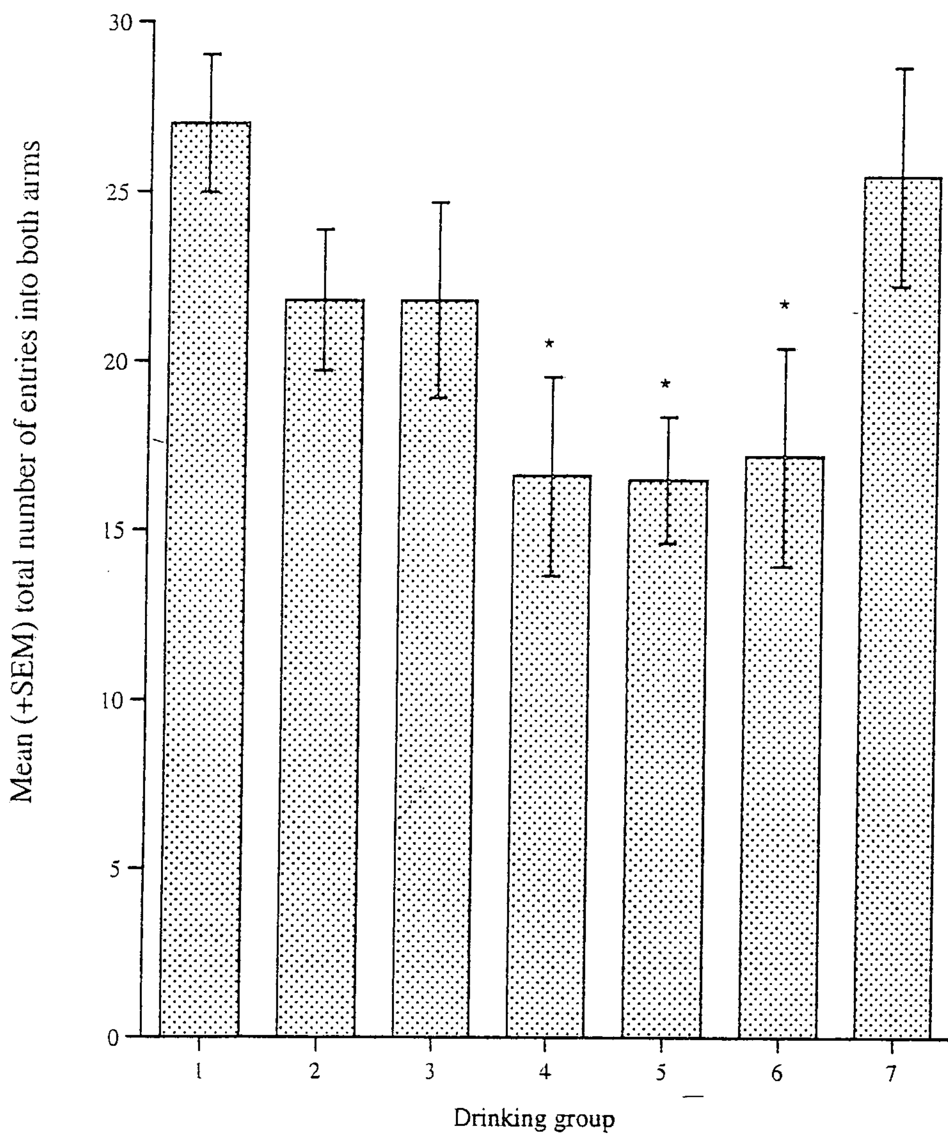
| Plus-maze parameter tested | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|---|---|---|---|---|---|---|---|
| Total number of entries made into closed arm | 12.7± 1.4 | 12.4± 1.0 | 14.1± 1.5 | 8.40± 1.4 | 11.7± 1.4 | 12.1± 2.4 | 16.0± 2.4 |
| Total number of entries made into either arm | 27.0± 2.0 | 21.8± 2.0 | 21.8± 2.9 | 16.6± 2.9 | 16.5± 1.9 | 17.2± 3.2 | 25.5± 3.2 |
| % of total time spent in the central section | 16.93± 1.8 | 16.02± 2.3 | 11.90± 2.1 | 8.549± 1.8 | 13.51± 2.4 | 12.88± 1.9 | 14.10± 2.2 |
| % of head dips which are unprotected | 96.93± 1.4 | 81.79± 10.5 | 65.64± 14.5 | 71.93± 12.9 | 57.50± 15.8 | 76.67± 13.2 | 85.63± 12.3 |
| % of stretched attend postures which are unprotected | 69.23± 12.6 | 76.17± 10.0 | 62.00± 14.7 | 82.17± 17.9 | 60.0±1 6.3 | 66.57± 13.8 | 76.04± 11.0 |
| Number of head-dips | 16.3± 2.7 | 6.80± 2.1 | 6.30± 2.0 | 6.80± 2.3 | 3.70± 1.4 | 3.20± 1.1 | 9.00± 2.7 |
| % of total time spent in closed arm | 48.80± 2.8 | 59.95± 5.2 | 71.29± 5.8 | 73.59± 5.2 | 74.94± 5.5 | 71.10± 6.3 | 62.61± 5.9 |
| % of total time spent in open arm | 33.60± 3.1 | 22.83± 4.2 | 16.50± 4.5 | 17.73± 3.7 | 11.39± 3.8 | 15.86± 5.2 | 23.18± 5.7 |
| % of total arm entries made onto open arm | 53.36± 2.9 | 40.57± 5.8 | 31.33± 6.4 | 42.19± 6.4 | 27.13± 5.6 | 25.01± 6.2 | 37.83± 4.4 |
| Number of closed arm returns | 0.60± 0.3 | 4.10± 1.7 | 3.40± 0.8 | 2.70± 0.9 | 3.60± 0.7 | 2.40± 0.7 | 3.375± 0.9 |
| Number of open arm entries | 14.3± 1.3 | 9.40± 1.9 | 7.70± 1.9 | 8.20± 1.8 | 4.80± 1.1 | 5.10± 1.6 | 9.50± 1.5 |
| Number of rears | 24.9± 3.2 | 19.8± 2.5 | 19.2± 2.4 | 17.4± 3.1 | 22.9± 3.0 | 21.0± 2.6 | 18.5± 2.8 |
| Duration of non-exploratory behaviour | 7.64± 3.5 | 8.40± 5.2 | 7.35± 1.4 | 29.9± 9.6 | 13.0± 2.2 | 6.61± 1.0 | 4.84± 1.4 |
| Mean net duration of stretched attend postures | 3.50± 0.9 | 4.70± 1.0 | 3.70± 0.7 | 2.70± 0.4 | 2.80± 0.8 | 3.70± 0.6 | 3.00± 0.3 |

Fig. 5.6 Median ranked percentage of total time spent in closed arm of plus-maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine-drinking TO mice.Second plus-maze, no subjects in withdrawal. ($P<0.05$, c.f. drinking group 1)



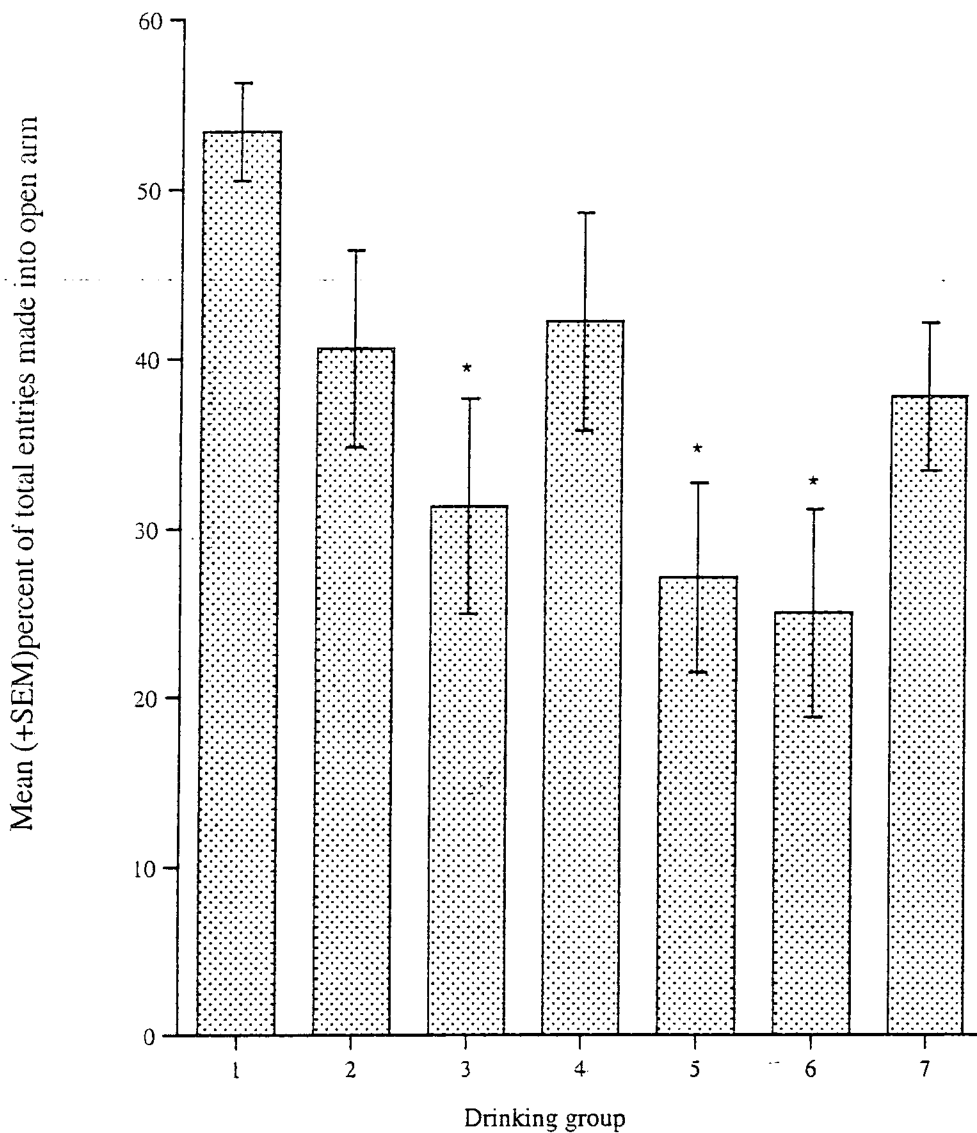

1=water
2=ethanol
3=ethanol/nicotine
4=nicotine
5=ethanol/nicotine
6=ethanol/nicotine
7=ethanol/nicotine

Fig. 5.7 Mean number of total entries into both arms of plus-maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine- drinking TO mice.Second plus-maze, ten days after the first. No withdrawal. ($P<0.05$, c.f. drinking group 1)




1=water
2=ethanol
3=ethanol/nicotine
4=nicotine
5=ethanol/nicotine
6=ethanol/nicotine
7=ethanol/nicotine

Fig. 5.8 Mean percentage of total number of entries made into open arm of plus-maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine- drinking TO mice.Second plus-maze, ten days after the first. No withdrawal. ($P<0.05$, c.f. drinking group 1)



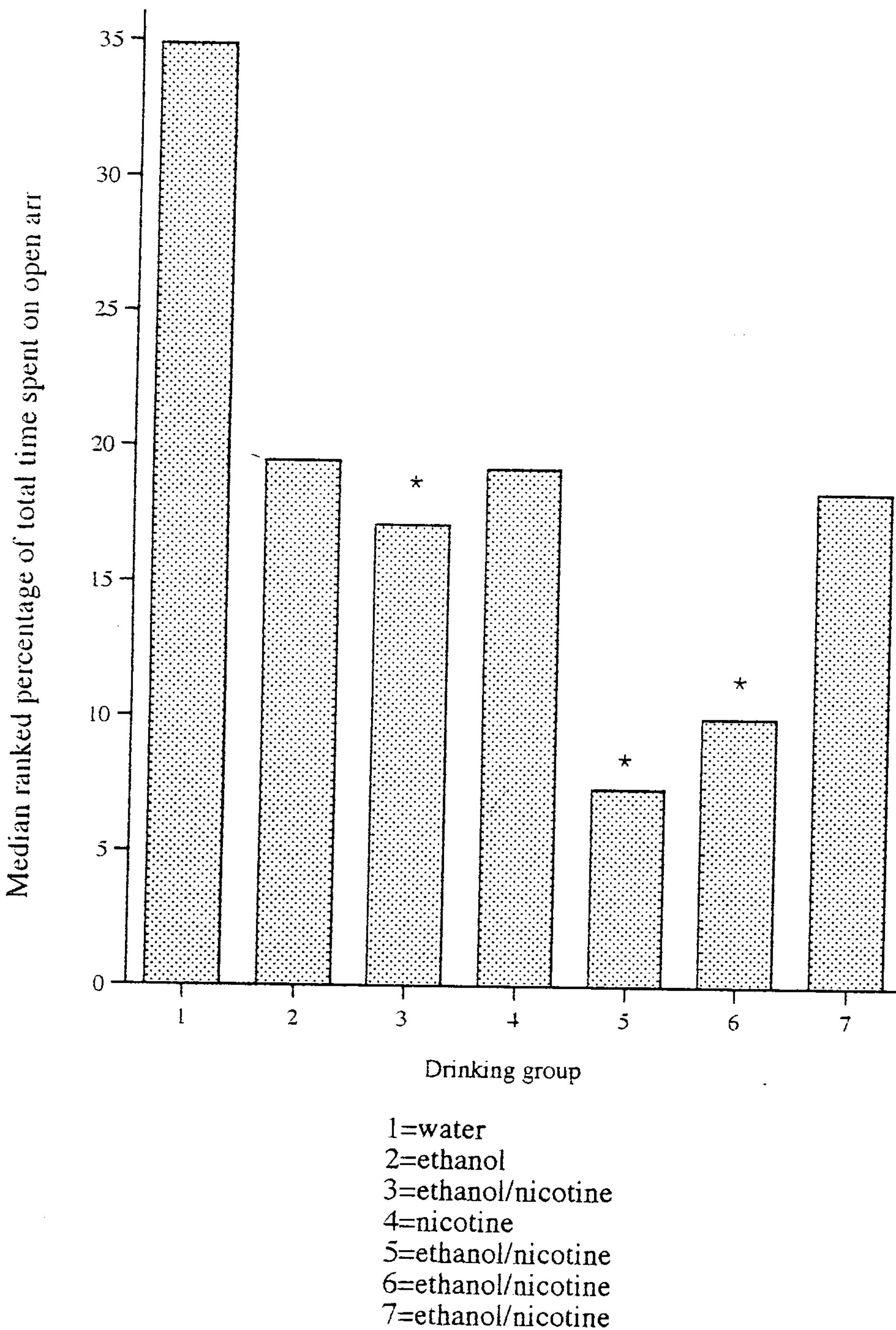

1=water
2=ethanol
3=ethanol/nicotine
4=nicotine
5=ethanol/nicotine
6=ethanol/nicotine
7=ethanol/nicotine

Fig. 5.9 shows the effect of drinking group on median ranked percentage of total time spent on the open arm of the plus-maze. Compared to group 1, all treatments appeared to decrease this percentage but only for groups 3, 5 and 6 was this decrease significant (all $P<0.05$, Dunn's test.) The following table gives a summary of the results stated above, and all other significant results from the second plus-maze session.

Table 9 Summary of all significant results from the second plus-maze session, days 107-108.

| Plus-maze parameter tested | Graph figure, if applicable | Drinking groups differing significantly from group 1 | Direction of difference (probability value, statistical test.) |
|---|---|---|---|
| Median ranked % of total time spent in closed arm | 5.6 | 3,4,5,6 | All increased (all $P<0.05$, Dunnett's.) |
| Total number of entries made into either arm | 5.7 | 4,5,6 | All decreased (all $P<0.05$, Dunnett's.) |
| % of total arm entries made onto open arm | 5.8 | 3,5,6 | All decreased (all $P<0.05$, Dunnett's.) |
| Median ranked % of total time spent on open arm | 5.9 | 3,5,6 | All decreased (all $P<0.05$, Dunn's.) |
| Number of closed arm returns | | 3,5 | Both increased (both $P<0.05$, Dunn's.) |
| Number of head-dips | | 5,6 | Both decreased (both $P<0.01$, Dunn's.) |
| Duration of non-exploratory behaviour | | 4 | Increased ($P<0.005$, Dunn's.) |
| Number of open arm entries | | 5,6 | Both decreased (both $P<0.01$, Dunn's.) |

Fig. 5.9 Median ranked percentage of total time spent on open arm of plus-maze. Long-term 10% (v/v) ethanol and/or 1 g/l nicotine- drinking TO mice.Second plus-maze, no subjects in withdrawal. ($P<0.05$, c.f. drinking group 1)



# IIII CONDITIONED PLACE PREFERENCE EXPERIMENTS

## i) Morphine/ Acamprosate

### 6.1 Introduction

The aim of this experiment was firstly, to determine whether morphine produces a place preference effect in TO mice, and secondly, to investigate whether acamprosate alters morphine's observed effect. Video analysis of the initial, drug-free pilot study showed that the clear side of the conditioned place preference box was the preferred side, so the morphine and acamprosate were paired with the non-preferred, black side of the box in conditioning sessions.

### 6.2 Place preference testing for morphine and acamprosate

Video analysis of the second, drug-free, baseline testing day showed that mice spent 60% of the total time on the clear side of the conditioned place preference box. 24h after the last of ten conditioning days, wherein the black side of the box was paired on alternate days with morphine 10 mg/kg, acamprosate 400 mg/kg, or both drugs together, all mice were tested in a drug-free state.

Fig. 6.0 shows the time spent by mice in the clear side of the conditioned place preference box on this drug-free test day, compared with their pre-conditioning time spent on this side, on the baseline day. The group of mice which had had saline injections every day, paired with both sides of the conditioned place preference box during the conditioning phase, exhibited a significant decrease in preference for the clear side of the box on the test day compared with their preference for this side on the baseline day ($P<0.05$, Student's t-test.) Mice in the groups wherein morphine or morphine and acamprosate had been paired with the black side of the conditioned place preference box did not exhibit any significant change in the duration of time spent in either side of the box on the test day compared to the baseline day.

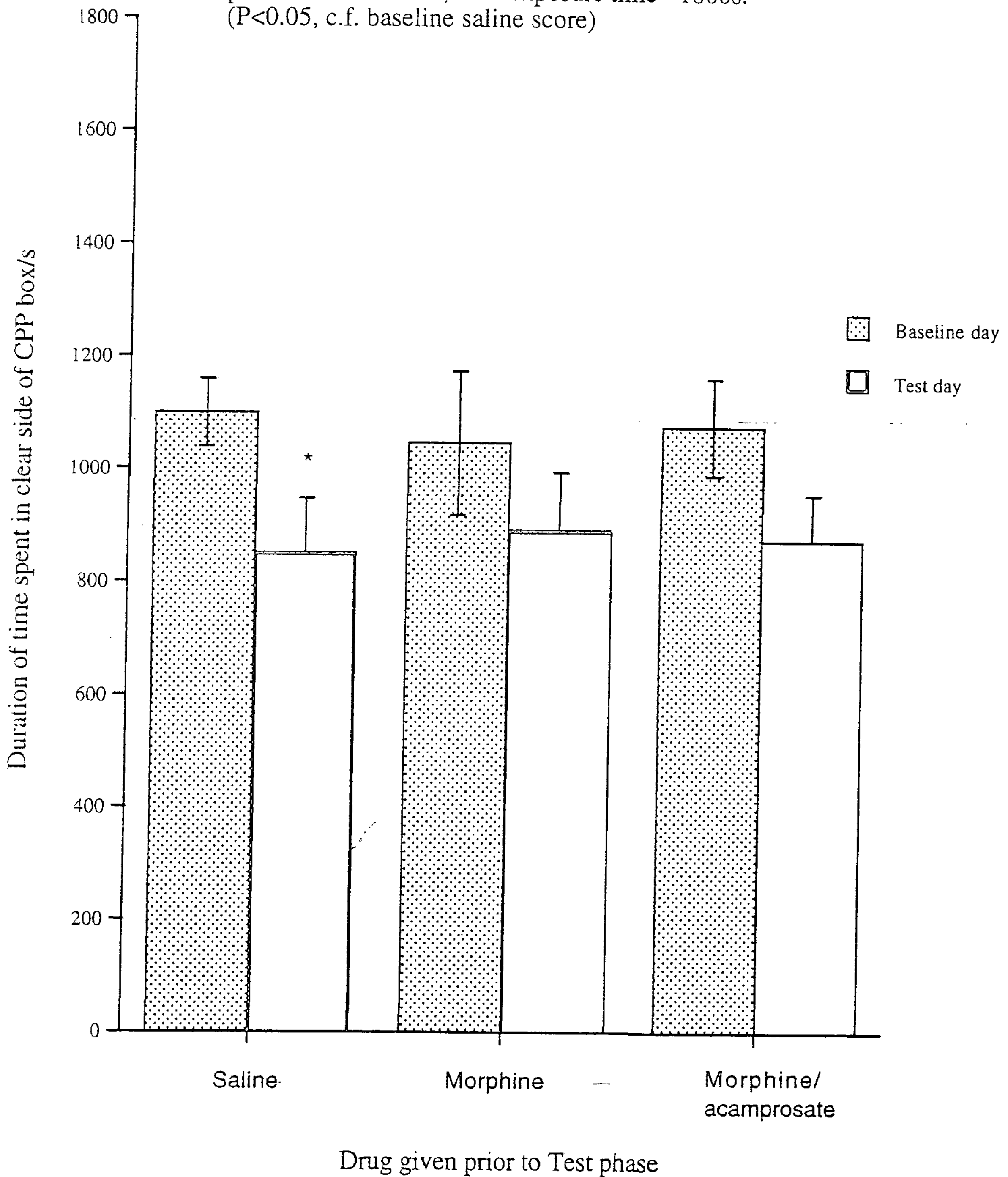



Fig.6.0 Effect of ten pairings of either saline (n=9), morphine (10 mg/kg, n=10) or morphine/acamprosate (10 mg/kg and 400 mg/kg respectively, n=10) with dark side of CPP box, on development of a conditioned place preference. TO mice, total exposure time= 1800s. ($P<0.05$, c.f. baseline saline score)

ii) Ethanol/Nicotine

## 7.1 Introduction

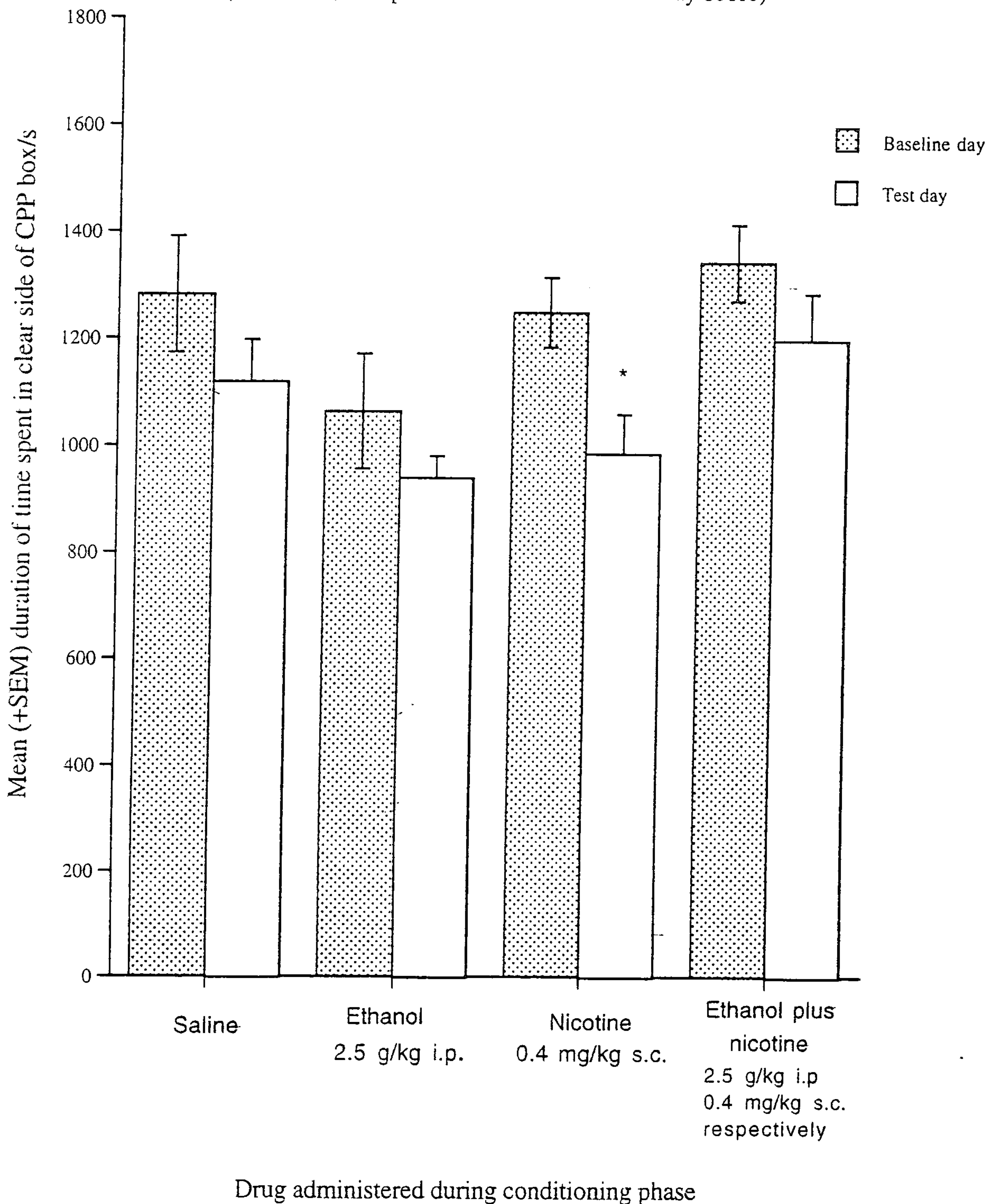
The aim of this experiment was to determine; firstly, whether ethanol produced a place preference effect in TO mice; secondly, whether nicotine produced the same effect; and thirdly, the effect produced when the two drugs were combined.

## 7.2 Place preference testing for ethanol, nicotine, and ethanol/nicotine

Analysis of videos of the drug-free baseline testing day showed that the preferred side of the conditioned place preference box was the clear side (69% of the total test time was spent on this side.) 24h after the last of ten conditioning days, wherein the black side of the box was paired on alternate days with either ethanol 2.5 g/kg, nicotine 0.4 mg/kg, or both injections together, all mice were tested in a drug-free state.

Fig. 7.0 shows the preference of the mice for the clear side of the conditioned place preference box on this drug-free test day, compared with their pre-conditioning preference on the baseline day. The group of mice which had had 0.4 mg/kg nicotine injections paired on alternate days with the black side of the conditioned place preference box during the conditioning phase, exhibited a significant decrease in preference for the clear side of the box compared with their preference for this side on the baseline day ($P<0.005$, Student's t-test.) Mice in the other three treatment groups did not exhibit any significant change in duration of time spent in either side of the box on the test day compared to the baseline day.

Fig. 7.0 Effect of ten pairings of drugs with one side of the CPP box on development of a conditioned place preference. Saline was paired with the clear side of the box, and the other drugs with the black side. TO mice. Total exposure time on test day =1800s. (*P<0.005, compared to nicotine baseline day score)



iii) Automated ethanol/nicotine-first attempt

## 8.1 Introduction

This experiment was intended as a modified repeat of the preceding ethanol/nicotine conditioned place preference experiment. The main modification was to automate the whole procedure and also to use an extra cue in the conditioned place preference box (chocolate.) As before, the eventual aim of this experiment was to determine; firstly, whether ethanol produces a place preference effect in TO mice; secondly, whether nicotine produces the same effect; and thirdly, the effect produced when the two drugs are combined. The experiment was planned and carried out in five stages, and to avoid confusion the results are also described in this way.

## 8.2 Setting up the automated conditioned place preference apparatus

The first objective was to set up and test the automated setup. Six TO mice were given free run of the conditioned place preference boxes to ensure that the computer was registering and recording beam-crossings accurately for each box. It was observed that sometimes beam-breaks would fail to register when the mice crossed betweeen the two sections of the box, and there could be problems when the mice groomed themselves or reared close to the boundary. However, the program always righted itself the next time the beam was broken.

## 8.3 Comparing results of the automated setup with those from video analysis

The conditioned place preference boxes were set up with the same extra differentiation cues as would be used in the main part of this experiment. This was a second one-day pilot study, using six further TO mice. The side preference of the drug-free mice in the boxes during a 30-minute period was monitored both by the computer and simultaneously a video camera. The results from these two sources were then compared.

Mice were found to spend 45% of the total time in the black, chocolate-paired side.

It was in fact necessary to conduct several 30-minute trials in order to obtain as accurate a figure as possible for the % total time duration value difference between the two methods. The final, mean discrepancy between the two methods for measuring time duration was found to be 5%.

8.4 Place preference testing for morphine, Fig. 8.0

The 5% discrepancy between time duration results, derived from using the video analysis or computer monitoring methods, was accepted. Consequently, in this next stage only the computer-generated results were used although video recording done simultaneously was analysed for the baseline day only to check that the score discrepancy was still averaging 5%. Twelve further TO male mice were used to try and test for the existence of a conditioned place preference effect with morphine. The standard procedure involved one drug-free baseline day, followed 24h later by ten conditioning days, followed 24h after the final conditioning trial by a final test day (drug-free.) On each of the ten conditioning days the treatment was as follows:-

n=6 mice: saline (0.9% i.p.) every day
n=6 mice: saline (0.9% i.p.) / morphine (10 mg/kg i.p.) on alternate days, such that each mouse received 5 saline pairings and 5 morphine pairings.

Both methods showed that 55% of the total time was spent on the clear side of the conditioned place preference box on the baseline day. Morphine was paired with the chocolate-smeared, dark side of the boxes in the ten conditioning days, whilst the saline was paired with the clear side.
Fig. 8.0 shows the duration of total time mice spent in the clear side of the conditioned place preference box on this drug-free test day, compared with their pre-conditioning time durations on the baseline day. Mice in neither of the two treatment groups exhibited any significant change in during of time spent in either side of the box on the test day compared to the baseline day.

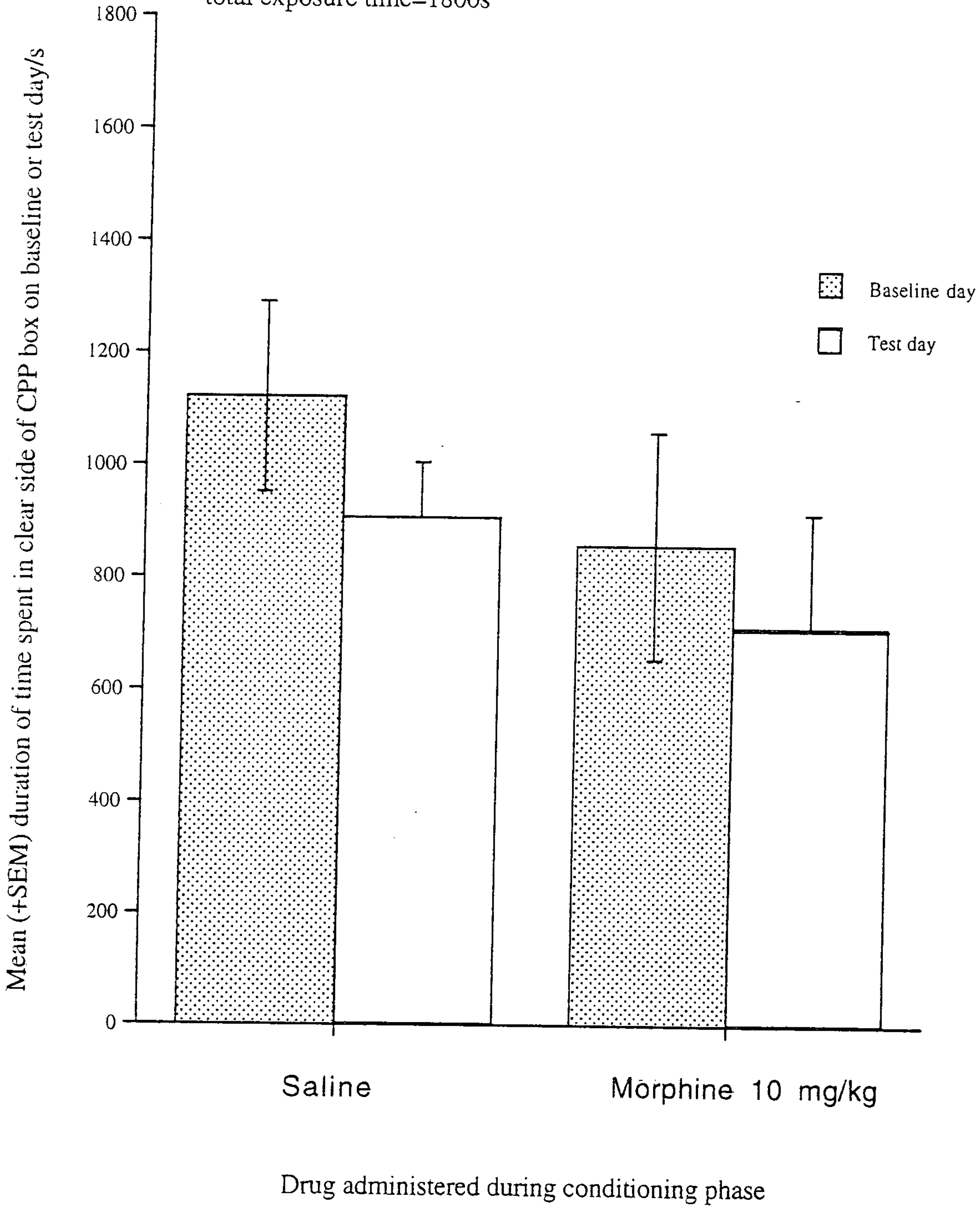



Fig. 8.0 Effect of ten pairings of either saline (n=6) or morphine (10 mg/kg, n=6) with one side of CPP box on development of a conditioned place preference on test day. Morphine was paired with the black side. TO mice, total exposure time=1800s

## 8.5 Place preference testing for morphine, second attempt, Fig. 8.1.

A further twelve TO mice were used in a repeat the experiment described above in section 8.4, in order to test again for the existence of a conditioned place preference effect with morphine. The standard conditioned place preference procedure followed was identical except on two counts: firstly, an additional differentiation cue (wet sawdust) was placed in the clear side of the conditioned place preference box, and secondly, the groups were matched for preference after the baseline day.

There was no significant difference between the duration of times spent in the two sides of the box on the baseline day. The decision to pair morphine with the black side was purely based on the fact that this was the morphine-paired side in the experiment described in section 8.4 above, otherwise the choice would have been arbitrary.

Fig. 8.1 shows the % of total time spent in the clear side of the conditioned place preference box on the drug-free test day, compared with their pre-conditioning time duration on the baseline day. Mice in neither of the two treatment groups exhibited any significant change in duration of time spent in either side of the box on the test day compared to the baseline day.

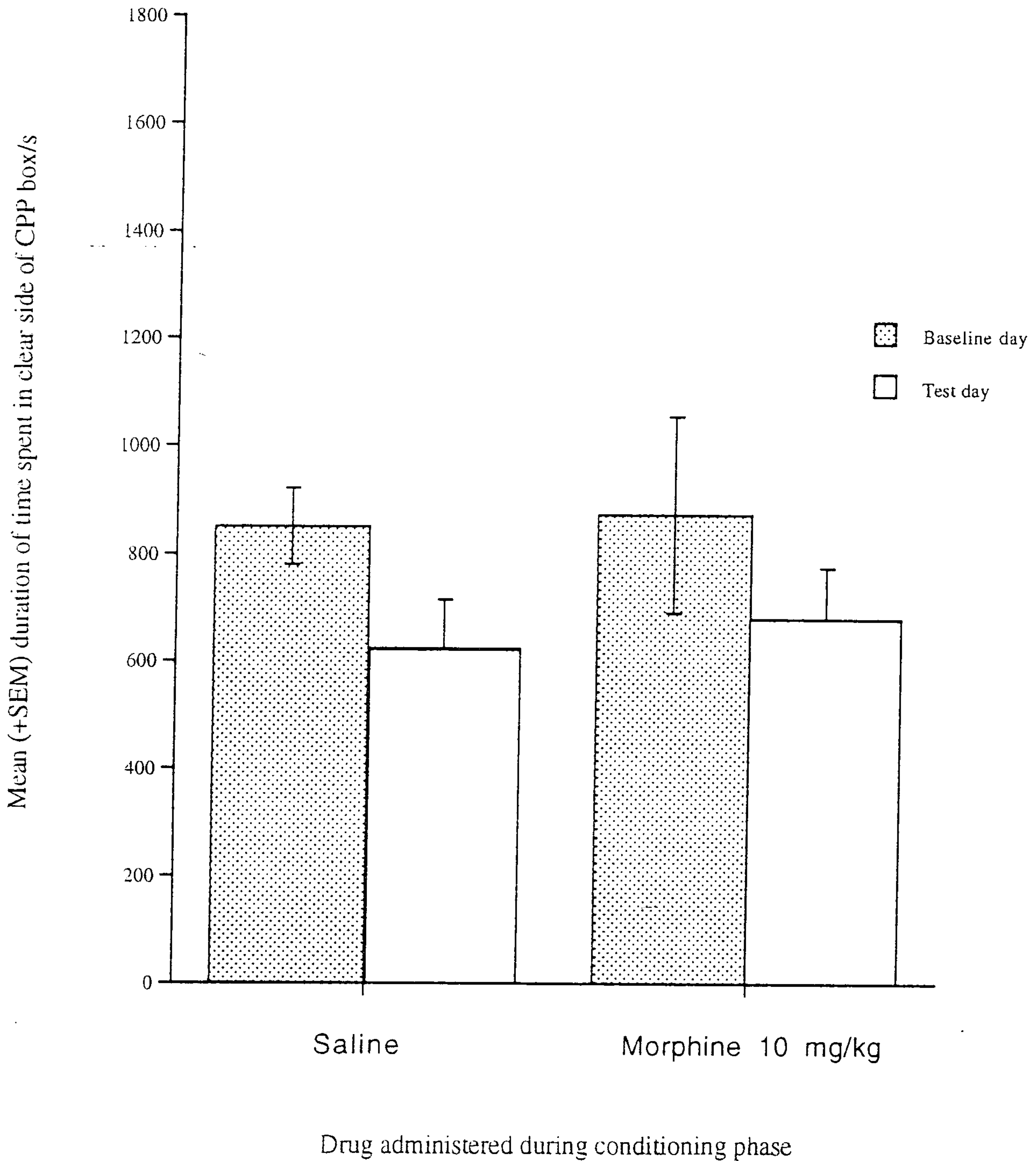
## 8.7 Place preference testing for haloperidol (planned)

A conditioned place preference experiment using haloperidol was planned to be carried out once a place preference effect for morphine had been achieved. This experiment was not carried out owing to lack of time to resolve problems with the preceding stage.

## 8.8 Place preference testing with ethanol/nicotine (planned)

If the results of the preceding stage showed an absence of a conditioned place preference effect with haloperidol, the experiment would be repeated using ethanol and nicotine. The procedure used would be a repetition of the earlier attempt, but this time using an automated setup instead of video monitoring. Again, this experiment was not carried out owing to lack of time to resolve problems with the preceding stages.

Fig.8.1 Effect of ten pairings of either saline (n=6) or morphine (10 mg/kg, n=6) with one side of CPP box on development of a conditioned place preference on test day. Morphine was paired with the black side.Second attempt, after methodological adjustments.TO mice. Total exposure time on test day= 1800s.



# DISCUSSION

The rewarding properties of a drug are considered the core cause of its addictiveness. This may seem paradoxical, since addiction can be defined as the compulsive use of a drug despite adverse consequences (Nestler, 1992.) One explanation for this problem could be that the drug is acutely rewarding and that reward occurs with repeated administrations such that the drive for the reward becomes the single most important factor in the individual's life. Another not entirely separate explanation is that repeated drug exposure produces adaptive changes in the brain of the addict such that discontinuation of the drug leads to a physical withdrawal syndrome that is eased by subsequent drug administration.

With reference to the first explanation, Bow Tong Lett (1989) found that repeated administrations of morphine produced sensitisation rather than tolerance to the rewarding effects of the drug. Cross-sensitisation was also seen, which could not be explained away by the alleviation-of-withdrawal theory; for example, amphetamine did not alleviate the symptoms produced by withdrawal from morphine, although it could sensitise its rewarding effects. Sensitisation to the rewarding effects of drugs is probably more important in causing an addiction to the drug rather than maintaining it. This is because in the long term, tolerance becomes the predominant adaptation to repeated exposures of the drug.

As to the second explanation, relapse may be caused by craving in which anticipation of the drug reward and subjective feelings similar to the early stages of drug withdrawal produce an intense desire for the drug (Littleton et al, 1996.) Craving is frequently elicited by "cues" (conditioned stimuli from the drug-taking past) and the mixture of positive and negative reinforcements for drug taking suggests that it may have a similar neurochemical basis to the acute drug reward and the neuroadaptation to this which induces withdrawal (Littleton et al, 1996.)

Studies centred around the experimental paradigms used to investigate reward have established the mesolimbic dopamine system as one important neural substrate of drug reward. The mesolimbic dopamine system consists of dopaminergic neurons in the ventral tegmental area and their various projection regions, especially the nucleus accumbens. The

ability of opiates and psychomotor stimulants to increase extracellular levels of dopamine in the nucleus accumbens is shared with a number of other drugs of abuse, notably ethanol and nicotine (DiChiara and Imperato,1988.)

It is important to consider how ethanol and nicotine work at all levels, from molecular to societal. The effects produced by ethanol and nicotine are partly psychological, e.g. depending on the learnt associations based on previous experience of the drugs; and partly physiological, e.g causing vasoconstriction (nicotine) or respiratory depression (ethanol.) To gain an understanding of how either drug works, one must consider both components. Both the physiological and psychological effects of drugs on mice will be manifested to some extent in their outward behaviour, which can then be observed and quantified. This principle formed the basis of this investigation. Since this investigation deals only with animal behaviour as modified by these two drugs, a brief overview of their believed modes of action of is now provided.

Starting with ethanol, it can be argued that the average social drinker has a fairly good idea of the mental and physical effects they can expect in relation to the dose of alcohol they imbibe, and the unpleasant consequences the morning after having "a few too many."

Considering ethanol's sites of action in living organisms, much of the earlier work postulated that ethanol induced non-selective fluidisation of cell membranes, thereby altering the cells' activity (for review, see Taraschi and Rubin, 1985.) To date, no specific ethanol receptor has been found but ethanol has been reported to have numerous actions at different receptor sites in the nervous system, including gamma-amino butyric acid (GABA), opioid, dopamine, N-methyl-D-aspartate (NMDA) and 5-hydroxytryptamine (5-HT) receptors (Grant, 1994.) The minimum effective dose threshold of ethanol varies between each of the receptor sites. Compared to the small doses required to elicit tissue responses to opioids, for instance, ethanol is a much weaker drug in terms of its potency. When taken in large amounts however, its profile of central depressant actions is similar to, but not identical, to that of other CNS depressants such as benzodiazepines (Koob and Bloom, 1988.)

The US Environmental Agency classified environmental tobacco smoke as a Class A carcinogen in 1992. Estimates put smoking as the cause of 90% of lung cancer deaths. Although tobacco smoke is a complex mixture of thousands of different chemicals, it is nicotine that produces most of the immediate effects of smoking on the body, and the addictive effects on brain and behaviour. A scientific consensus has emerged that nicotine in cigarettes causes and sustains addiction. Thousands of pages of internal documents from tobacco companies have also been disclosed, revealing that the companies know that nicotine causes significant pharmacological effects and that they have designed their products to provide pharmacologically active doses of nicotine (Wise, 1997.)

Nicotine is absorbed into the bloodstream within seven seconds of being inhaled. It increases the heart rate and blood pressure and causes vasoconstriction. It acts biphasically; as a stimulant in lower and as a depressant in higher doses. The effects of nicotine last for about two hours. Nicotine exerts its effects by acting directly on nicotinic acetylcholine receptors in the body. Like ethanol, nicotine has been shown to increase the levels of dopamine in reward-associated areas of the brain. 6-hydroxy-dopamine lesions of the mesolimbic dopamine system produced weakened responses for nicotine in self-administration and locomotor activity studies in rats (Stolerman and Shoaib, 1992) and systemic nicotine or infusion of nicotine into the nucleus accumbens has been shown to lead to dopamine release.

One study examining the effects of chronic nicotine on group-housed rats was conducted by Fung (1986) using implanted osmotic minipumps. The pump administered 1.5 mg/kg/day nicotine for 14 days. (This produced a plasma nicotine levels equivalent to the mean dose received by a person smoking 20 cigarettes per day.) After 14 days, there was a significantly higher level of dopamine in the nucleus accumbens of the nicotine-treated rats compared with saline-treated rats. This may be because nicotine increases the activation of tyrosine hydroxylase, the rate-limiting enzyme in the formation of dopamine.

Nicotine causes a feeling of euphoria but this in itself is not a 'big' enough effect to maintain reinforcement. As with ethanol withdrawal, the prevention or termination of the nicotine withdrawal syndrome may be an indirect source of positive reinforcement. Human smokers were deprived overnight of cigarettes and reported significantly higher levels of stress, irritability and depression compared to nondeprived smokers and nonsmokers. After

access to cigarettes had been restored, the mood levels of all three groups were broadly similar (Parrott et al, 1996.) It is, as always, important to distinguish rigorously between real benefits due to nicotine and the mere alleviation of declines in performance caused by nicotine withdrawal (Stolerman, 1990.)

Studies linking the use of ethanol and nicotine include those by Covernton and Connolly (1995.) They found that agonist responses at one particular nicotinic receptor subtype (the $\alpha3\beta4$ one) can be both inhibited and enhanced by ethanol, with enhancement dominating at high concentrations. In 1996 the same researchers found that as well as the $\alpha3\beta4$ subtype, two further subtypes of the same receptor could also be mediators of the synergistic addictive processes involving both alcohol and nicotine addiction.

From conversations with drinkers that also smoke, there is some consensus that drinking is a most satisfactory way of relieving the slight dryness of the throat experienced from inhaling cigarette smoke. There is also agreement that drinking alcohol complements the taste of cigarettes. Of course, the validity of this anecdotal evidence is limited, but is nonetheless interesting.

Investigating both the physiological and psychological effects of drugs on systems at the same time may be sound in principle but produces problems when interpreting results. For example, is the mouse sitting motionless on the plus-maze doing so out of fearfulness or fatigue? Interpretative difficulties such as this may justify the separation of research on ethanol's effects into either purely physiological or psychological studies. However, this separation cannot be clearly made, since psychological changes have a fundamental physiological basis. Particularly in the elevated plus-maze experiments, the behaviour exhibited by mice whilst under the influence of drugs is an expression of both underlying psychological and physiological changes, not just one or the other. The experimenter should always bear this in mind.

Results of the first two-bottle choice experiment indicated a profile of drinking patterns and preference for 8% (v/v) ethanol in male TO mice when housed in groups or isolated from the same groups, and when subject to routine maintenance procedures, such as cleaning-out of cages.

The TO mouse strain is only one of a vast number of strains of mice used to date in research. It should be borne in mind that many physiological and behavioural measures have been shown to be profoundly influenced by the strain of mouse used (Brain, 1975; Cunningham et al, 1992a.) Growing evidence indicates that genetic factors influence the predilection to drug addiction (Phillips et al, 1994.) In humans such an influence is well established for alcoholism and is presumed to exist for other addictions. Genetic factors possibly influence the neurochemical responses the drugs elicit in the brain acutely and/or longer term adaptations to chronic drug use. In spite of this evidence, parallels often had to be assumed between TO mice and other strains for the purposes of choosing appropriate drug doses to administer in experiments.

The number of mice housed together per 'group' was fixed at five in this experiment. As mentioned in the Methods section, it appeared that the rationale behind choice of group size made by the researchers can be quite arbitrary. It would have been useful to conduct a further 'baseline' study investigating the relationship between mean ethanol preference and number of subjects per group. Particularly if the size of the cage remained constant, I would anticipate the stress due to heightened dominant/subordinate relationships to alter preference for ethanol. Here it is important to consider the artificiality of traditional group cages, in which the restrictive size usually means subordinate mice cannot actually escape from the territory of the dominant mice (Brain, 1975.) One modification which might get round the restrictive space could be to house groups of rodents in colonies ('naturalised,' large enclosures with hiding-places and varied sensory stimuli.) Results of one of the very few studies where this type of housing was used reported that rats housed in colonies with access to food, water and a 10% ethanol solutions (one sweetened, one unsweetened) ingested significantly less total ethanol than group-housed or singly-housed rats (Kulkosky et al, 1980.)

The daily fluctuation on both graphs of mean preference for ethanol for group-housed mice (Figs. 1.0, 1.1.) was probably due to the ethanol bottle position. For both single and group-housed animals, the position of the drinking-bottles containing water and 8% ethanol were swapped daily. Ethanol preference of group-housed mice was generally highest on days when the bottle containing ethanol was placed on the left side of the cage- the side associated with the water-bottle position in the holding-room cages. It must be

remembered that the ethanol preference value obtained will always be the average value of the five mice in that cage. The position of the mouse within that group's "pecking order" might well affect the accessibility it is allowed to both drinking-bottles, and subsequent ethanol preference. Wolffgramm and Heyne (1995) found that the latter was true for rats; those categorised as subordinate by ethological classification were found to consume 30-100% more ethanol, opiate and benzodiazepine than dominant mice in a free-choice situation.

On the five occasions when cages were cleaned out, effects on preference for 8% ethanol the following day of were not found to be significant, in any of the three groups of mice (singly-, group- or newly-singly-housed.) This results contrasts with the findings of Smith et al (1994) who examined the effect of cage-cleaning on ethanol drinking in singly-housed C57 mice. Subjects from the highly ethanol-preferring strain were offered a choice between 8% ethanol or water. On the day after cage-cleaning had taken place, preference for ethanol was decreased compared to a control group where no cleaning-out was done.

The results of the experiment where mice were habituated to either single or group housing for 17 days and then were newly-exposed to 8% ethanol showed that on the fourth and sixth days after first presentation of ethanol, the singly-housed mice had a significantly higher preference than those housed in groups (Fig. 1.1) A 'mirror-image' version of this experiment was also carried out, where group-housed mice were introduced to single housing when already accustomed to ethanol. It was found that this act of isolation significantly increased their ethanol preference, compared with mice remaining group-housed or mice accustomed to single housing.

The point at which ethanol was introduced appeared to be crucial. At the very beginning of the experiment, mice were isolated or placed in groups and on the same day were newly exposed to ethanol in a two-bottle choice situation. It was found that the ethanol preference for the group- and singly-housed mice did not differ significantly. However, when mice accustomed to ethanol choice in a group were isolated, their ethanol preference significantly increased. This may well be a phenomenon where learning is important. Mice which had been exposed to ethanol prior to isolation would at least have had the chance to learn an association between drinking ethanol and its psychoactive effects. Ethanol may even have been used by some mice to help relieve some of the stress

of subordination. When isolated, mice might use this "knowledge" of ethanol's effects to help them adapt to the perceived stress of their changed environment. Neill and Costall (1996) carried out studies on rats to determine the effects of isolation rearing on preference for ethanol over water. 'Isolation rearing' means that animals are housed singly from weaning onwards, as distinguished from my experiments in which adult mice were housed in isolation for variable periods of time, so only limited comparisons can be made between my results and those of Neill and Costall (1996.) Their experiment showed that rats which had been reared in isolation were found to have a reduced preference for 5% and 10% ethanol over water in a two-bottle choice test compared to those reared in groups (it was not stated that this reduction was significant.) Rats were housed singly or in groups of 5 for 17 weeks, then all were housed singly for preference studies. The fact that all rats were ultimately preference-tested in isolation is interesting;- perhaps isolation after long-term group-living is relatively more stressful than long-term single housing (magnitude of 'stress' being indicated by ethanol preference.)

Animals can be made dependent on ethanol by giving alcoholic liquid diet as the only food source. That the mere induction of physical dependence is not sufficient to promote ethanol intake in animals, is demonstrated in studies where rats will, under certain conditions, refuse to consume ethanol despite the fact that such consumption could alleviate their state of withdrawal distress (Meisch et al, 1994.) Again, with reference to this experiment, it seems likely to me that it is first necessary for the rats to actually learn the association between alleviation of abstinence symptoms through consumption of ethanol (also suggested by Cicero, 1980.)

Wild strains of house mice are territorial. Isolating male mice may result in changes characteristic of territorial dominance. There is evidence that rats reared in social isolation exhibit a range of behavioural and neurotransmitter abnormalities including reduced place preferences for morphine (Wongwitdecha and Marsden, 1995.) This indicates a dysfunction in the reward mechanisms in isolation-reared rats. Support for the theory that isolation constitutes a stress to laboratory rodents comes from hormone level studies, from which there is evidence that variations in housing conditions influence the production and release of sex steroids and increase adrenocortical activity (Brain, 1975.) However, the idea that the increased ethanol preference after isolation may be due at least partly to the

rodent's predisposition for drug taking is interesting and mirrors a school of thought in human addiction research. Wolffgramm and Heyne (1995) showed that the initial ethological classification of rats into dominant or subordinate types remained unchanged even after long periods of drug intake and social isolation. In fact, social isolation was found to have no effect on the ethanol intake of subordinate rats, but the intake of isolated dominant rats was found to increase until it reached the levels of subordinate rats.

When using animals to understand better drug intake in humans, experimental models must be based on the reality of drug presentation. The reality is that drugs of abuse are nearly always taken out of choice, at least initially. Only perhaps if drinking-water was of particularly dubious quality or scarce could the drinking of alcoholic beverages be envisaged as life-supporting, rather than life-enhancing. The two-bottle choice test is therefore a useful model of drug intake because it measures voluntary intake. In this test, the voluntary daily intake of drug solution is inferred to give an index of its rewarding properties.

The second important fact about drug presentation is that drugs taken orally as liquids are often made more palatable by adding sweeteners and spices. Tea and coffee are customarily made less bitter with the addition of milk and sugar. Soft drinks containing caffeine, such as coca-cola, tend to contain large amounts of sugar and other sweeteners. 'Congeners,'which include sugars and herbs, are substances added to alcoholic beverages to increase their palatability (Kessel and Walton, 1965.) In recenttimes, the sweet, fruity flavours of so-called "designer drinks" (a new range of fortified wines and strong white ciders) have been developed specifically to make them particularly appealing to 13-16 year olds, according to industry sources (Hughes et al, 1997.) There are grounds for supposing there to be a 'higher' link than palatability between the two. Experiments attempting to localise the genes affecting alcohol drinking in mice revealed that several genetic markers were associated with ethanol consumption levels, some of which were closely associated in turn with loci thought to determine saccharin detection (Phillips et al, 1994.) A realistic model of oral drug intake must therefore include both the voluntary aspect and take into account the palatability of the fluid.

It was thought necessary to determine first what effects, if any, the chosen congeners themselves had on the experimental subjects' behaviour, before using them to flavour drug solutions. The converse question- what effect the drugs have on intake of congeners- is more commonly addressed; for example the finding that nicotine may reduce the consumption of high-calorie foods (Stolerman and Shoaib, 1992.)

Sucrose was anticipated to be used to improve the palatability of nicotine in future experiments, and it is a common congener. This second experiment therefore examined the behavioural effects of sucrose on TO mice, alone and in conjunction with ethanol. After being exposed to 10% (w/v) sucrose for four weeks in a two-bottle choice test, mice were tested once on the elevated plus-maze after an ethanol or saline injection.

After daily sucrose consumption monitoring, it was clear that there was a wide range of individual sucrose consumption levels. After a few weeks, it was clear that individual mice maintained a level of consumption that varied little from day to day. This phenomenon is known in rats as well, and it is possible to selectively breed lines of rats which are either high- or low-saccharin consumers (Badia-Elder et al, 1996.) Humans, too, seem to vary in their intake and preference of sweet-tasting substances; some admit to being particularly 'sweet-toothed.' Interestingly, Moles and Cooper (1995), when investigating sucrose intake among mice, found no significant differences between subordinate and dominant males, unlike reports for ethanol intake (Wolffgramm and Heyne, 1995) mentioned above.

All mice used in this study were habituated to single housing for two weeks prior to presentation of the sucrose choice. Results from Moles and Cooper (1995) indicate that if this experiment were to be repeated in the future, this habituation period would be unnecessary, since thay found no evidence to suggest that sucrose intake by CD-1 mice was affected by social isolation. This habituation period was not 'isolation rearing', where animals are housed singly either from birth or immediately after weaning, but the fact that mice were housed singly may have had similar effects to isolation rearing on sucrose intake throughout the experiment. There has been recent interest in the effect of isolation-rearing on sucrose intake in rodents. Rats isolated 21 days from birth can exhibit behavioural disturbances such as being spontaneously hyperactive and having an enhanced response to reward-related stimuli. Jones and Marsden (1990) carried out operant studies on isolates,

compared to group-housed rats, where 10% sucrose was the reward. There was no significant difference between the results for the two groups of rats. A possible explanation for this finding is that social isolation does not alter the acquisition of the association between the stimuli and the reward, or cause a general disruption of normal behaviour. However, carrying out operant studies necessitated all rats to be tested away from their home cages, effectively isolating the group-housed rats prior to testing, which may have 'flattened out' subtle differences between group-and singly-housed subjects. Neill and Costall (1996) found that in a two-bottle choice test, there was no difference in the preference for 0.005%-0.05% saccharin over water between isolation-reared and group-reared subjects, although all rats were ultimately preference-tested in isolation, and importantly, had not been exposed to saccharin prior to the preference test. Similarly, a recent study by Parker et al (1996) using a different strain of rat, showed that social isolation after weaning does not alter sucrose consumption in rats. Again, I am not totally convinced by the validity of this result, as all rats were food and water deprived for a total of 5 hours, and placed singly in test cages for one hour prior to a preference test with 1% sucrose or water. From familiarity of the Animals in Scientific Procedures Act (1986), I know that food and water deprivation is considered a stressful procedure and I wonder whether these combined pre-test conditions may have 'flattened' the differences due to isolation or group-rearing which might otherwise have exerted an effect on sucrose preference? However, this experiment did include a pre-test exposure (duration of exposure not specified) of rats to sucrose and water in the home cage, which from the results of my first experiment should have provided some opportunity for the subjects to experience the effects of the sucrose.

A distinct advantage of using the elevated plus-maze to test putative anxiolytic substances , rather than, for example, giving the animal electric shocks or depriving it of food and water (both of which can interfere with drug action), is that the method relies solely on spontaneous activity and the stress of testing it engenders is relatively mild. Mice were injected with ethanol (1.75 g/kg or 1.0 g/kg) or saline prior to testing on the elevated plus-maze. Some workers proposed that the behaviour of animals exposed to a novel situation, such as a plus-maze, results from a competition between an exploratory tendency (motivated by curiosity or boredom) and a withdrawal tendency (motivated by fear.) The

elevated plus-maze can help to elucidate which drive, fear or curiosity, is the supreme one, as manifested in the animal's behaviour during a set length of time. Looked at another way, the maze is sensitive to the effects of anxiolytic as well as anxiogenic drugs. Factors altering the relative strengths of these drives can include the complexity of the situation, its degree of novelty, and the internal state of the animal.

Administering 1.75 g/kg ethanol significantly decreased the percent of time spent in the closed arm, net duration of stretch attend postures, and number of closed-arm returns compared to saline-treated mice. The same dose of ethanol significantly increased the percent of total time spent on the open arm, the total number of entries into either arm, the number of head-dips (both protected and unprotected) and the number of open arm entries. The only significant effect of administering 1.00 g/kg ethanol was to increase the number of protected head-dips. To summarise, the effect of 1.75 g/kg ethanol prior to plus-maze testing of mice which had been chronically exposed to 10% sucrose was to increase exploratory behaviour and the general level of behaviour compared to mice treated with saline. These behaviours were indicative in most cases of an anxiolytic effect of the ethanol.

The percent of the total time spent on the open arms gives an index of the anxiety experienced by the animal. Compounds causing anxiety in man, such as caffeine and pentylenetetrazole, have been found to significantly decrease the percent of total entries into either arm made onto the open arms and the total time spent on the open arms. Reluctance to explore the open arms results from a combination of the rodent's aversion to open spaces and the elevation of the maze. It has been shown that confining animals solely to the open arms results in an approximate doubling of the plasma level of the stress hormone, corticosterone, compared to the level measured when the mouse is in the closed arms (Pellow et al, 1985.) 1.75 g/kg ethanol could therefore be assumed to have anxiolytic effects on the mice since it caused a significant increase in the time this group spent on the open arm.

If a treatment increases the number of open arm entries without altering the total number of entries into either arm, this can be inferred to reflect that the treatment has anxiolytic actions. Similarly, if a treatment decreases the number of open arm entries without a change in the total number of arm entries, the treatment could be said to be anxiogenic (Balfour et al, 1986.) Interpretation is harder if both the total number of arm

entries and the number of open arm entries is increased, as was the case for the mice injected with 1.75 g.kg ethanol. This could be indicative of a stimulatory or anxiolytic effect, or both. There is evidence that anxiolytics selectively increase exploration in animals, rather than their general level of activity. Mice treated with anxiolytics in a uniformly lit, non-compartmentalised box were not significantly more active than vehicle-treated mice under the same conditions (Treit, 1985.) Both the behavioural and neurochemical effects of ethanol may be related to its anxiolytic properties. Socially isolated rats, injected with 1.2 g/kg ethanol i.p. showed increased exploratory behaviour and a higher preference for the white side of the box compared to untreated controls (Parker and Morinan, 1987.)

Head-dipping is another exploratory behaviour indicator, and both head-dipping from the relative security of the central section and closed arms, and from the relative exposure of the open arms, was significantly increased after administering 1.75 g/kg ethanol, indicating an anxiolytic effect of the drug. The lower dose of ethanol only significantly increased the number of head-dips made from the security of the closed arms and central section, indicating a relatively weaker anxiolytic effect than seen with the higher dose of ethanol. Closed-arm returns are practised by animals experiencing a degree of anxiety. The animal always retains its hind legs in the relative security of the closed arm, ready to retract immediately if necessary. The mice administered 1.75 g/kg ethanol had a significantly decreased number of closed-arm returns compared with the saline-injected group, indicating that their level of anxiety was lower.

When practising scoring plus-maze behaviour, some difficulty was experienced in discerning when stretch attend postures 'ended' and 'began'again because they tended to occur consecutively. Therefore, in my analysis of this behavioural measure, I decided to measure their mean net duration rather than the more usual frequency score. Anxiolytics generally reduce the stretch attend posture frequency (Pollard and Howard, 1988) and if frequency is correlated to net duration, it would appear that 1.75 g/kg ethanol had an anxiolytic effect as it significantly reduced the mean net duration of stretch attend postures.

Mice which were administered saline prior to plus-maze testing were compared across the five sucrose consumption groups. None of the plus-maze behavioural measures

varied significantly between subjects in the five different sucrose consumption groups, indicating that the level of anxiety associated with very variable sucrose consumption levels was equivalent. Perhaps the fact that each mouse had continuous free access to as much sucrose as they wanted, be it a negligible or considerable quantity, kept their anxiety to a basal level. It is of course possible that there may have existed differences but this test was not sensitive enough to detect them, or this test was not in fact the correct one to use. Animal models measuring behaviour, such as the elevated plus-maze do possess several weaknesses, often because it is difficult to obtain quantitative and objective data. Also, many designs do not differentiate between anxiety, stress, fear and the effects of sedatives, for example (Lal and Emmett-Oglesby, 1983.) The plus-maze can at best indicate the anxiolytic/anxiogenic properties of drugs as represented in rodents' behaviour, and give some indication of the sedative or stimulatory effects of drugs as reflected by the subjects' locomotor activity. It may be that the effect of different sucrose consumption levels on mice is to alter some other behavioural modality, such as affective state (Sucrose preference has been suggested by Willner (1987) to be a simple method for identifying anhedonia.)

Weiss and Lorang (1993) measured dopamine release in the nucleus accumbens of rats using intracranial microdialysis. In a free-choice operant task, saccharin solution or water was available. No significant increase in dopamine efflux was measured post peak intake of saccharin, unlike results measured when 10% ethanol was ingested in the same setup. However, saccharin and sucrose are reported to have other effects, most notably on endogenous opioidergic systems. It has been reported that access to palatable foods leads to increased release and breakdown of hypothalamic β-endorphine in rats (Dum and Herz, 1983.) From the experience of humans, in a book which summarises the experience of recovering alcoholics, it is emphasised that,

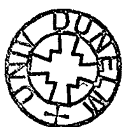
"many of us, even many who said they had never liked sweets- have found that eating and drinking sweets allays the urge to drink." (From 'Alcoholics Anonymous- Living Sober', 1987.)

This anecdotal evidence has been confirmed in clinical reports, which report that alcoholics who stay sober in treatment for more than thirty days consume significantly more sucrose than those who relapse within the same period (Yung et al, 1983, cited in Kampov-Polevoy

et al, 1995.) This evidence mainly points to the influence of sucrose/saccharin on subsequent ethanol effects. However, these sweeteners are usually mixed with alcoholic drinks as congeners and are therefore ingested simultaneously. I think it is correct to say that this experiment does measure the effects of ethanol and sucrose in combination (although the blood level of sucrose was not verified) because mice had continous access to sucrose at all times except when on the plus-maze. With hindsight, it would have been useful to have an extra experimental group of mice which were not offered sucrose. It might then have been shown that the sucrose was blocking most significant effects of the lower dose of ethanol (1.00 g/kg) which, in the absence of sucrose, might exert significant anxiolytic effects.

There are two major groups of calcium channel inhibitors: dihydropyridine derivatives, (for example, nimodipine) and phenylalkylamine derivatives (for example, verapamil.) All tested so far produce a dose-dependent suppression of ethanol intake in ethanol-dependent rats (Engel et al,1988), and they also appear to protect against ethanol withdrawal symptoms (Little, 1991.) Some evidence suggests that these drugs may alleviate disorders such as anxiety (Pucil and Kostowski (1991.)

The effect of a course of daily Tween-80 or nimodipine injections (5 or 50 mg/kg) on sucrose drinking was investigated. Mice used in this part of the experiment were those which had had only saline injections so far. The mean sucrose intake of mice in the 50 mg/kg nimodipine dose group decreased significantly below that of the Tween treatment group on the first and second days after the initial injection. The mean sucrose intake of the lower-dose (5 mg/kg) nimodipine group did not differ significantly from that of the Tween group throughout the experimental phase. The curse of leaking bottles affected not one but both nimodipine groups on the two days after the initial injection, reducing the group sizes to four mice in each. These results were included because they might well reflect the expected change in sucrose consumption after nimodipine treatment. Calcium channel antagonists have been shown to decrease preference for both caloric solutions (ethanol, sucrose) and non-caloric solutions (saccharin) over water (Pucilowski et al 1992, 1994.) It is stressed that a repeat of this phase of the experiment would have been preferable had time allowed, because it is difficult to attach credence to results when the groups consists of less than six subjects.



Again, the endogenous opioidergic system may provide an explanation for nimodipine's effect on sucrose intake. As mentioned above, intake of palatable foods has been reported to increase the release of β-endorphines in rats and humans. Glucose ingestion also potentiates and prolongs the analgesic effect of exogenous opioids such as morphine (Blass, 1987.) Similarly, nimodipine administered daily to cancer patients is reported to reduce the dose of morphine required for pain relief (Santillan et al, 1994.) It is possible that because nimodipine decreases tolerance to opioids, the daily intake of sucrose can be reduced because the decreased level of β-endorphines correspondingly released has a potentiated and prolonged effect.

Nicotine was offered to mice in a two-bottle choice test and the daily intake was measured. It was found that TO mice would drink nicotine in a 1 g/l solution without the need for an added sweetener, with an average intake of 37 mg/kg nicotine per day. Following a 5-day period of forced nicotine consumption (the water bottle was removed), the average daily intake of nicotine decreased to 34 mg/kg/day (not a significant change.) The reason for removing the water-bottle was to force all mice to experience nicotine and learn to associate its psychotropic effects with its taste. A sharp weight drop among animals given nicotine as their sole fluid prompted the return to the two-bottle choice paradigm after 5 days.

The behavioural effects of nicotine on mice, both alone and when in conjunction with ethanol, were investigated using the plus-maze, the first time after twenty-nine days of nicotine exposure. Half the mice were injected with 1.75 g/kg ethanol and the other half with saline, prior to being placed on the plus-maze. It was found that none of the behavioural parameters measured in this plus-maze test varied significantly between subjects from either the water/water or nicotine/water choice group. Neither were there any significant differences between subjects injected with saline or ethanol, from either drinking group, for any of the behavioural parameters measured. The data associated with each behavioural parameter were mainly normally distributed, but differences between groups failed to reach significance. Perhaps the stress of the first exposure to the plus-maze, first injections and change of environment masked the more subtle behavioural changes induced by the chronic drug treatment and interaction with the injected drug. However, the first and

only plus-maze carried out for the sucrose/ethanol experiment produced some significant results under similar, novel conditions. In experiments with rats, it was noticed that variations in the extent to which rats are handled and/or whether they are naive or experienced as regards exposure to the plus-maze may give rise to different sensitivity to pharmacological agents. For example, diazepam was found to have a significant anxiolytic effect when administered to rats which were placed on the plus-maze twice, each time for 10 minutes. However, when the second exposure to the maze was reduced to 5 minutes, no significant anxiolysis was observed (File et al, 1993.) These findings are contrary to earlier work by Pellow et al (1985), who found that neither the effect of novelty nor illumination was a significant contributor to behaviour of rats on the elevated plus-maze.

The second and final plus-maze test was an exact repeat of the first test in so far as the same drugs were administered to the same mice. Controversy exists concerning the effects of repeated exposure to the elevated plus-maze. Shilliam et al (1996) compared effects of repeat exposures to the elevated plus-maze in male and female rats. Females did not show any significant change in behaviour irrespective of the number of exposures, whilst males exhibited significant decreases in open arm activity with repeated exposures. Pellow et al (1985) made the observation that the behaviour of undrugged animals repeatedly exposed to the plus-maze does not significantly change. There could be several explanations for this finding; perhaps the subject habituates to the anxiogenic effect of novelty but does not habituate to the fear of open spaces.This time however, mice exhibited some significant behavioural differences between drinking groups and drug treatment groups as observed on the plus-maze. First, the significant behaviour changes of mice in both drinking groups (water/water or nicotine/water choice) following ethanol injections, compared to water-drinking, saline injected mice are considered. Both drinking groups were found to spend a decreased percent of total time in the closed arm, an increased the percent of total time on the open arm, and an increased number of open arm entries. Reluctance to explore the open arms results from a combination of the rodent's aversion to open spaces and the elevation of the maze, but both drinking groups engaged in a signifcant level of exploratory behaviour, indicative of an anxiolytic effect of ethanol (Pellow et al, 1985.) The finding that both drinking groups spent an increased time on the open arms is partly in accordance with a study by Balfour et al (1986) which concluded that neither acute

nor chronic nicotine altered the preference of rats for the open arms of the plus-maze. However, the group also found that chronic nicotine (administered on 7 consecutive days) increased the total number of entries into either arm.

Considering next those mice in the water/water drinking group only when injected with ethanol, in addition to the changes reported above, this group made an increased percent of total arm entries into the open arm, made fewer closed-arm returns and displayed fewer rears. The percent of the total number of arm entries made onto the open arm and the percent of total time spent on the open arm measures are highly correlated (Pellow et al, 1985.) One behavioural marker of the natural aversion of rodents for the open arms is the percent of total entries into either arm made onto the open arm. An increase in the frequency of this parameter in the absence of modifications in the number of total entries made onto either arm, as was found for this drinking group when treated with ethanol, can be interpreted as an anxiolytic effect on the rodent of the drug being tested (Reiband and Bohme, 1993.) Fewer closed-arm returns also points towards an anxiolytic effect of the drug. Although rearing is an exploratory activity, and a decrease in the frequency of exploratory behaviour would suggest an anxiogenic effect of the ethanol, it should be remembered that rearing occurs almost exclusively in the closed arms, so reduction in this parameter would be a logical consequence of reduced closed arm time (Cole and Rodgers, 1994.)

The importance of interindividual variations within a session and also variations between experiments is stressed. One study examined specifically the variation of one measure, the percent of total entries into either arm made onto the open arm, for the same rats throughout the year. The variation of the scores for this one measure was between 5 and 15% from month to month (Reiband and Bohme, 1993.)

Nicotine is not used in the treatment of anxiety disorders in the clinic but there is some evidence that it can act as an anxiolytic (Warburton et al, 1987.) Both alcohol and nicotine have been shown to be active in rat and primate models indicative of anxiolytic activity (Costall et al, 1989.) The results from the second plus-maze did not however indicate significant differences between mice which had had chronic exposure to nicotine prior to testing, and those which had hd a water/water choice. The anxiolytic effects on plus-maze behaviour of an injection of 1.75 g/kg ethanol was significant for both drinking

groups, but more indicators of anxiolysis were evident in the water/water choice drinking group than the nicotine choice group.

A range of nine ethanol concentrations (0-20% v/v) were presented to singly-housed TO mice in a two-bottle choice paradigm in order to find the optimum concentration of ethanol to use in a future combined ethanol/nicotine drinking experiment. The group which had 12.5% (v/v) ethanol in the second bottle of the two-bottle choice had the highest mean daily intake of ethanol (g/kg/day); those in the 2.5% (v/v) ethanol group the lowest mean daily intake. 10% (v/v) ethanol was selected to be used in the combined nicotine/ethanol drinking study. Mice in this group had an appreciable average daily intake of ethanol of over 6 g/kg/day, and the standard error of the mean was not as large as for the 12.5% ethanol group. After three weeks of the two-bottle choice study, all mice were tested once on the elevated plus-maze whilst undergoing withdrawal (of variable durations) from ethanol. Only one behavioural parameter measured on the plus-maze varied significantly between the subjects from one of the eight ethanol concentration groups compared with those in the water-only group. Mice in the 20% ethanol drinking group exhibited a significantly lower mean total number of arm entries compared to mice in the water group. This measure indicates a decrease in the general level of activity of the mice in the 20% ethanol group compared to those drinking solely water. Interestingly, behaviour indicative of anxiety (such as increased percent of total time spent in the closed arms, and decreased exploratory behaviour) was not significantly different in mice withdrawing from ethanol compared with those in the water-drinking mice, although anxiety is known to be a prominent and often debilitating component of drug withdrawal, especially withdrawal from opiates, benzodiazepines and alcohol (Victor and Adams, 1953, cited in Nutt, 1990.)

The fact that mice were housed singly rather than in groups might well be another important factor. As explained before, "isolation rearing" is certainly not the same as housing mice singly (as in my experiment,) but results on the effects of isolation-rearing on rodent plus-maze behaviour will be compared in a limited way to those that would be expected from singly-housing subjects. Parker and Morinan (1986) found that isolation-rearing rats lead to a decrease in their level of exploration of the elevated plus-maze, a decrease which can be reversed by chlordiazepoxide. The same workers measured the

effects of ethanol on exploratory behaviour in a further repeat of the same experiment. Socially isolated rats, injected with 1.2 g/kg ethanol i.p. showed increased exploratory behaviour compared to untreated controls (Parker and Morinan, 1987.)

There are obvious differences between my study and Parker and Morinan's, above. My study used not isolation-reared rats but singly-housed mice. The route of ethanol administration in my experiment was oral and voluntary, compared to intraperitoneal and forced. My experiment measured the effect of ethanol withdrawal rather than acute effects of ethanol. What can be applied from Parker and Morinan's study is that isolation rearing itself caused a decrease in exploration. Anxiety of withdrawal might have been manifested in my study by such a decrease in exploration. However, this effect was not seen for any of the ethanol concentration group subjects when in withdrawal. One explanation could be that the effect of single housing had more influence on subjects' behaviour than drug withdrawal. Thus subjects in the 'control' group would be affected to an equal degree by social isolation, and any additional effects due to ethanol withdrawal would not cause significant discrepancies between control and ethanol groups' behaviour.

Mice were exposed to 1 g/l (v/v) nicotine, or 10% (v/v) ethanol, or a mixture of the two, for fourteeen weeks in a two-bottle choice paradigm. After fourteen weeks, all mice were exposed to the elevated plus-maze after undergoing exactly 4h withdrawal from their respective drug solutions. Five days later, all mice were re-exposed to the plus-maze but this time none had their drug solutions withdrawn prior to testing.

Considering first the ethanol intake of mice with and without the addition of nicotine to the drinking solution, it was found that the mean daily ethanol intake of these two groups did not differ significantly throughout thefourteen weeks. This contrasts with the results of the two nicotine drinking groups, where the mean daily intake of nicotine alone was significantly lower than the intake of nicotine when ethanol was added to the solution.

Cage-cleaning was not found to produce a significant change in either ethanol intake or nicotine intake the day after this procedure (compared with the day before) throughout the fourteen weeks. However, the day after the stale drug solutions were replaced by fresher solutions, the ethanol intake of those mice in the solely 10% ethanol

solution group significantly increased compared with their mean intake the day before on one occasion. No such effect was seen for the nicotine drinkers when solutions were renewed.

After fourteen weeks of two-bottle choice all mice except those in the 'control' group underwent a 4h-withdrawal period, followed by exposure to the plus-maze. ('Control' group mice were drinkers of a mixture of ethanol and nicotine and this mixture was not withdrawn.) The behaviour on the maze of the mice from different treatment groups was compared with that of group the water/water choice group.

Considering plus-maze results after withdrawing ethanol from mice which had had a chronic ethanol/water choice first, none of the behavioural parameters measured on the plus-maze differed significantly from the water/water group. Withdrawing ethanol could be inferred from this result to produce behaviour on the plus-maze no different to removing one of two water bottles from the water/water group. Alcohol acts as an anxiolytic in some situations, hence the conception of alcohol as a drink which 'drowns your sorrows.' However, it can also increase the anxiety of a subject performing a task requiring skill because of its psychomotor impairing effect (Lister, 1991.) This may be one explanation as to why no significant behaviours indicative of anxiety were seen in this group.

Considering now results of the group which had undergone withdrawal from both ethanol and nicotine, the only behavioural parameter which differed significantly from the water/water group was the percent of total arm entries made into the open arm. This exploratory behaviour measure was reduced indicating an increase in anxiety (Pellow et al, 1985.) Comparing this result with the ethanol/water group above (withdrawal from solely ethanol) it could be inferred that withdrawal from both chronic ethanol and nicotine simultaneously is more anxiogenic than withdrawing from chronic ethanol.

Considering results from the group which had had chronic access to a solely nicotine choice up to 4 h before plus-maze testing, the only behavioural parameter differing significantly from the water/water group was that the percentage of total time spent in the closed arm was increased, indicating a reduction in exploratory behaviour, and, by inference, an increase in anxiety of this group. By comparing this result with the group where chronic ethanol was withdrawn it could be inferred that withdrawing chronic access

to 1 g/l nicotine is a more anxiogenic practice as reflected in mouse behaviour than withdrawing chronic access to 10% ethanol.

The plus-maze results of the group where ethanol only was withdrawn from mice which had had chronic access to a mixture of nicotine and ethanol alongside water, is now considered. Behavioural parameters differing significantly from the water/water group were: the total number of entries into either arm was decreased, the percentage of total time spent in the closed arm was increased, the number of open arm entries was decreased, the percentage of total entries made onto the open arm was decreased, the percentage of total time spent on the open arm was decreased and the number of head-dips was decreased. The first measure indicates a general decrease in the level of behaviour of mice in this group, but each of the other five measures indicate a reduction in exploratory behaviour, and, by inference, an increase in anxiety of this group. Comparing the results of this group with the group in which solely ethanol was presented chronically, then withdrawn, it can be seen that withdrawing ethanol, when it has been presented chronically in a mixture with nicotine, produces five significant behavioural changes indicative of anxiety, whereas none are seen when withdrawing ethanol presented chronically on its own.

Now comparing the chronic intake of the ethanol group with and without the addition of nicotine, it is recalled that the intake of ethanol did not differ significantly between the two groups. There is an anomaly: reward as an index of ethanol intake during a two-bottle choice test did not differ between the two ethanol groups, but if reward is taken as an index of withdrawal anxiety (discussed in the Introduction) it can be said to be greater when ethanol is withdrawn from a mixture of ethanol and nicotine than when ethanol is the sole drug used and withdrawn.

Results from the group which had nicotine only withdrawn after having chronic access to a mixture of nicotine and ethanol alongside water are considered next. Behavioural parameters differing significantly from the water/water group were: the percentage of total time spent in the closed arm was increased, the number of open arm entries was decreased, the percentage of total entries made onto the open arm was decreased, the percentage of total time spent on the open arm was decreased and the number of head-dips was decreased. Each of these five measures indicate a reduction in exploratory behaviour, and, by inference, an increase in anxiety of this group. The only

difference between the results of this group and those of the group where ethanol only was withdrawn from a mixture of nicotine and ethanol group, was that the latter group exhibited a decrease in the total number of entries into either arm, indicative of a general decrease in the level of behaviour.

Comparing the chronic intake of the nicotine group with and without the addition of ethanol, it is recalled that the intake of nicotine significantly increased when ethanol was added to the nicotine solution. Reward as an index of nicotine intake during a two-bottle choice test differed significantly between the two ethanol groups. If reward is taken as an index of withdrawal anxiety (again, see Introduction), reward can be said to be greater when nicotine is used in, or withdrawn from, a mixture of ethanol and nicotine than when nicotine is the sole drug used and withdrawn.

It is interesting to compare the results of groups where only one drug was withdrawn from a drug mixture, with the results of the group in which both ethanol and nicotine were withdrawn simultaneously. Where only one drug was withdrawn from the mixture instead of both at once, an increased number of separate anxiety-related behaviours were observed on the plus-maze. Interpreting reward as an index of withdrawal anxiety, reward was lower when the drug mixture was completely withdrawn, compared to when just one drug was withdrawn from the mixture. This result is contrary to findings of the pre-test chronic intake study; intake of the ethanol and nicotine stayed constant or increased (respectively) when the other drug was added, therefore reward as an index of intake did not alter or increase (respectively) with a mixture of the drugs. From the results of this first plus-maze test, it would appear that withdrawing either one of the drugs, when mice have had long-term access to a mixture of ethanol and nicotine in free-choice situation, causes very similar behavioural patterns indicative of anxiety as measured on the plus-maze. Taking withdrawal anxiety to be an index of reward, it could be added that the reward obtained from the individual drugs when administered together was comparable.

The plus-maze results of the 'control' group, (in which mice had had access to the ethanol/ nicotine drug mixture but did not undergo withdrawal at any point) are considered next. Had not this group been included, it might well have been assumed that changes in behaviour exhibited on the plus-maze were due to withdrawal from ethanol or nicotine or mixtures of the two. Although drug withdrawal probably played a part in

behaviour exhibited, this 'control' group demonstrated flaws in jumping to the withdrawal anxiety conclusion above all others. Mice in this group differed significantly from the water/water group in their behaviour on the plus-maze in the following parameters: the percentage of total time spent in the closed arm was increased, the number of open arm entries was decreased, the percentage of total entries made onto the open arm was decreased and the percentage of total time spent on the open arm was decreased. All of these parameters indicated a decrease in the group's exploratory behaviour, which indicates an increase in anxiety compared to the water/water group.

The one measure which differed from groups the results of groups in which a single drug was withdrawn from a mixture was that no significant decrease in head-dips was seen for the 'control' group. This aside, there were far more similarities than differences between these two withdrawal groups and the 'control' group in terms of their behaviour on the plus-maze, indicating that the anxiety displayed was predominantly a product of factors other than drug withdrawal.

One week after the first plus-maze test, the whole procedure was repeated using the same mice. This time, none of the solutions were withdrawn or changed at any time, i.e. all mice had continuous access to their respective drugs except when they were placed on the maze.

Considering the results for the ethanol/ water choice group, none of the behavioural parameters measured on the second plus-maze differed significantly from the water/water group. In addition, the results from the second plus-maze did not differ significantly from the results of the first, when ethanol was withdrawn. It could be inferred from these results that chronic exposure to a water/ethanol choice and withdrawal from the ethanol produced behaviour on the plus-maze not significantly different to that of mice in the water/water group. The finding that mice withdrawing from ethanol did not differ behaviourally from those not in withdrawal is contrary to the results of Rezazazdeh et al (1990), who examined the behaviour of rats undergoing withdrawal from ethanol using the elevated plus-maze. They found that these rats (compared to rats not in withdrawal ) spent less time on and made fewer entries onto the open arms of the maze. When the anxiolytic

buspirone was administered to the rats in withdrawal, the total time and entries made onto the open arm increased to scores similar to those seen for rats not in withdrawal.

The group which had had both ethanol and nicotine withdrawn before the first exposure to the plus-maze was considered next. In the first plus-maze, the percent of total arm entries made into the open arm was decreased compared to the water/water group. This time, when the ethanol and nicotine were not withdrawn, significant behavioural changes were: the median ranked percent of total time spent in the closed arm being increased, the percent of total entries made onto the open arm being decreased, the median ranked percent of total time spent in the open arm being decreased, and the number of closed arm returns being increased. All these measures showed that mice in this group exhibited significantly decreased exploratory behaviour, indicative of increased anxiety. There were four such measures seen in the second plus-maze, when the drugs were not withdrawn, compared only one in the first, when subjects were undergoing withdrawal. Therefore it can be inferred that the second exposure to the plus-maze produced a higher level of anxiety in the mice than the first. It could be argued that exactly the opposite results would be expected, because the second exposure to the plus-maze was not whilst the subjects were experiencing the unpleasant sensation of drug withdrawal, and the subjects had already been exposed to the plus-maze once before, so the second time it might be expected that the familiarity would lower their anxiety. However, these results are somewhat supported by the recent findings of Shilliam et al (1996.) They showed that the plasma corticosterone levels of rats increased with repeated exposure to the elevated plus-maze (without confinement to an arm.) They also found that this increase in this stress-linked hormone was both sex and strain specific (male Lister hooded rats were not affected by repeated exposures.) Conversely, earlier work by Pellow et al (1985) concluded that the behaviour of undrugged animals repeatedly exposed to the plus-maze does not significantly change. Several explanations for this finding were offered; perhaps the subject habituates to the anxiogenic effect of novelty but does not habituate to the fear of open spaces.

Considering next the group which had had nicotine withdrawn in the first plus-maze and at all other times had free choice of nicotine or water, the only behavioural parameter differing significantly from the water/water group in the first plus-maze was that the percentage of total time spent in the closed arm was increased. In the second plus-maze

this same parameter again significantly increased, again indicating a reduction in exploratory behaviour, and, by inference, an increase in anxiety of this group. Additional results for the second plus-maze were that the total number of entries into either arm was decreased, and the duration of non-exploratory behaviour was increased. The former parameter indicates a general decrease in the level of behaviour, whilst the second indicates a reduction in exploratory behaviour, and, by inference, an increase in anxiety of this group. It would seem that the level of anxiety was higher in the second exposure to the plus-maze than the first, because there were two behaviours indicative of increased anxiety the second time compared with only one the first time. However, the lower level of exploration in the second exposure could just have been the result of the significantly decreased level of behaviour rather than a change in anxiety level.

Prior to the first exposure to the plus-maze, mice in one group had ethanol withdrawn from a mixture of nicotine and ethanol alongside water. Considering this same group, no drug withdrawal preceded the second plus-maze exposure. Significant behavioural results of the first exposure showed that one measure indicated a general decrease in the level of behaviour of mice in this group, but each of the other five measures indicated a reduction in exploratory behaviour, and, by inference, an increase in anxiety of this group. All six behavioural parameters differing significantly in the first exposure, including the one measure indicating a decrease in the general behaviour, were found to differ significantly, in the same directions, on examining the results of the second exposure. There was one additional behavioural parameter change in the second exposure which did not reach significance the first time: the number of closed-arm returns was increased, indicative again of an increased level of anxiety. It can be concluded from a comparison of the two plus-maze tests that the level of anxiety was significantly higher than the water/water group both times, but the differences between the same groups when in withdrawal and when not were minimal.

The group considered next had nicotine only withdrawn prior to the first plus-maze after having chronic access to a mixture of nicotine and ethanol alongside water. No drug withdrawal preceded the second plus-maze exposure. The five behavioural parameters differing significantly from the water/water group in the first plus-maze exposure were all indicative of a reduction in exploratory behaviour, and, by inference, an increase in anxiety

of this group. All five behavioural parameters differing significantly from the water/water group in the first exposure were found to differ significantly, in the same directions, in the second exposure. The only difference between the results of this group and those of the same group in the second exposure was that on the latter occasion, mice also exhibited a decrease in the total number of entries into either arm. It can be concluded from a comparison of the two plus-maze tests of this group that the level of anxiety was significantly higher than the water/water group both times. The second exposure 'anxiety' may have been partly due to a general decrease in the level of behaviour, indicated by the decreased total number of entries into either arm. Apart from this parameter, the behaviours on the two days were similar.

Mice in the 'control' group had had access to an ethanol/ nicotine drug mixture but did not undergo withdrawal at any point, neither prior to the first nor the second plus-maze exposure. In effect, the two results for this group indicate the effect of repeating the plus-maze test on mice allowed continuous free choice between a mixture of ethanol and nicotine, and water. In the first exposure to the plus-maze, results differed significantly from those of the water/water group in four behavioural parameters, all of which were indicative of an increase in anxiety compared to the water/water group. In the second exposure to the plus-maze, mice from the same group did not exhibit any significant differences in behaviour compared to the water/water group. The comparison of the two days' tests indicates that repeating plus-maze exposures under the same conditions is associated with a decrease in anxiety compared with the first exposure. This statement only applies to mice which had had uninterrupted access to an ethanol and nicotine mixture and water throughout the experiment.

This decrease in anxiety-related behaviour for the 'control' group on repeating the plus-maze test was not the general finding considering all the other results of the second-plus maze. Either the level of anxiety remained constant (in the ethanol/water group) or increased (in groups drinking a drug mixture and withdrawing from both drugs or solely ethanol) or the general level of behaviour decreased (groups where nicotine was withdrawn from a drug mixture or solely nicotine) but the level of anxiety never decreased as it was for the 'control' group. It could be that having undergone the first plus-maze test whilst in withdrawal, some degree of anticipation of this unpleasant state might promote

heightened anxiety, or fearfulness, in the mice on the second exposure. Increased fear would lead to a decrease in exploration.

The importance of interindividual variations within a session and also variations between experiments is stressed. One study examined specifically the variation of one measure, the percent of total entries into either arm made onto the open arm, for the same rats throughout the year. The variation of the scores for this one measure was between 5 and 15% from month to month (Reiband and Bohme, 1993.) It is difficult to speculate how much of the change in anxiety-related behaviour in the 'control' group could be due to temporal and interindividual variations, and how much could be due to the actual drugs tested, but this factor should be borne in mind.

Withdrawal from benzodiazepines and ethanol in rats has been demonstrated to substitute fully for the effect of the anxiogenic pentylenetetrazole, (Lal et al, 1988) but I am not convinced of the validity of this result since the experimenters had to use some of the rats for more than one trial. Withdrawal from nicotine has been shown to substitute for the same effect (Lal et al, 1988.) Stephens (1995, personal communicatiom) commented that the severity of alcohol withdrawal is increased depending on the length of time the subject has been drinking. In addition, if the subject undergoes several separate withdrawals from alcohol prior to the last withdrawal, (with access to alcohol between each withdrawal episode) this will also increase the severity of the most recent withdrawal response. This phenomenon was also shown to be true for withdrawal from diazepam (Ward and Stephens, 1996.) Costall et al (1987) ran experiments examining withdrawal from long-term alcohol and nicotine in mice. Mice were either given two daily i.p. injections of 0.1 mg/kg nicotine, or 8% (w/v) ethanol in their drinking water for 14 days. During this time, the anxiolytic actions of both were inferred from results of black/white box tests carried out daily, wherein the mice preferred the white over the black side. Within 24 hours of withdrawal of the drugs, the mice when tested showed a preference for the black side of the box. When 0.01 μg/kg diazepam was injected into the amygdalae of the mice, this was found to antagonise the anxiety caused by drug withdrawal. It may be that a changed function in the amygdala leads to the anxiogenesis seen during withdrawal from addictive drugs (Costall et al, 1987.)

Although anxiety is experienced as an affective state, it is accompanied by behaviour that might be characterised in animal models. The elevated plus-maze is just one example of the various behavioural paradigms used as models of anxiety; others include conflict procedures and social interaction studies. Animal models of anxiety based on interoceptive stimuli are of quite recent origin and were first proposed by Lal in 1979. Behavioural responses that are reliably produced by interoceptive stimuli producing anxiety in man and that are also antagonised by anxiolytic drugs are accepted as analogues of anxiety. However, there is a fundamental problem here in that there is little data about the behavioural aspects of anxiety in man. Clinical reports describe almost wholly the disruptive qualities of affect related to anxiety. Most animal models were developed with a rationale for understanding the effects of anxiolytics rather than anxiety-related behaviour.

Because anxiety is a concept describing a subjective state, it has been considered to be an exclusively human trait, therefore at best it can only be modelled and not reproduced in animals. (Schweitzer and Adams, 1979.) Also, it should be borne in mind that anxiety is only one of the many effects of drug withdrawal, and may not always be the most important or noticeable symptom, depending on the extent of drug use prior to withdrawal.

One set of experiments used the conditioned place preference paradigm to measure reward. A general version of the test is where animals experience two distinct neutral environments subsequently paired spatially and temporally with distinct drug states. Later, the animal is given free run of both environments. The duration of time spent in either environment is seen as an index of the reinforcing value of the drug (Schechter and Calcagnetti, 1993.)

Experiments testing the reinforcing properties of drugs using conditioned place preference paradigms are quick (usually lasting only 1 or 2 weeks) and relatively easy to carry out. One or two pairings of drug and environment can be carried out each day without a reduction in the associative strength of conditioning. The i.p. or s.c. route of the drug administration used cancels out the problems of differences in taste sensitivity and other preabsorptive differences which can occur with oral administration. It is possible that either

the rewarding or the aversive properties of a drug can be determined using the same behavioural technique.

The testing phase is carried out when the animal is drug-free. This should mean that results obtained are not influenced by anything other than the rewarding or reinforcing properties of the drug, e.g. motor depressant effects do not have an effect. The apparatus used can be automated but when kept simple is inexpensive. There is potential to investigate a wide range of reinforcing or aversive stimuli, (such as the company of another animal, or a sexually receptive female) and not solely psychotropic drug effects. Moreover, the predictions made from the results of conditioned place preference experiments have been shown to consistently compare well with the results obtained using other behavioural paradigms, when testing drugs for their rewarding/reinforcing properties.

The aim of the first in this set of experiments was to determine whether 10 mg/kg morphine i.p. produced a place preference effect in TO mice, and to investigate whether 400 mg/kg acamprosate i.p. altered morphine's observed effect. Morphine is an opiate drug. Evidence suggests that opiate receptors in the ventral tegmental area and nucleus accumbens may mediate the reinforcing actions of opiates (Koob and Bloom, 1988.) This stems from studies where opioid antagonists were administered into these areas and self-administration of the opiate, heroin, subsequently increased. Dopamine release has been suggested to be the primary rewarding action of opiates (Kuzmin et al, 1992.)

Acamprosate (calcium homotaurinate) is a derivative of the natural inhibitory amino acid taurine (Whitworth et al, 1996.) As already stated, it has been shown in clinical trials to reduce relapse and/or its severity in alcoholics undergoing detoxification programmes and is suggested to work by inhibiting the conditioned negative reinforcements for alcohol drinking (Littleton et al, 1996.) It is its actions at calcium channels which are of particular interest when considering its interactions with opiates such as morphine. Increasing evidence suggests that changes in calcium channel function play an essential role in opioid tolerance and development of dependence. It has been shown that synaptosomal calcium content and uptake are decreased after acute morphine administration and enhanced after development of tolerance (Harris et al, 1977.) Biochemical studies have demonstrated that chronic exposure to morphine increases the number of dihyropyridine binding sites in the rat brain (Zharkovsky et al, 1993) and that in tolerant animals, acute treatment with

calcium channel blockers provides protection against morphine withdrawal (Bongianni et al, 1986.) Furthermore, dihydropyridine-sensitive calcium channel antagonists administered daily in long-term morphine treatment prevent the upregulation of L-type calcium channels observed in tolerant animals as well as inhibiting the withdrawal syndrome (Zharkovsky et al, 1993; Kuzmin et al, 1992.)

The results from the initial pilot study and drug-free baseline testing day showed that the 60% of the total time was spent in the clear side of the conditioned place preference box. This was not a significant preference. The morphine and acamprosate were paired with the black side of the box on alternate days of a ten-day conditioning phase. On the test day, the group of mice which had had saline injections every day exhibited a significant decrease in preference for the clear side of the box compared with the time spent in this side on the baseline day. Mice in the groups wherein morphine or morphine and acamprosate had been paired with the black side of the conditioned place preference box did not exhibit any significant preference for either side of the box on the test day compared to the baseline day.

A consistent bias (not a significant preference) of subjects for the side of the conditioned place preference box with the mesh floor (during baseline preference tests) was found in my experiments and was also reported by Cunningham et al (1991b.) This could have ben because the mesh was more 'interesting' than the smooth floor in the other side of the box, so that more examination was made of it when mice were given free run of both sides of the box. In the post-conditioning preference test, mice which had received morphine and/or acamprosate in the other side of the box did not alter their exhibit a significant preference for the mesh-floored, clear side compared with the baseline test score; only those injected with saline did so significantly. Either the strength of the rewarding effect of the morphine was not sufficient to overcome the initial baseline preference for the mesh-floored, clear side or mice were not forming an association between the injection-paired side during conditioning and the drug effects experienced therein.

As to the dose of morphine used, the absence of a significant place preference effect for 10 mg/kg morphine is contrary to previous results obtained by Cunningham et al

(1992a) and Schechter et al (1995), where 10 mg/kg was in fact found to be the most reliably reinforcing dose in the same behavioural model and using mice. Morphine does not only have analgesic and euphoriant actions, though. Unpleasant side-effects such as nausea and hypothermia are often associated with morphine therapy, particularly in the short term (British National Formulary, March 1996.) If the administration of 10 mg/kg morphine produced more of these side-effects than euphoric effects, the 'net' effect of the drug would not have been rewarding and place preference effects due to morphine would not then be expected. As mentioned before, many physiological and behavioural measures have been shown to be profoundly influenced by the strain of mouse used (Brain, 1975; Cunningham et al, 1992a), and the dose chosen was necessarily based on the optimum doses for strains other than TO mice. Although all these factors had a bearing on whether a conditioned place preference was seen for morphine, it should be realised that the paradigm was still being set up at this point. Therefore, it is more probable that imperfect test conditions rather than unusual pharmacological phenomena were the reason why morphine was not seen to produce a conditioned place preference.

As to the procedure used, it is quite usual to pair the drug expected to be rewarding (e.g. morphine) with the less-preferred side of the conditioned place preference box, as determined by the baseline test (Kuzmin et al, 1992.) Acamprosate, being a relatively new drug, is not often used in conditioned place preference studies, but recent work demonstrated the importance of time of testing on the effects of acamprosate. Watson et al (1996) found that male TO mice treated with 400 mg/kg acamprosate exhibited motoric impairment on the elevated plus-maze in the dark phase, when only 200 mg/kg had a comparable effect when mice were treated in the light phase. Since exactly the same strain of mice were used, one would expect there to be some degree of motoric impairment induced by the 400 mg/kg acamprosate used, which may well have affected the strength of conditioning of the drug (Schechter et al, 1995.)

Considering the procedure used when testing for baseline preference, it will be recalled that mice were given free run of both environments. Looking at this another way, they were exposed to the compartment which would later be paired with an unconditioned stimulus (the drug injection) and at the same time also had free access to the compartment which would later be paired with the neutral stimulus (the saline injection.) This baseline

test could result in producing 'latent inhibition' in the mice (the learning to ignore irrelevant stimuli) which, according to Mackintosh, (1974; cited in Martin-Iverson and Reimer, 1996) should result in less effective conditioning. This phenomenon might go some way to explain why a place preference effect was not seen for morphine- the drug which is normally the very "litmus-test" of rewarding drugs.

Considering now the conditioning phase of the experiment, carrying out a drug-paired conditioning phase after a drug-free baseline test may mean that the novelty of the injections interferes with the strength of association formed. This latter problem might be got round by giving a vehicle injection prior to the baseline test (Cunningham et al, 1992.)

The second experiment employing the conditioned place preference paradigm aimed to determine; firstly, whether 2.5 g/kg ethanol produced a place preference effect in TO mice (in the conditions used in our laboratory); secondly, whether 0.4 mg/kg nicotine produced the same effect; and thirdly, the effect produced when the two drugs are combined. Again, a non-significant slight preference for the mesh-floored, clear side of the conditioned place preference box was seen after baseline testing. Both drugs were paired with the black side of the box during the conditioning phase. Mice paired with nicotine alone were the only treatment group to exhibit a significant decrease in preference for the mesh-floored, non-drug paired side of the box compared with their mean baseline preference for this side.

The finding of a conditioned place preference for nicotine-treated mice is in accordance with reports by Risinger and Oakes (1995) and Schechter et al (1995); the latter group also pairing the nicotine with the less-preferred side of the conditioned place preference box. Why a conditioned place preference effect was found for nicotine but not for ethanol or ethanol/nicotine could have been due to the phenomenon of behavioural sensitivity. This is an increase in the behavioural effects of drugs, observed when psychomotor stimulants (such as nicotine) are repeatedly administered. Previous results have shown that intermittent administrations of psychomotor stimulants with a spacing of two or three days between injections (two days in my experiment) leads to the most robust sensitisation (Robinson and Berridge, 1993.) Work done by Burger and Martin-Iverson

(1994) indicated that the sensitisation to cocaine, when it was paired with one compartment of a two-compartment conditioned place preference box , was more robust when it was administered every other day rather than daily.

According to Cunningham et al (1992) it is more usual to find ethanol-induced conditioned place aversion in rats, whereas conditioned place preference is more usual for ethanol-treated mice. However, a significant preference effect with ethanol alone was not found in my study. This 'negative' finding is in agreement with ethanol preference studies on rats by Asin et al (1983.) Most groups report that ethanol does produce a significant place preference (De Witte, 1984; Cunningham et al, 1990; 1991b; 1992a; Ali et al, 1995.) Throughout the conditioning phase, it was obvious that the dose of ethanol chosen depressed locomotor activity. Ethanol-treated mice became increasingly tolerant to this effect as pairings were repeated, as was manifested in their increasing activity in the conditioned place preference boxes, but this initial motor depression may have affected conditioning. Cunningham et al (1991b) reported that ethanol's effect in conditioned place preference studies were most rewarding when the subjects experienced the least severe hypothermic effects from the drug, and mice remaining very still during the conditioning trials may have been experiencing a degree of hypothermia.

Another possible problem may have manifested itself in the final preference test. If, due to the motor depressant effects of the ethanol, mice did not learn to correctly associate the drug with the environment in which it was experienced during condiditoning trials, the environment itself might have become the discriminative stimulus in the test phase. For example, the mouse might gravitate towards the most familiar environment (that paired with the vehicle) instead of the environment associated with the preferred drug. Consequently, results inferred from the final preference test would not give a true indication of the effect the drug had had on the mice. Drugs with additional anxiolytic/ anxiogenic or stimulative properties may cause similar problems.It can be seen that even though preference testing takes place when the subject is in a drug-free state there is some doubt as to the extent of preference/aversion measurement possible. Particularly in experiments where the drugs under investigation have actions other than solely rewarding or aversive, it is difficult, if not impossible, to define the controlling stimulus which leads to the observed place preference or aversion.

If this experiment were to be repeated with TO mice, a lower dose of ethanol would be recommended. The dose of ethanol chosen (2.5 g/kg) was based on studies using BKW mice (Ali et al, 1995) even though a study by Cunningham et al (1992a) reported 3 or 4 g/kg ethanol to be the lowest in a range of doses to induce a place preference in DBA/2J mice. These examples just go to show yet again the degree to which the various strains of mice differ in their responses to the same drug dose.

The importance of considering the length of the conditioning trial duration was highlighted by Cunningham et al (1990) in reference to conditioned place preference studies for ethanol in mice. Mice received four pairings of 2 g/kg ethanol with one side of a CPP box, and four pairings with saline in the other side of the box. Conditioning sessions were either 5, 15 or 30 minutes in duration. In the drug-free test phase, mice which had experienced the shortest conditioning sessions demonstrated a relatively higher preference for the ethanol-paired side compared with mice which had had longer conditioning sessions. (This result might suggest that ethanol produces an initial, short-lived excitatory reward effect, which is replaced by a longer-lasting (inhibitory) aversive effect.) The number of conditioning trials is another question; De Witte (1984) obtained a conditioned place preference effect in rats for ethanol after only one pairing of the conditioned place preference box with the ethanol prior to the test phase.

A significant place preference was not found in TO mice treated with both ethanol and nicotine. No other studies examining conditioned place preference in mice with ethanol and nicotine have been seen. However, two groups report that the locomotor stimulation induced by nicotine is enhanced by ethanol: Lapin et al (1995) and Johnson et al (1995), and, as has been mentioned before, Schechter et al (1995) have suggested a link between the locomotor-stimulating and rewarding effects of drugs.

There are a great many variations on theme in the design of conditioned place preference experiments. The breed of animal used, light phase in which the animal is tested, type of cues used in the preference boxes, number of compartments in the apparatus, number and duration of conditioning trials and state of the animals pre-test usually vary widely between experiments and research groups. Which of these variations has more influence over the final preference score is debatable, but some investigations have been

done on individual aspects of this paradigm which could be useful when planning future repeats of my experiments (Costall et al, 1989; Cunningham et al, 1990.)

The last conditioned place preference experiment carried out was intended as a modified repeat of the preceding ethanol/nicotine experiment. The five-stage procedure was automated and incorporated chocolate as an extra olfactory cue in the black compartment of the conditioned place preference boxes. Baseline testing with both video and automated monitoring showed the mesh-floored, clear side of the conditioned place preference box to be the slightly (but not significantly) preferred side when mice were allowed free run of both compartments. 10 mg/kg morphine was paired with the other, chocolate-paired side of the box during conditioning trials. As was found in the first conditioned place preference experiment after ten conditioning days, mice treated with morphine exhibited no significant preference for either side of the box on the test day compared to the baseline day. The same result (i.e. no significant change in preference) was obtained after further modifying the apparatus by placing wet sawdust underneath the mesh in the clear side of the conditioned place preference box.

Further planned stages of this experiment were not carried out because it was necessary first to have a conditioned place preference setup which was sensitive enough to reliably detect the 'standard' rewarding effects of drugs such as morphine or amphetamine. Only once the paradigm had been modified sufficiently to achieve this aim could further drugs be tested; drugs which would be expected to be either non-rewarding in a conditioned place preference test (e.g. haloperidol) or have subtler rewarding effects than morphine (such as ethanol and ethanol combined with nicotine.) It would be completely unrealistic to assume from the results of my experiments that morphine does not have rewarding effects on TO mice, partly because Cunningham et al (1992) carried out conditioned place preference experiments on various strains of mice (unfortunately not including the TO strain), using a range of morphine doses, and all mice showed a place preference effect with all doses of morphine tried. A far more likely explanation could be that mice of different strains vary not in their response to the motivational effects of drugs but in their ability to learn associations between the test drug and the environment with which it is paired. This is especially true when drugs have effects other than simply rewarding/aversive ones. The results of these experiments using the conditioned place preference paradigm show the

importance of a methodical approach: testing the apparatus itself should be done before testing the effects of drugs using the same apparatus.

Two-bottle choice tests and conditioned place preference experiments are not the only ways to obtain an index of the rewarding effects of drugs. Other models of drug-seeking behaviour, such as self administration paradigms, could be said to have greater validity as a model of drug use and abuse because the animal, like a human, controls drug intake. This paradigm has the advantage, like the two-bottle-choice test, of producing a graded dose-response function. A graded dose-response relationship is not really obtainable using the conditioned place preference paradigm; there is often a definite step-up point where one dose does not produce place preference but the next dose up produces a positive and maximal effect. However, the self-administration model does mean that the subject is affected by any non-specific actions of the drug, such as sedation, whereas in conditioned place preference the subject is tested in the drug-free state. Also, I am not wholly sure that the operant model of drug administration is easily related to human behaviour- people do not have to do physical work in order to receive alcohol or nicotine, although they have to find the money to buy the drugs. Then again, in the conditioned place preference paradigm, the whole concept of conditioning subjects in one context (confining the animal to one side of the preference box and giving it an injection of a drug) and testing in another (giving the animal free access to both sides of the box and not injecting it) may also be inherently 'wrong.'

Measuring withdrawal anxiety as an index of reward can be criticised easily as a paradigm, mainly because it does not have the weight of research behind it as, say, self-administration paradigms have. To use the former paradigm again, it would need to be refined somewhat, for example to control for the repeated plus-maze test more rigorously. I think the paradigm could be fundamentally valid, whereas the frequently-used experimental practice of administering ethanol chronically in a calorie-controlled liquid diet as a model for addiction has been soundly criticised (Wolffgramm and Heyne, 1995.) Obviously, there are fundamental arguments for and against all behavioural models purporting to measure some aspect of the rewarding effect of drugs.

How closely we can draw parallels between wild strains of mice and laboratory mice, and furthermore, between laboratory mice and humans, is too important a question to be avoided, since mice were used throughout this experiment. Experiments which purport to be miniature representations of human behaviour and physiology should provide a reasonable analogy to the ordinary conditions under which humans perform. The problem of excessive anthropomorphism is then hit upon. How can we recreate in the laboratory the parental influences, education, sociology, work stresses and relationship problems that can all have a bearing on why some people abuse drugs and others do not? When designing experiments, it is essential to reconcile the 'normal' ethology of the species being used with what is known of human behaviour as regards initial and continued intake of drugs. Equally, the dose, presentation and normal availability of the drug to humans must be carefully considered, as it was in this investigation. This does not imply just 'scaling down' doses of drugs to allow for the reduced size of mice compared to humans. There appears to me to be a dearth of fundamental research on how laboratory mice behave 'normally'; for example, the size of a 'normal' social group and cage conditions which allow the maximum expression of thier 'normal' range of behaviours. Certainly important advances have been made in elucidating some genetic, psychological and psychopharmacological factors which can precipitate or increase the probability of one person going on to develop alcoholism where another would remain a social drinker. However, the same experiments, (which every so often spawn new theories on human drug-taking habits) are usually based around the behaviour and drinking habits of isolated rodents living in small, standard cages and often deprived of food and/or water (Kulkosky and Zellner, 1980.)

On the other hand, I appreciate the use of animals in research into drug addictions. Many people I have spoken to condone the use of animals for research into heart disease, diabetes and cancer, but draw the line at drug addiction research, because they see addiction as somehow the 'fault' or 'weakness' of an individual. Alcohol and nicotine addiction is widespread across all ages, socioeconomic groups, and both sexes. The cost of alcoholism is huge, not only in simple monetary terms but also in terms of indirect and direct morbidity, years of potential life lost, related crime and accident costs and productivity losses. In spite of this, many people still appear not to consider alcohol and

nicotine to be "drugs"- a misconception perpetuated daily by journalists, even those from leading broadsheet newspapers.

It is highly unsatisfactory to continue research into drug abuse with the aim of formulating improved pharmacological treatments which aid individual addicts' withdrawal and recovery from the effects of the drugs. In fact, I consider this approach morally questionable. This is not to say that drug addicts should not receive the highest standard of care when they are facing the enormous physical and mental task of weaning themselves off an ingrained habit. It is just that investigating and attacking the source of the problem is the more logical, although infinitely more complicated approach. The 'force' pushing people into drug addiction in the first place is likely to be a combination of a host of additional 'forces' of variable sizes. Social scientists, medical practitioners, pharmacologists, philosophers, geneticists, community leaders, journalists, politicians, teachers, parents and the addicts themselves all have valuable knowledge and views as to the catalysts which spur the onset of a drug addiction in a person who previously maintained a controlled level of the drug(s.) An encyclopaedic investigation which aimed to collate the experience of these groups would conceivably shed some light on the root of the problem. The 'force' I have been examining is reward. Central to all addiction is the way the drug itself reinforces behaviour. Mechanisms of reinforcement can include the reduction of withdrawal symptoms; the production of a state of wellbeing in the drug-taker and secondary reinforcement derived from drug-associated cues.

Drug-related behaviour is the consequence of interaction between the drug, the individual and society. The keyword here is interaction. None of the component factors alone is sufficient to cause drug dependence and their relative importance is different in different circumstances.

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