

PHARMACODYNAMICS AND PHARMACOKINETICS OF CENTRALLY
ACTING DEPRESSANTS IN MAN.

by

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DECLARATION

I declare that this thesis is of my own composition and is a record of my own research, not having been presented in any previous application for a degree. Help given by others is acknowledged and all sources of information are indicated in the text.

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ABSTRACT

A "steady-state" constant plasma concentration paradigm was used to assess: a) whether rapid acute tolerance to the central nervous system effects occurred following administration of three centrally acting drugs and, b) the profiles of psychomotor impairments obtained under these constant conditions. The issue of differential test sensitivities at steady-state was also addressed. The drugs used were chlormethiazole, ethanol and nitrous oxide. These were administered by the intravenous, oral and inhalation routes respectively. Central nervous system effects were assessed using a battery of standard and new psychological tests and subjective measures which were first assessed for reliability.

In terms of cognitive and psychomotor performance, there was no evidence of acute tolerance to chlormethiazole or to ethanol at steady-state. There was, however, evidence of acute tolerance to some peripheral effects of chlormethiazole, and to some of the subjective effects of nitrous oxide. The patterns of effects were consistent with the view that the drugs had broadly the same profile of effect on the tests used, but at different points on the same basic dose response curve of impairment. The exception was critical flicker frequency which was less affected than expected with chlormethiazole (a hypnotic) and nitrous oxide.

The nitrous oxide data suggested that some of the dose-response curves for different processes may cross over. Thus body sway which was substantially impaired with all three drugs, was one of the least sensitive tests at the lower concentrations of nitrous oxide. Tapping, on the other hand, was sensitive at the lower concentrations, but the magnitude of impairment was smaller than for other tests at the higher concentrations.

It is suggested that the pattern of impairment changes with dose, and that this is probably a reflection of differences in test sensitivities rather than specific effects of the drugs. Thus drugs with non-specific actions in the central nervous system could have specific-looking patterns of effect as an artifact of the methodology.

It is concluded that the steady-state paradigm is an extremely useful method for assessing the pattern of impairments produced by drugs, and that the benefit in terms of cleaner methodology outweighs the disadvantage of increased effort.

CHAPTER 1 - INTRODUCTION

INTRODUCTION

In recent years there has been a rapidly expanding interest in measuring the effects of drugs on the central nervous system in man. A variety of factors have spurred this interest including increasing regulatory requirements for data on new drugs, and concerns over the side-effects of prescribed drugs and drugs of abuse. Thus the number of experiments on the effects of central depressants on performance testing has increased dramatically. Due to major differences in methodologies, however, the overall increase in real scientific knowledge from these experiments has been rather disappointing.

Many of the test methods employed to assess brain function have been borrowed from psychology and have been evaluated in that context. Many others have been invented by researchers who have not attempted to demonstrate their sensitivity or to validate them in any way. Hindmarch (1980) reviewed the literature on tests used to assess psychomotor function after psychoactive drugs and found the techniques used to be

"....diverse, often complex, frequently insensitive to drug-induced changes and sometimes inconvenient to enact or replicate".

Over ten years later, despite concerted efforts, there is still no general consensus on the most appropriate and reliable tests and testing strategies. There have, however, been a number of putative candidates. Critical flicker frequency, choice reaction time, body sway, peak velocity of saccadic eye movements, electroencephalograms, auditory vigilance and measures of information processing such as the continuous performance test are examples of tests which have been recommended as tests of choice (Smith and Misiak, 1976; Hindmarch 1980; Swift, 1984; Linnoila, 1983; Griffiths et al., 1984; Tedeschi et al., 1985; Saletu, 1987; Wilkinson, 1968, 1970 and Mirsky, 1988).

In selecting a "best test" there is an implicit assumption that different drugs produce qualitatively the same impairment, and that the same test should be able to detect performance changes with all drugs. This is probably a reasonable assumption if central depression is a global phenomenon. However, it is at odds with the concept of different drugs having different mechanisms of action in different receptor, biochemical or membrane systems. If drugs have specific mechanisms of action on different systems then the patterns of gross impairment might be expected to differ, and this would only be observed by using a variety of tests. Thus benzodiazepines acting on the benzodiazepine/gamma-aminobutyric acid receptor complex might not necessarily produce the same qualitative pattern of central depression as anticholinergics acting on muscarinic receptors. It has been argued that this may be the case with functions such as memory (Bartus et al., 1987; Curran et al., 1988; Sunderland, et al., 1989).

Benzodiazepines have been shown to interfere with acquisition of both verbal and visual information into the memory store without appreciably impairing retrieval of already well learned information i.e. knowledge memory (for a review of this subject see for example Taylor and Tinklenberg, 1987). Indeed, information learned just prior to administration of the drug may even be remembered better (Ghoneim et al., 1984). The anticholinergic drug scopolamine has also been shown to interfere with memory, but it has been suggested that it does so in a slightly different way. That is, in addition to interference with acquisition of new information, the drug may also cause a temporary impairment of knowledge memory (Weingartner., 1987). This has prompted some workers to propose that benzodiazepines produce an impairment resembling Korsakoff's syndrome (Wolkowitz et al., 1987), whereas anticholinergic drugs such as scopolamine produce effects like normal ageing in the young (Drachman and Leavitt, 1974; Flicker et al., 1990), and Alzheimer's disease in

the elderly (Sunderland et al., 1986). It is hard to imagine that one single test could ever discriminate this kind of subtle difference between drugs.

One of the difficulties in choosing tests for evaluating the psychomotor effects of new drugs especially is that there may be no way of predicting a priori what effects there might be, and therefore no way of knowing which functions to test for. This again suggests that a battery of tests is preferable to a single test. However, as with the "best test", there is still no consensus on the best battery of tests for general use, or the most appropriate rationale for choosing the tests.

The methodological approaches to psychomotor testing are many and varied and span several disciplines including psychology, neuropsychology, chronobiology, neurotoxicology, undersea biomedical research, anaesthesia and psychopharmacology. For reviews of psychomotor testing see Cronbach, 1970; Fitts and Posner, 1973 (psychology), Strub and Black, 1977; Lezack, 1983; Walsh, 1987 (neuropsychology), Annau, 1987 (neurotoxicology), Fowler et al., 1985 (undersea biomedical research), Hindmarch and Bhatti, 1987 (anaesthesia), Wittenborn, 1979, 1987; Hindmarch, 1980; Johnson and Chernick, 1982 (psychopharmacology).

Not all researchers use a structured or consistent strategy for testing, but some do. These strategies include for example a hierarchical approach (Strub and Black, 1977), an information processing approach (Berry et al., 1965; Welford, 1968; Senders et al., 1967; Tharp et al., 1974; Michon, 1976; Wesnes et al., 1987), an abilities approach (Fleishman 1967, 1972; Levine et al., 1975), a deficit measurement approach (Lezack, 1983), a standardised approach (Hindmarch, 1980), a real driving approach (Biehl, 1979; de Gier, 1981; O'Hanlon and de Gier, 1986), a driving skills approach using simulators (Ashton et al., 1972; Linnoila, 1973b) a pseudo-driving skills approach (Moskowitz, 1973; Linnoila, 1973a) an

ethanol impairment model approach (Donaldson et al., 1980; Linnoila, 1983; Baker et al., 1985) and a random selection approach (until recently, most authors).

The hierarchical approach involves first assessing basic processes or "mental activity variables" (Lezack, 1983), comprising level of consciousness, attention, and vigilance along with motor speed and activity rate. The rationale is that since the "efficiency" of behavior and intellectual performance depends critically on the state of the mental activity variables, these variables should be assessed first. If the basic variables are impaired then it can be inferred that higher level functions such as memory or abstract reasoning dependent on the basic variables would also be impaired. If the basic functions are intact, however, it does not necessarily mean that the higher functions are unimpaired. Thus functions at the next level should be tested, and so on.

In the information processing model components of human behaviour are analysed in terms of processes that would be relevant for analysing the efficiency of a computer. The aspects of performance investigated are usually selection (or input), processing, storage, and output of information. In behavioural terms these can be roughly translated into attention, cognition, memory and co-ordination.

Application of the abilities classification involves the determination of the extent to which a task requires a particular ability for its performance. The ability "domains" are cognition, perceptual-sensory and psychomotor performance. In this particular classification attention comes under the cognitive domain, and co-ordination comes under the psychomotor domain.

The deficit measurement approach basically involves testing all of the main aspects of psychomotor function in all of the various modalities, and then narrowing

down on particular aspects of impairments once these have been identified.

In the standardised approach, a standard set of tests are administered which isolate the major variables likely to be affected according to a well-defined behavioural model. Any changes are assessed in terms of deviations from this model.

In the driving, driving simulator and pseudo-driving skills approach, subjects are tested on driving skills or on skills related to them. This methodology is perceived by many to have good face validity since epidemiological studies have shown that even modest ethanol consumption may lead to driving accidents (British Medical Association, 1987). Pseudo-driving tests allow driving-type skills to be evaluated in the laboratory.

The ethanol impairment model involves using tests which have been shown to be sensitive to ethanol. The rationale here is that since ethanol is known to impair driving skills, anything that detects the effects of ethanol would be an indicator of possible driving impairment.

The random selection approach was, until recently, the most common approach. This involves using any tests that are available, that the authors have previously heard about, or that have been found to be sensitive.

The information processing, the abilities classification and the standardised approach have much in common. In all of these models standard sets of functions are tested, and the only difference is in the functions chosen or the tests recommended. This type of model of performance is considerably easier to test than the hierarchical or deficit measurement models since there is usually a fixed number of tests on which performance is evaluated. The information processing model of behaviour is becoming extremely popular as it provides a logical

framework for the assessment of psychological skills.

Despite having a considerable amount of face validity, there are problems with the driving skills approach. Even using large doses of drugs with well known side-effects it may not be possible to detect performance impairments on driving. For example Fargus and Hindmarch (1974) were unable to detect impairment in car driving performance in volunteers acutely after 30 mg temazepam, a dose more than adequate to produce deep sleep ordinarily in most individuals and with effects that are easy to detect in the laboratory. These authors suggested that the driving task was sufficiently motivating and interesting to overcome any such impairment. However, it may also be that the task is underloaded and overlearned, so that it is a relatively insensitive skill in experienced drivers. The following example illustrates this point.

In a series of experiments Senders and co-workers (1967) examined the relationships between road characteristics, the amount of time a driver has to look at the road, the interval between such observations and driving speed. Driver vision was controlled by the face shield of a remotely activated protective helmet which could be rapidly lowered over the driver's eyes. The authors showed that 0.5 sec viewing times were long enough to provide nearly all the information needed to drive at any speed (within road limits) in a real driving situation, and that for most individuals, 1.0 sec visual occlusion times in a 1.5 sec cycle did not require a reduction in speed at all. Indeed one subject drove safely and with complete control at speeds in excess of 70 miles per hour with only 1.0 sec looks at the road separated by 4.0 sec intervals of complete occlusion. Even when the road was unfamiliar and twisting drivers were still able to give error-free performances as long as they could reduce speed. Since car driving is also an overlearned task the demands of the motor component would also be expected to be low (Linnoila, 1983).

It therefore seems probable that the magnitude of the impairment produced by 30 mg temazepam in the Fergus and Hindmarch (1974) experiment was relatively small in comparison to the "allowance" of the task. However, the experiment clearly demonstrated that even with seemingly valid complex tasks and large doses of central depressants it may not always be possible to detect impairments.

Other psychophysical factors believed to influence performance of the individual (and performance after drugs) are age and sex (Spring et al., 1982/83), memory and intelligence (Hindmarch, 1980), interest and motivation (Ayd, 1972; Hindmarch, 1980), the expectations of the subject and of the investigator (Ayd, 1972), the surroundings and the subject's previous experience and conditioning (Staiger and White, 1988), the temperature of the test room, rewards for performance (Ayd, 1972), knowledge of results including feedback (Fitts and Posner, 1973), time of day (Colquhoun, 1971; Okawa et al., 1984; Tilley and Warren, 1983), diet (Spring et al., 1982/83), the subject's mood, stage of the menstrual cycle in females (Rubinow et al., 1984), noise (Broadbent, 1971; Fitts and Posner, 1973), right or left handedness (Irwin, 1985, different responses after drugs), sleep loss (Johnson, 1972; Angus et al., 1985), fatigue (Lubin et al., 1976), bed rest (Lubin et al., 1976; Kjellberg, 1977 cited by Angus et al., 1985), illness and hospitalization (Cole and Zarit, 1984), psychosocial background, personality including introversion/ extroversion and the degree of anxiety, neuroticism or psychoticism (for review of personality and performance see Eysenck and Eysenck, 1985). Many of these factors may interfere with the measurement of drug effects. As pointed out by Glaister (1981), a weak effect is

"..... difficult to demonstrate in measures which characteristically exhibit wide variations between subjects, and which are sensitive to diurnal changes, subject mood and so on".

The pharmacological and pharmacokinetic aspects of experiments with psychomotor performance and psychoactive drugs further complicate the situation, although this does not always appear to be obvious to psychologists working in the psychopharmacology area. Figure 1.1 shows a simple information processing model of psychomotor behaviour likely to be affected by central nervous system depressant drugs (Hindmarch, 1983). The whole of the drug contribution in this behavioural system is represented by a single arrow labelled "psychoactive drug".

Presumably because of this kind of oversimplified view of drug action, single doses of drugs have often been compared despite the fact that equivalent doses of the drugs were typically unknown. However, although they are preferable, dose response curves are often complex and do not allow easy comparison between tests, testing strategies or drugs. Typically, there are multiple doses and multiple testing times. Different formulations and routes of administration are used. The plasma concentrations constantly change, and there is often no clear relationship between plasma concentration and effect. For example, the depressant effects may only be apparent above a certain brain concentration indicating a possible threshold effect (Gibaldi, 1984), or the drugs may have a weak excitatory effect in low doses, which may lead to improved performance. Alcohol, barbiturates, anticholinergic drugs and some anaesthetic drugs such as nitrous oxide appear to be excitatory in low doses (Bowman and Rand, 1980). This is generally interpreted as disinhibition or inhibition of inhibition.

Small amounts of ethanol have been demonstrated to improve performance on some tasks (Palva et al., 1979; McManus et al., 1983). Similarly, speeding of reaction times after low doses of benzodiazepines such as diazepam (Palva et al., 1979; Steiner-Chaskel and Lader, 1981); brotizolam (Saletu et al., 1983), lopirazepam (Saletu et al., 1980), and quazepam (Lee and Lader, 1988) have

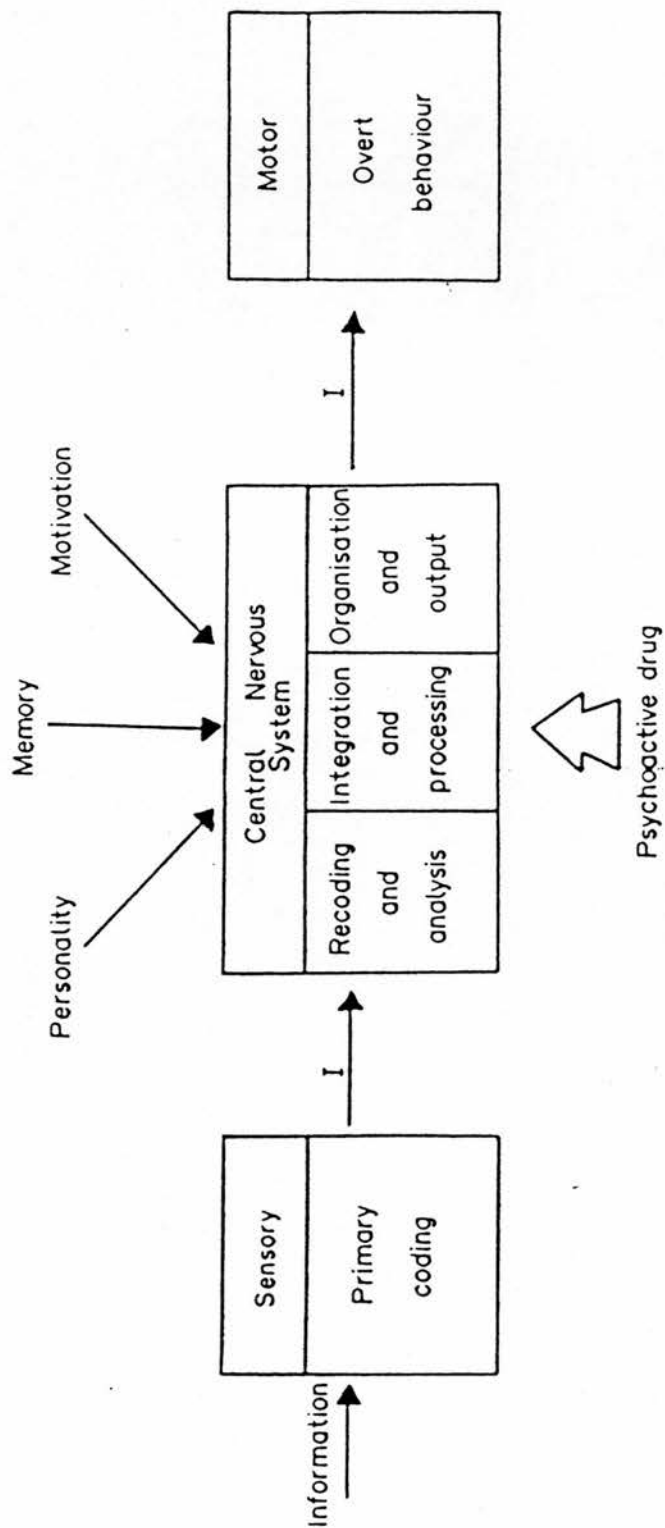


Fig.1.1. An information processing model of psychomotor behaviour. Sensory information is analysed, processed and organized by the CNS to provide appropriate information to the effector systems. Should a psychoactive drug be administered then the integrity of the relationship between sensory and motor systems will be changed.

been reported. Saletu et al., 1983 reported that attention and mental concentration were impaired at benzodiazepine concentrations where improvements in reaction time were seen. Similarly, Lee and Lader (1988) reported that, for quazepam, a motor task with a more complex cognitive component (symbol copying) was impaired when simple reaction times and tapping rates were improved. Korttila et al., (1978) showed a significant negative correlation between the end tidal concentration of trace amounts of halothane and driving time in a driving simulator indicating improved task performance with increasing halothane in a group of theatre nurses.

Lee and Lader (1988) suggested that with respect to the benzodiazepines, the improvement may have been due to reflex muscle relaxation allowing freer repetitive movements. However, the faster reaction time may also have been due to a change in the speed/accuracy trade-off function as proposed for ethanol (Rundell and Williams, 1979). This latter hypothesis is not easy to test since the error rate on reaction time tasks is generally so low to begin with that decreased accuracy can not always be measured.

Beta-adrenoreceptor blocking drugs including propranolol, metoprolol and atenolol have also been shown to be capable of producing improvements as well as impairments in reaction time tasks (Harms, 1985; McDevitt, 1985), and in other tasks involving precision motor skills and attention, though the majority of studies with beta-blockers have not demonstrated any change in performance at all. Improvements, when they occur, seem to be found at low doses, impairments then occur with middle range doses where they reach a maximum, and the highest doses sometimes then have a lesser effect (Glaister 1981; McDevitt, 1985). The anxiolytic action of these drugs (Baldessarini, 1990) may account for the lessening of effect in higher doses.

There have been reports of differences in effect at the same plasma concentration between the rising and falling phases of the plasma concentration curves after single doses of some drugs (see Goldberg 1943; Baird and Hailey 1972; Ellinwood et al., 1981a, 1981b, 1983; Saletu et al., 1982, Schulz et al., 1983 and Ellinwood and Heatherly, 1985). This may be caused by acute tolerance (tachyphylaxis), by delayed penetration to the effector site, by sensitization at the effector site, or may simply be an artifact due to the blood sampling site used.

Mellanby (1919) described a shift in effect on the downswing of the blood ethanol compared to the upswing. He noted that psychomotor function was more impaired when the blood concentration was rising compared to when it was falling, at the same blood ethanol concentrations. Goldberg (1943) confirmed these findings and derived a theory for the relationship between the degree of ethanol intoxication and the blood ethanol concentration. More recently, a number of authors have tried to repeat these findings, with equivocal results. For example Ellinwood et al., (1981b), Linnoila and Mattila (1973b) and a group including Goldberg himself several years later (Ekman et al., 1963, 1964) were unable to demonstrate these differential effects, while others such as Kalant et al., (1971), Hurst and Bagley (1972), Jones and Vega, (1972), MacLeod et al., (1977), Vogel-Sprott (1979) and Haubenreisser and Vogel-Sprott, (1983) have not only agreed with Mellanby's original findings, but have postulated compensatory mechanisms to explain the tolerance. Other authors such as Palva et al., (1979), Mills and Bisgrove (1983) and Fagan et al., (1987) could only demonstrate impairments on some skills using very large doses of ethanol, of the order of a quarter to a third of a bottle of vodka given in a short period of time to an average sized man.

Differences in performance or in the extent of central nervous system effects between the rising and falling

phases of the plasma concentration curve have been demonstrated for a variety of other centrally acting drugs including diazepam (Baird and Hailey, 1972; MacLeod et al., 1977; Ellinwood et al., 1983; 1985; 1987), thiopentone (Dundee et al., 1956; Toner et al., 1980), chlordiazepoxide (Greenblatt et al., 1977), clorazepate (Greenblatt et al., 1977), alprazolam (Ellinwood et al., 1985, 1987), midazolam (Al-Khudhairi et al., 1982; in dogs), pentobarbital (Ellinwood et al., 1981a), glutethimide (Curry and Norris, 1970) trazodone (Bayer et al., 1983), nomifensine (Saletu et al., 1982), cocaine (Van Dyke et al., 1978; Fischman et al., 1985), fluvoxamine and clovoxamine (Saletu et al., 1983), cinnarizine (Golding et al., 1989) and amitriptyline (Schulz, 1983). In all but the last four cases, the effects were greater during the rising than the falling phase.

For several drugs (e.g. diazepam, chlordiazepoxide and ethanol) the absolute concentration of drug was shown to be less important in producing psychomotor impairment than the rapidity of rise of drug in the plasma (Linnoila and Mattila, 1973a; Bliding, 1974; Greenblatt et al., 1977; Ellinwood et al., 1981a, 1981b). Dose was also shown to be an important factor since greater tolerance appeared to develop after a larger than a smaller dose of diazepam (Orr et al., 1976). This latter finding was confirmed for diazepam, and also demonstrated for pentobarbital (Ellinwood et al., 1981b, 1981a), though only for complex tasks. Simple tasks did not demonstrate tolerance suggesting that the effect was task specific (Seppala et al., 1980). Task specificity was also noted by Goldberg (1943), Mitchell (1985) and Nagoshi and Wilson (1987) for ethanol, by Aranko et al., (1983) for diazepam and lorazepam, and by Handel et al., (1988) for midazolam.

Aranko et al., (1983) suggested that, for benzodiazepines, different receptors may have different capacities for tolerance, and so different tasks develop

tolerance depending on the receptors involved. However, the fact that it appears to be a general phenomenon may point to a general mechanism. Importantly, the degree of adaptation observed after ethanol was insufficient to abolish impairment completely (Hurst and Bagley, 1972). This has also been demonstrated in animals given cocaine (Wood and Emmett-Oglesby, 1986). The maximum tolerance effect which could be reached with cocaine was about half the magnitude of that produced by d-amphetamine.

Bayer et al., (1983) showed that, with the antidepressant trazodone, young subjects had earlier onset of impairment and faster recovery while plasma concentrations were still high, whilst in older subjects impairments followed the blood concentration curves. These data suggest that acute tolerance may be age dependent. Differences have also been shown for sex. Radlow and Hurst (1985) demonstrated that the peak effect of ethanol tended to be earlier in males than females, though the timing of the peak effect appeared to be dose dependent. Timing of doses may be important. For example, Fischman and Schuster (1982) showed that the euphoric effects of a moderate (32 mg) dose of cocaine were diminished if the dose was given within one hour of a previous dose. Similarly, a single pretreatment with an opioid has been demonstrated to induce rapid acute tolerance on subsequent injection of the same drugs (e.g. Kalant, 1980). For ethanol, acute metabolic tolerance to ethanol was reported to occur such that a second dose was metabolised faster than an initial dose (Wilson et al., 1983). Previous drinking habits were also noted to be important for acute ethanol tolerance as well as chronic tolerance, habitual drinkers demonstrating greater acute tolerance (Golberg, 1943).

It has been postulated that genetic factors may be involved in acute sensitivity and acute tolerance to ethanol in rats (Khanna et al., 1990). However, the evidence for this in humans is less clear. In a study between first degree relatives of alcoholics and age

matched controls Nagoshi and Wilson (1987) found no differences in either acute sensitivity or acute tolerance to ethanol.

Subject motivation may influence results since knowledge of results and monetary incentives were demonstrated to raise the threshold for ethanol impairment, and to facilitate acute tolerance (Haubenreisser and Vogel-Sprott, 1987).

Many other aspects of experimental design have been shown to be important in the outcome between the development of tolerance or its opposite effect, sensitization. Post (1980) reviewed this subject and identified the following aspects as being important to the outcome; intermittent versus continuous stimulation, route of administration, dose, duration of treatment, environmental context, conditioning, genetics, the nature of the parameter measured and its relationship in time to the experimental treatment.

Several mechanisms of rapid tolerance after single doses of drugs have been proposed. The involvement of a compensatory response associated with learning and task practice was suggested for ethanol (e.g. Haubenreisser and Vogel-Sprott, 1983, 1987). Rapid uptake of highly lipid soluble drugs into the grey matter during absorption, and central nervous system adaptation have been proposed for diazepam and other benzodiazepines (Ellinwood et al., 1981b; Ellinwood and Heatherly, 1985). The possibility of variable responsivity to diazepam in the brain areas involved in different tasks has also been raised (Nikaido et al., 1987).

Differences in drug receptor dissociation rates, affinities, kinetics and adaptation have variously been proposed to explain differences in acute tolerance characteristics between different benzodiazepines (for references see Ellinwood and Heatherly, 1985; Ellinwood et al., 1987). The involvement of different types of

benzodiazepine receptors in varying combinations for different tasks has also been postulated to explain task specificity of the phenomenon (Aranko et al., 1983; Nikaido, 1987). Goldstein (1983) suggested, however, that for ethanol, the phenomenon was partly or wholly an artifact due to the differences in arterial/brain and venous sampling, and thus could be explained pharmacokinetically.

In the experiments described above blood was sampled, in general from peripheral veins, most commonly the antecubital or forearm veins. It was probably assumed that the plasma concentrations were homogeneous throughout the body, or that equilibrium occurred very rapidly. For example, Toner et al., (1980) rejected the notion that arterial/venous differences could be the reason for acute tolerance to thiopentone on the grounds that venous mixing would have been complete in a short time. In the last few years, however, these assumptions have been challenged. Barrat et al., (1984a, 1984b) argued that venous concentrations must reflect the concentrations in the tissues which they drain, and that these may not always correspond with the concentrations at the sites of action within the brain. Being a well perfused organ, the tissue concentration in the brain should be close to that of the plasma perfusing it (allowing for a suitable partition coefficient), and the effluent plasma concentration should reflect the tissue concentration to a significant extent. The tissue concentration in less well perfused regions such as in the arm, may take longer to equilibrate, and so the peak venous concentration would occur later. The differences in times to peak at different sites would also be exacerbated by uptake.

Differences in plasma concentration and different times to peak concentration at different sampling sites have been shown for a number of drugs including ethanol (Haggard and Greenberg, 1934; Forney et al., 1964; Payne et al., 1966; Dundee et al., 1971; Gostomzyk,

1971; Sedman et al., 1976), local anaesthetics (Tucker and Boas, 1971; Tucker and Mather, 1975), thiopentone (Barratt et al., 1984a in sheep; Barratt 1984b) and propofol (Major et al., 1983). Though some of these differences lasted for only a short time (<2min, Major et al., 1983), the differences sometimes lasted for a considerable time. For example, Tucker and Boas (1971) showed that, following cuff release after intravenous regional anaesthesia of the arm with lignocaine, the concentration in the brachial artery of the other arm was greater than that in the antecubital vein for over 15 minutes. Tucker and Mather (1975) also found that arterial plasma concentrations of etidocaine and lignocaine were considerably higher than venous concentrations up to an hour after epidural injection of these drugs. For lignocaine, the arterial concentration was three times higher than the venous at fifteen minutes, and double the concentration at 30 minutes. Arterial concentrations were still 50% higher at an hour.

Route and rate of administration appear to be important factors for arterial/venous differences. Forney et al., (1964) showed that arterial blood concentrations of ethanol were 50-100% higher than venous concentrations during absorption, and that equilibrium was faster after intravenous infusion than after oral intake. Gostomzyk (1971) further demonstrated in rabbits that, for ethanol, the rate of intravenous infusion greatly influenced the extent of arterial/venous difference. The more rapid the infusion rate, the greater the arterial/venous difference, but the shorter the time of difference. This was confirmed by Dundee et al., (1971) in patients. Using capillary and venous blood in two human volunteers, Sedman (1976) showed that there was only one overlap point when the capillary and venous concentrations were the same. This was when the absorption and elimination rates were equal i.e. at steady state. Before this point the capillary concentration was always greater than the venous. After

this point the capillary concentration was always less than the venous.

Barrat et al., (1984b) demonstrated that after the initial period during which the arterial blood concentration was higher than the venous, the jugular vein concentration in patients was considerably higher than the arterial, mixed venous or peripheral venous concentrations after an intravenous dose of thiopentone. This difference was still apparent at twelve minutes after the intravenous dose was given. Christensen et al., (1982) noted that the arterial/venous differences with thiopentone were more pronounced in elderly than in young males. For propofol, however, there was little difference in concentrations between sampling sites after 1 minute (Major et al., 1983).

Other proposed factors affecting arterial/venous differences are; decreased blood flow due to drugs or disease (Major, 1983), pulmonary first pass metabolism (Chiou, 1979), poor peripheral perfusion, changes in limb temperature and altered acid-base status (Major, 1983). Tucker and Mather (1988) have emphasised that blood cannot be considered a homogeneous drug-containing compartment if drug administration or elimination is taking place. Drug concentrations in peripheral blood can be homogeneous only under steady-state conditions and caution is necessary when interpreting absolute drug concentrations from arterial or venous blood. Apparent acute tolerance to the central nervous system effects of drugs may therefore be an artifact of differential drug distribution throughout the body.

The time course and the magnitude of the distribution artifact appears to be in sufficient agreement with the time course and magnitude of the acute tolerance to provide an adequate explanation for the phenomenon. However, it may not be the whole explanation. LeBlanc et al., (1975) showed in rats that acute tolerance was still present even after the distribution artifact was

taken into account. These authors measured brain levels of ethanol directly, and tested each animal's performance only once after an acute dose was given. The animals tested at one hour after ethanol were less impaired than those tested at ten minutes although the brain concentrations were the same. Thus the acute tolerance could not be attributed entirely to the distribution artifact or to task practise. Similarly, Maynert and Klingman (1960) showed that dogs became free of ataxia at higher plasma concentrations of ethanol and other intravenous anaesthetics (using samples from the jugular vein) after large doses than after smaller doses. Testing was carried out at a time when the arterial/venous differences should have been minimal.

Differential rates of recovery on tests of differing complexity cannot be explained adequately by pharmacokinetics, though the lack of complete recovery might be explained in this way. In support of a non-pharmacokinetic explanation is evidence produced by Campanelli et al., (1988) that acute tolerance to the motor effects of ethanol in rats was abolished when the median raphe nuclei was lesioned.

For delayed drug effects, Paalzow (1981) and Schulz et al (1983) suggested the presence of a pharmacokinetic deep (brain) compartment which had to be filled before the physiological effects became noticeable. However, delayed penetration to the brain may also have been involved.

Some attempts have been made to relate plasma drug concentrations to responses using mathematical models. Wagner (1968) proposed that the intensity of pharmacologic responses could be related to the concentration of drug in the body by one basic equation, the Hill equation (Hill, 1910), which could be modified as required to suit the pharmacokinetic model. A number of authors have developed this idea further including Holford and Sheiner (1981) and Ritschel and Hussain

(1984). The models proposed by these authors generally assume that the effect, or at least the response, can be adequately measured. However, the responses assessed in neuropsychological assessments are far removed from the likely primary effects on receptors, membranes and biochemical systems. The concentration at these effect sites may also be far removed from the concentrations as measured in the blood from peripheral veins, and the sensitivities of the assessment measures may be variable or unknown. To account for differences in the time course of action between plasma concentrations and responses, "effect compartments" are usually postulated (e.g. Holford and Sheiner, 1982), the number of effect compartments required depending on the data.

More recently, Ellinwood and Nikaido, 1987, have taken single tests with cognitive and motor components, factored out the contribution of these components to performance using computer techniques, and used these to calculate effect half-lives for the components. These were then compared to the pharmacokinetic distribution and elimination half-lives for different drugs. Using this method the authors showed that for the test used (digit symbol substitution test), the effect half-life was longer for the cognitive component than for the motor component, and that the rate limiting process for impairment was relatively independent of the differential pharmacokinetic and receptor kinetic properties of these drugs. The authors also expressed the degree of acute tolerance in terms of a rate constant relating the pharmacokinetic to the behavioural offset rates. This may be a more generally useful approach than the mathematical modelling approach at present.

One of the problems with this area of research is that a great deal of the work is generated by drug companies studying new compounds, whereas many of the old drugs that are in common use (and therefore of great interest) have hardly been studied. Equivalent doses of the new drugs compared to the old drugs is often unknown. Also,

there tends to be a reluctance in medical research to replicate experiments in sufficiently similar formats to allow a broad consensus of opinion to be built up on a new finding (Goodwin and Roy-Byrne, 1987). Unreproduced work with dubious results may therefore effectively be written in tablets of stone. This is probably exacerbated by the well known tendency for positive results to be promptly published in journals whilst negative and inconclusive results fail to be published (publication bias). Inevitably, this must distort our cumulative knowledge of the effects of drugs and the techniques used to measure them (Gotzche, 1987). Aside from the literature on benzodiazepines and ethanol, the amount of work extensively repeated is small. However, there have been several papers on the effects of beta-blockers on performance in recent years, a few on antihistamines and neuroleptics and the data on anaesthetic drugs is accumulating.

It is, nevertheless, believed that many drugs affect psychological performance in ways that are reproducible, and which ought to provide a basis for the selection of tests (Gruneberger and Saletu, 1980). Thus drugs with sedative actions usually decrease critical flicker frequency (considered to be a measure of arousal), while stimulant drugs increase it (Smith and Misiak, 1976). Sedative drugs usually also slow reaction time (Hindmarch, 1980). Some effects appear to be specific e.g. benzodiazepine and anticholinergic effects on memory (Dundee and Wilson, 1980; Sunderland et al., 1987; Wolkowitz et al., 1987), the impairment of judgement and divided attention with small doses of ethanol (Mitchell, 1985; Bloom, 1987) and the impairment of reaction time with large quantities (e.g. Palva et al., 1979).

There are, however, a number of general assumptions in the psychopharmacology area which might be challenged. One is that significant impairment of performance on psychological tests by drugs necessarily represents

specific and selective underlying deficits. It is feasible that functions which are most often significantly affected are more easily measured, that the tests used to measure them are more sensitive or that the tests are used more often. Another assumption is that bigger doses of drugs must produce qualitatively identical (although larger) effects than smaller doses. Substantial doses of depressant drugs may lead to profound central nervous system depression/sedation (Curran et al., 1986, 1988) and gross impairment, and so any specificity might be lost with bigger doses given to get a "good effect". Yet another widely held view appears to be that dose response curves from these experiments are necessarily complex, and that there is little that can be done to simplify them. However, modern pharmacokinetic methods allow plasma concentrations of drugs to be held constant allowing the production of much simpler dose-response curves.

Only a few studies have focussed on the acute, within session, effects of central nervous system depressants on psychomotor performance during steady-state. Of the studies designed to assess acute tolerance to stable ethanol concentrations, the results have been contradictory, and only one appears to have been double-blind and placebo controlled (Kaplan et al., 1985).

Kaplan and co-workers reported acute tolerance in the number of words recalled in a memory task but not to body sway or reaction time. The lack of effect of ethanol on reaction time was confirmed by Klotz et al., (1986), despite a large effect on this test being demonstrated. Wilson and Plomin (1985), however, reported acute tolerance on a number of tests including sway and reaction time. Similarly, Nagoshi and Wilson (1987, 1989) noted differences in indices of acute sensitivity and acute tolerance for different tests after steady-state ethanol achieved using oral loading and top-up doses. However, testing was only carried out

during the ascending limb of the blood ethanol concentration curve after each dose of the drug in the last three studies, and the results from these studies may not be directly comparable with those of the other steady-state ethanol studies.

Korttila et al., (1981) were unable to show acute tolerance on a reaction time test during 30 minutes of steady-state inhalation of 30% nitrous-oxide, or on re-challenge, despite reports of acute tolerance to the analgesic effects of the drug (Kripke and Hechtman, 1972; Whitwam et al., 1976).

Klotz and Reimann (1984) found evidence of a trend to recovery of test scores on reaction time when midazolam was infused for 26 hours. However it is possible that different tolerance or diurnal processes were involved over this longer time scale. In another steady-state experiment with midazolam, Handel et al., (1988) found that subjects recovered substantially on a reaction time test and on subjective sedation over a shorter time scale (within 2 hours of the start of infusion), but showed no recovery on electroencephalographic measures during 6 hours.

The evidence for acute tolerance to the effects of a constant infusion of triazolam was inconclusive since the timecourse of improvement could not be separated from diurnal effects (Breimer, 1985). However, Stone (1984) compared the sensitivities of a number of psychological tests (paper and pencil) using the data from Breimer's study. Using level of statistical significance as the criterion of sensitivity she found quite large differences between tests which appeared to be broadly similar. Thus letter cancellation, arithmetic and digit symbol substitution appeared to be more sensitive than logic tests or cognitive processing tests.

The concept of testing psychological performance at steady-state with constant plasma concentrations is an interesting and exciting one. Repeated testing under these conditions should make the observation of any acute tolerance easy to detect. Larger batteries of tests involving more functions may also be used to evaluate impairment profiles since fewer testing times are required. Additionally, it may be possible to compare the sensitivities of the tests at the same plasma concentration. The data for looking at plasma concentration-effect relationships should also be simpler (Sheiner et al, 1989).

Aims:

The studies in this thesis were designed to explore the following issues;

1. Evaluation of the reproducibility and reliability of the tests in a battery chosen to assess a range of psychological and motor functions.
2. Evaluation of the effects of constant plasma concentrations of centrally depressant drugs on psychomotor performance to assess whether rapid tolerance develops to the behavioural effects during a single session.
3. Comparison of the sensitivities of the tests under constant conditions with respect to placebos and centrally depressant drugs.
4. Simplification of the methodology for looking at "profiles of impairments" after centrally acting drugs.
6. Simplification of the methodology for looking at dose-response relationships in human behavioural studies of the central effects of drugs.

CHAPTER 2 - METHODS OF ASSESSMENT

INTRODUCTION

The aim of psychological performance testing in the study of drugs is to assess qualitative and quantitative changes in behaviour to determine one or more of the following:

- (i) whether a drug produces any effect(s) on the brain,
- (ii) the nature of the effect(s),
- (iii) the magnitude of the effect(s),
- (iv) the time-course of the effect(s),
- (v) the threshold level of the effect(s).

In order to be able to detect these effects a sensitive test battery is required which is administered under well controlled conditions.

The test battery chosen for the experiments in this thesis was compiled over some time, and since no standard methodology has been established, the approach used was an amalgam of several of the philosophies outlined in chapter 1. The mental activity variables of attention and activity rate were assessed, several aspects of psychomotor function were always observed, and some of the tests used were from the standardised set recommended by Hindmarch (1980).

The tests and rating scales used in the battery are outlined in the present chapter along with a description of the equipment used to run the tests and the experimental conditions under which they were administered. The statistics used to analyse results of experiments using the battery are also described.

Finally, demographic and other relevant data pertaining to the participating subjects is included in this Chapter.

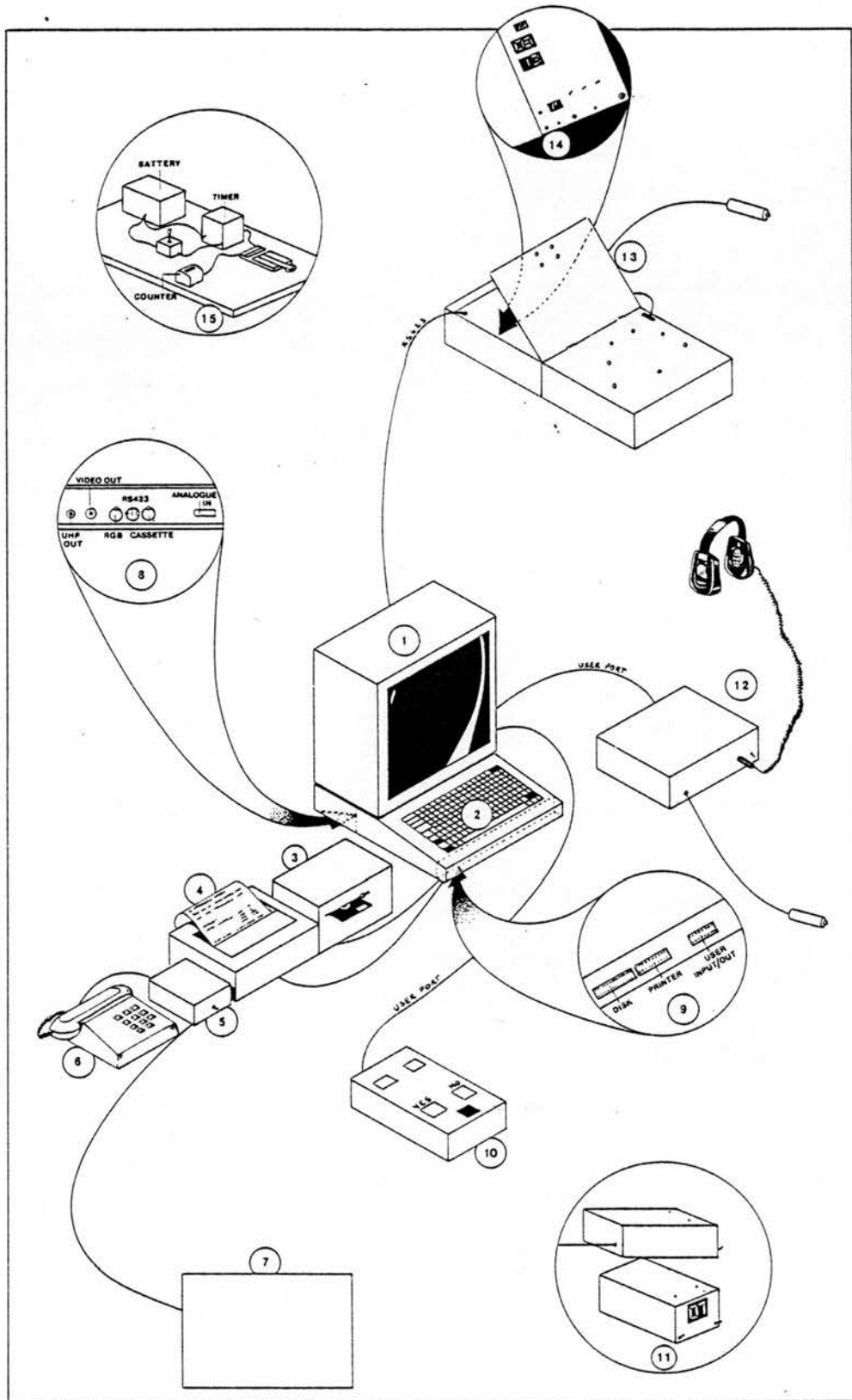


Figure 2.1. The test apparatus (not to scale). Key: 1 - CUB colour monitor, 2 - BBC microcomputer, 3 - disk drive, 4 - printer, 5 - modem, 6 - telephone, 7 - mainframe computer (for downloading data and data analysis), 8 - BBC microcomputer rear connections, 9 - BBC microcomputer underside connections, 10 - response box, 11 - sway meter, 12 - auditory vigilance box, 13 - Leeds Psychomotor Tester, 14 - Leeds Tester detail, 15 - tapping apparatus.

Body sway

This was measured in the upright position using a Wright-Codoc ataxiometer, a modification of the mechanical ataxiometer described by Wright (1971). The apparatus measures sway in the anterior-posterior plane. The meter consists of a photoelectric cell separated from a light source by a transparent disc. The disc is calibrated with opaque markings at fixed intervals. The swaymeter is placed on a bench or table and is attached at a standard distance by a thread and clip to the subject's waist. As the thread moves it rotates the disc. Movements of the disc in either direction causes the interruption of light to the photoelectric cell. The interruptions are summed on a cascade counter for one minute and the total is read from a digital display.

The posture adopted is rather like that of a soldier "at ease". The feet are placed comfortably, shoes off, a slight distance apart, and the arms are at rest at the sides. Subjects are instructed to fix their gaze straight ahead and to stand as still as possible while the measurements are recorded. Two measurements over one minute each are made at each test run.

This method was chosen for its reasonably good sensitivity, ease of use, lack of discomfort or interference with the subject's performance and low cost. Changes in postural stability after a variety of central depressant drugs have been detected using this ataxiometer (for review see Swift, 1984).

Choice reaction time

There are many techniques for measuring reaction times utilizing auditory and/or visual cues. One convenient method for measuring "choice" or complex reaction time to a visual stimulus is the Leeds Psychomotor tester (Hindmarch and Parrot, 1978).

In the Leeds tester the subject has to scan a horizontal board containing a "home" button on which the forefinger of the dominant hand is rested. A series of "target" buttons are arranged about the arc of a circle equidistant from the home button. The buttons are touch-sensitive. Associated with each button is a small light emitting diode. The lights come on one at a time at random. The subject's task is to move the forefinger as quickly as possible to the appropriate target button whenever the light associated with it comes on. The finger is moved back to the home button and the subject waits for the next stimulus.

Three components of reaction-time are measured; the time to leave the home button from the light coming on (the stimulus latency), the total time to react from the stimulus onset to arrival at the target and by subtracting these the motor movement time.

In the present series of experiments the Leeds tester was placed on a table while the subjects sat on a comfortable chair adjusted, if necessary, to an appropriate height for unrestricted arm movement. The chair was then used at this height for the rest of the session. A minimum of 10 and a maximum of 30 reaction times were used throughout.

Choice reaction time measured using the Leeds tester has been shown to be sensitive to a variety of centrally acting drugs (for review see Hindmarch, 1980).

Critical flicker frequency

This was also measured using the Leeds psychomotor tester.

In this test the subject views four light emitting diodes arranged vertically in a square in foveal fixation at a

distance of 1 metre. The diodes are programmed to flicker with square wave pulses starting at 10 hertz (Hz), and increasing in frequency up to a maximum of 50 Hz. The subject presses a button when the light no longer appears to be flickering. Once the button has been pressed the system resets and the process happens in reverse. With the descending frequency, the subject responds when the light appears to start flickering. Two to 6 pairs of flicker frequencies were used throughout according to the psychophysical 'method of limits' (Ott et al., 1982).

Critical flicker frequency has been shown to be sensitive to the effects of many psychotropic drugs (for reviews see Smith and Misiak, 1976; Hindmarch, 1980; Bobon et al., 1982 and Curran, 1990).

Decision making time

This is a computerised reaction time test (Tiplady, 1985). The subject holds a box with 5 buttons on it. One is a 'home' button, and the nearest two buttons equidistant to the home button are marked YES (left button) and NO (right button). The set up is as shown in Figure 2.2. Pictures of animals or objects appear one at a time on the monitor screen, and the subject responds by pressing the appropriate button as quickly as possible in answer to the question 'Is it an animal?'. Total, latency and motor times are recorded to the nearest 10 msec. There are 5 different sets, each having 32 pictures (16 animals, 16 objects). Two of the pictures presented in decision making time are shown in Figure 2.2. This test has been shown to be sensitive to phenytoin (Thompson et al., 1981).

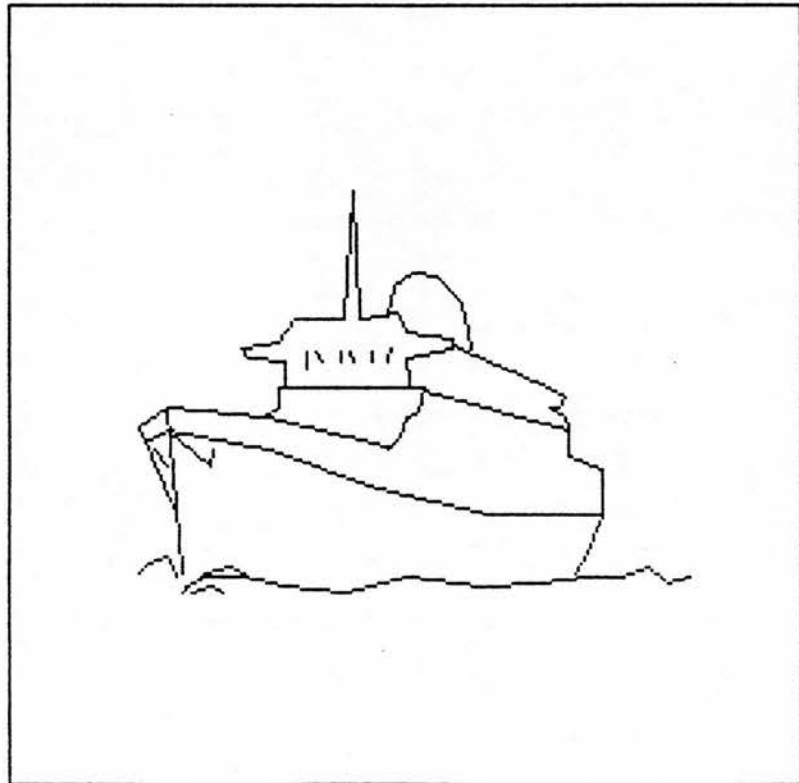
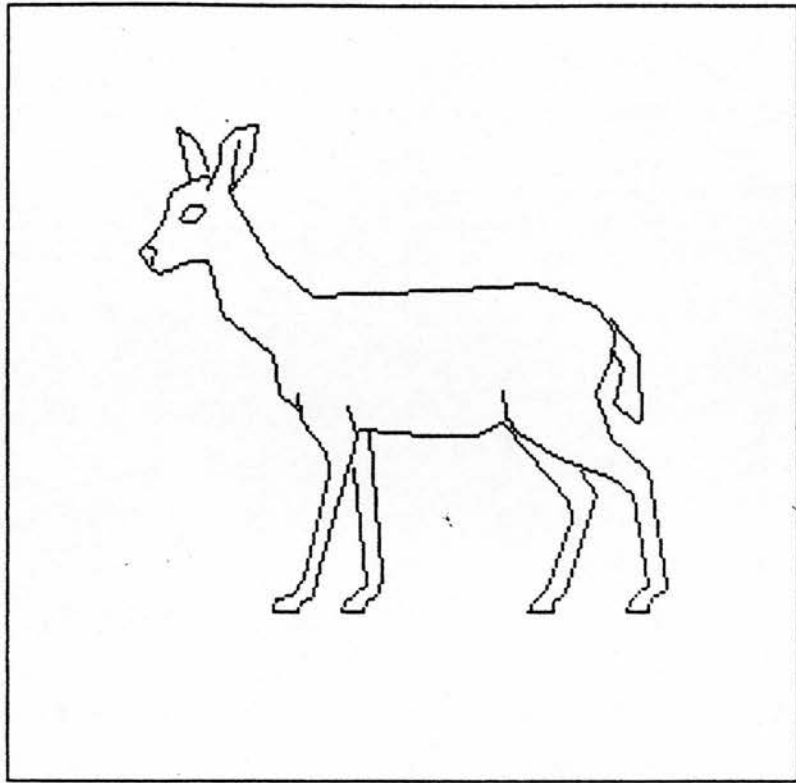


Figure 2.2. Examples of decision making time pictures as presented on the microcomputer screen (not actual size).

Continuous attention

Continuous attention also comes from the Tiplady battery (Tiplady, 1988). This test is based, in part, on a continuous performance test described by Rosvold et al., (1956). The major difference between these tests concerns the type of stimuli presented. In the Rosvold test the stimuli are letters or sequences of letters, whereas in the Tiplady test the stimuli are abstract shapes (Figure 2.3).

In the continuous attention test the subject is briefly presented with a series of random block patterns, each of 0.1 seconds in duration, on the monitor screen at intervals of 1.5-2.5 seconds. Every so often two consecutive stimuli are identical. It is the task of the subject to identify these duplicates and to respond by pressing a button whenever they occur. The responses are not timed, but the number of correct and incorrect responses are recorded. The total error score (omissions plus commissions) is then computed. The sets are randomly generated each time. The task typically involves 240 stimuli of which 40 are test signals. This takes 8 minutes to perform.

The continuous attention test has been shown to be sensitive to mianserin (Swift et al., 1988) and to chlormethiazole (Fagan et al., 1990).

Digit symbol substitution test

This is a well-established paper and pencil test which was part of the original United States Army Performance Tests at the beginning of the century and became part of the Wechsler Adult Intelligence Scale (Wechsler, 1944). The subject is given a 'key' consisting of a grid of digits (1-9) with a symbol below each number (Figure 2.4). Below this is a grid, with random digits (1-9) but no symbols.

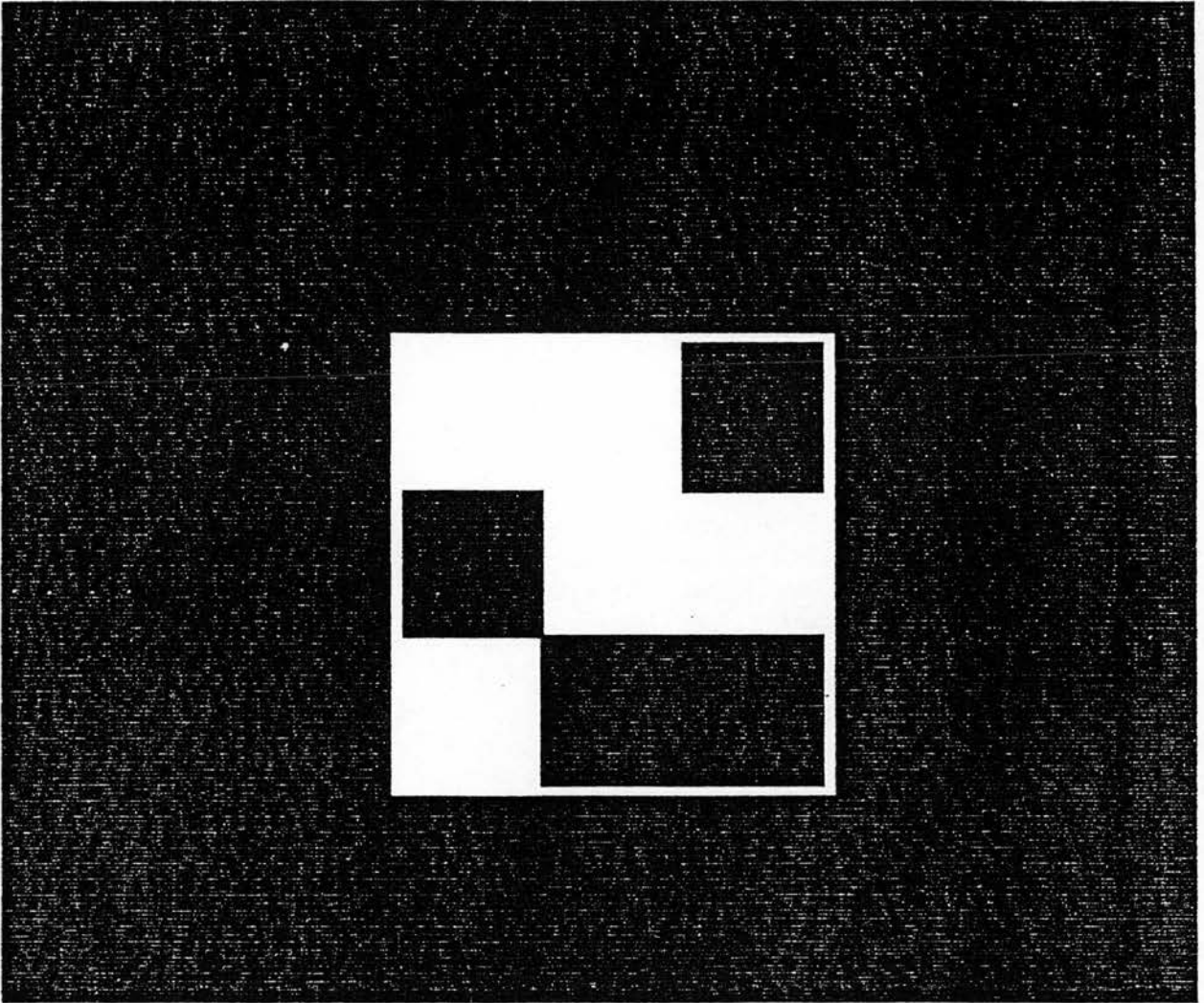


Figure 2.3. Typical continuous attention test pattern.

D.S.S.T.

1	2	3	4	5	6	7	8	9
×	I	∧	○	∩	≡	▽	∪	□

Time

Practice

1	9	4	2	7	8	3	6	5	2	2	9	8	3	1	7	5	6	4	1

4	7	6	2	1	8	5	3	2	9	3	5	2	4	8	1	6	7	9	5

5	2	1	6	3	9	8	4	7	3	6	1	7	2	8	9	4	5	4	3

3	7	9	2	4	1	6	8	5	1	6	1	2	3	5	4	7	6	9	8

8	1	7	6	4	2	5	3	7	9	2	6	8	7	9	4	3	5	1	9

2	9	8	3	1	7	5	6	4	1	5	2	1	6	3	9	8	4	7	3

Figure 2.4. Typical digit symbol substitution test grid (reduced in size).

The subject has to copy the corresponding symbols from the key on to the grid as quickly as possible. The subject is given 90 seconds to do this after a practice of 10 symbols.

The Wechsler version of the digit symbol test had a total of 90 items, whereas the present set has 110. The symbols used in the present experiments were also different. The symbol presentations were balanced over the grid in a pseudo-random fashion. 9 different test forms were used. 5 practice runs were always performed prior to subjects taking part in experiments.

This test has been shown to be sensitive to the residual effects of hypnotic drugs (e.g. Peck et al., 1975a), to beta-blockers (McDevitt, 1985), to antihistamines (Peck et al., 1975b), to antidepressants (Bye et al., 1978) and to the acute effects of a variety of other central depressants (Hindmarch, 1980; Wittenborn, 1987).

Gibson spiral maze

Gibson's spiral maze is a psychomotor test of speed and accuracy (Gibson, 1967). It is a paper and pencil test. The subject starts at the centre of a maze printed on a card (Figure 2.5), and draws round the path to the end of the maze. The subject has to complete the task as quickly and as accurately as possible without lifting the pen and without touching the walls of the maze or the obstacles (circles) in the path. The time taken to complete the maze, and a total error score based on the number and extent of collisions are recorded (touch = 1, overlap = 2, each inch of touch = 1, each inch of overlap = 2). Two mazes are completed at each test run.

Because of the known liability to practice effects on repeated administration over a short period of time (Hindmarch, 1980) a minimum of 10 practice runs were performed by each subject before entering into any studies.

THE GIBSON SPIRAL MAZE

Copyright © H. B. Gibson 1961

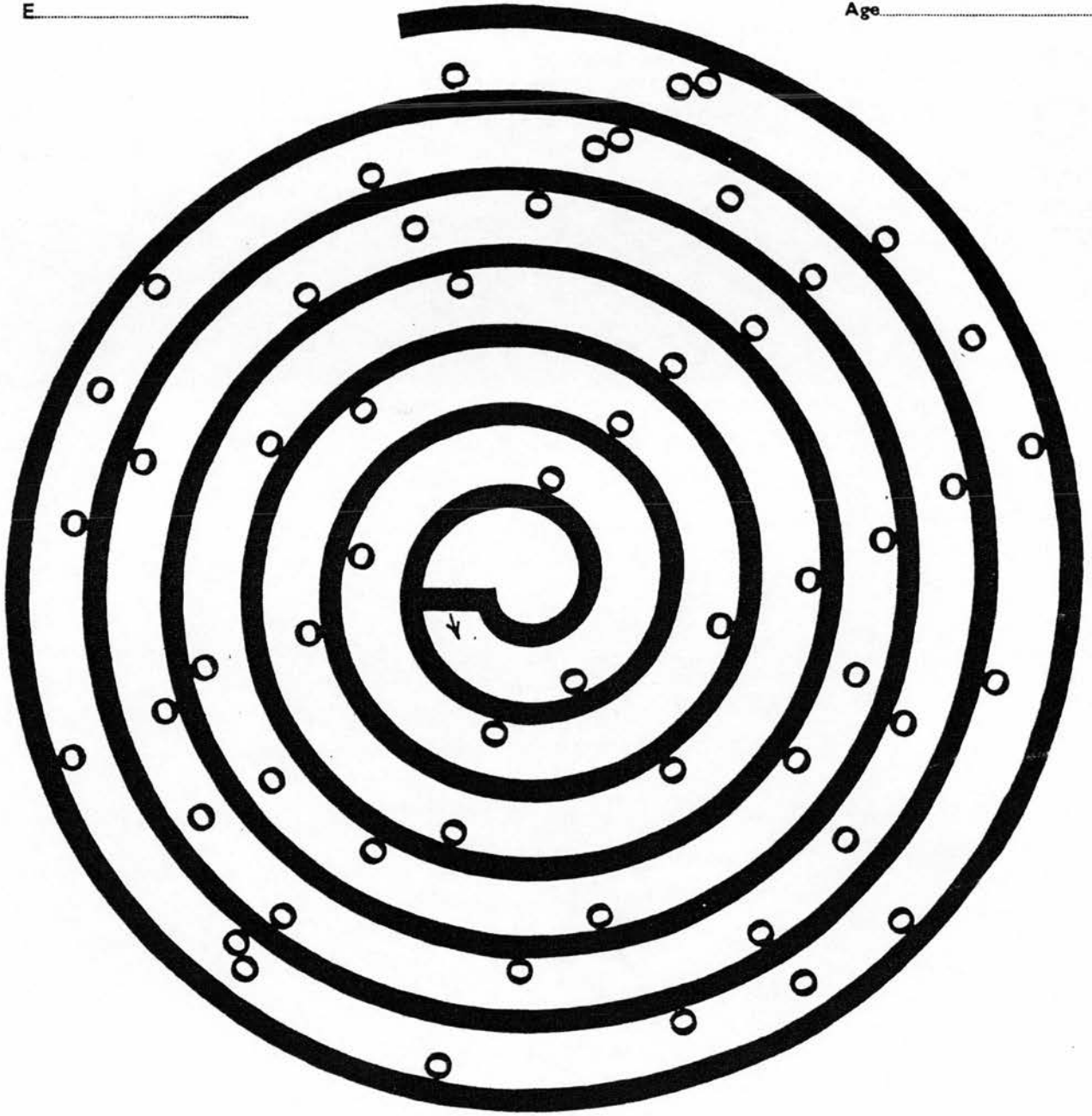
Eighth impression 1984

T.....

Name.....

E.....

Age.....



HODDER AND STOUGHTON, Mill Road, Dunton Green, Sevenoaks, Kent TN13 2YD

Figure 2.5. Gibson's spiral maze (reduced in size).

The Gibson spiral maze has been used to assess the effects of anticholinergic drugs (Anderson et al., 1985) and has been shown to be sensitive to central nervous system depressants (Zimmerman Tansella et al., 1979; Morgan, 1985; Fagan et al., 1990).

Paired word association

Paired word association forms a component part of the Wechsler memory scale (Wechsler, 1945). The tester reads aloud pairs of associated words. The subject has to reply with the correct word of the pair when one of the words is read aloud at random.

The computerised version of paired word association from the Tiplady battery, based on Isaacs and Walkey's modifications (1964) was used. In this test there are 3 groups of 3 word pairs, each consecutive group having less association, and therefore being progressively more difficult. The subject reads the word pairs from the computer screen for three seconds each. The subject has to respond verbally, within 15 seconds, with the second word of the pair when one of the words appears on its own on the computer screen. Each stimulus word is presented 3 times, in random order. After each reply the subject is shown the word pair again (2 seconds for a correct reply, 4 seconds otherwise) to enable learning. Set 1 of paired word association is shown in Figure 2.6. Correct, incorrect and null responses are recorded. The maximum possible correct score and the maximum error score is 27.

As there were too few word pairs in the battery originally, the number of sets of word pairs was extended. The words were chosen using the 'Norms of Word Association' (Postman and Keppel, 1970). and the 'Teachers book of 30,000 words' (Thorndike and Lorge, 1944).

writer-book

forest-tree

soldier-army

bed-pillow

eagle-nest

silver-brass

cabbage-pen

knife-chimney

sponge-trumpet

Figure 2.6. Paired word association set.

All words had frequencies of at least 50 occurrences per million words. Pairs of words in level 1 were "first associations", in level 2 associated, but not the first association, and at level 3 had no recorded association. 12 sets were compiled in this way, making a total of 16 sets. Paired word association has been shown to be sensitive to the effects of clonidine (Frith et al., 1985), to chlormethiazole (Fagan et al., 1990) and to nitrous oxide (Biersner, 1972).

Visual vigilance

This is also in the Tiplady battery. A random pattern resembling a snowstorm is generated on the monitor. Every so often a dark square slowly appears in one of the 4 quadrants of the screen (Figure 2.7). The subject holds the same box as is used in the decision making time test (Figure 2.1). Four of the buttons are arranged in a square. The subject presses the appropriate button according to the quadrant in which the space is clearing. The test takes 5 minutes to perform. Correct and incorrect scores are recorded.

Tapping

There are several techniques for looking at tapping rate (e.g. Korttila et al., 1981; Peck et al., 1975a; McClelland, 1987). The present apparatus involves using a morse key attached to a timer and digital counter. The subject starts to tap as fast as possible, and the timer is started. The subject continues to tap until the timer stops (after 1 minute). The score is recorded from the digital display at that point.

Tapping has been shown to detect the effects of anaesthetics (Korttila et al., 1981), hypnotics (Peck et al., 1975a; Zimmerman Tansella et al., 1979), sympathomimetics (Bye et al., 1974) and stimulants (Fagan et al., 1988; Tiplady et al., 1990).

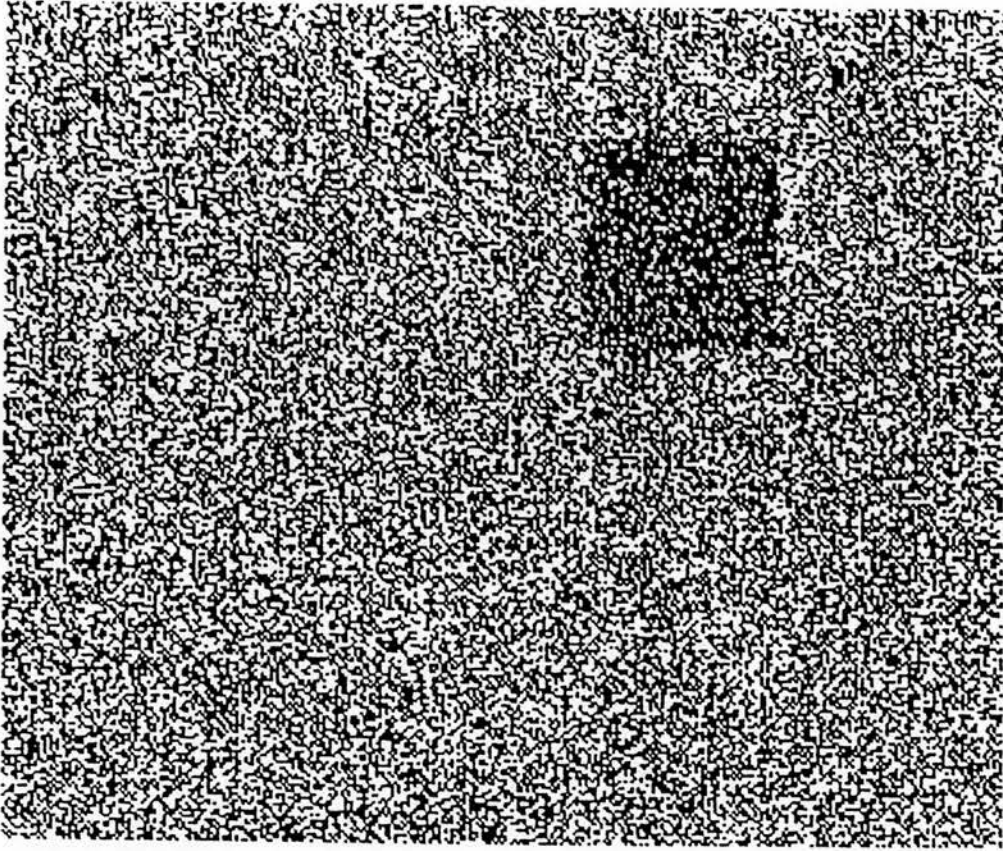


Figure 2.7. Typical visual vigilance display.

RATING SCALES

Visual analogue scales

Visual analogue scales have been used to collect subjective information since the early twenties (Hayes and Patterson, 1921), and have been reviewed many times since (e.g. Aitken, 1969; Bond and Lader, 1974; Lundberg, 1980). The scales used in the present experiments were bipolar, horizontal, 100 mm lines with semantic opposites at either end. Several scales were on a single page, and the directions of the scales were randomised. There were no normality lines. Some of the scales were taken from those of Norris (1971). A set of visual analogue scales used in one of these studies (Chlormethiazole experiment, Chapter 5) is shown in Figure 2.8.

Visual analogue scales have been used in very many studies, and have been shown to be sensitive to the effects of a wide variety of drugs including stimulants (e.g. Peck et al., 1979; Fagan et al., 1988) hypotensives (Ashton and Rawlings, 1978) and depressants (e.g. Bond and Lader, 1972; Parrot and Hindmarch 1978; Carrington and Hindmarch, 1980; Wesnes et al., 1988).

Severity rating scale for side effects

The easiest and probably most commonly employed method of collecting data on the type, number and severity of side-effects is to write down the symptom, time of observation and, if possible, the severity as well. The severity rating scale most often employed is "mild", "moderate" and "severe".

In an attempt to increase the sensitivity and adopt a more rigorous approach to the collection of this 'soft' data, the number of points on the severity scale was increased to eight, and the progress of the symptoms

Please rate the way you feel at the moment by marking clearly across each of the lines below.

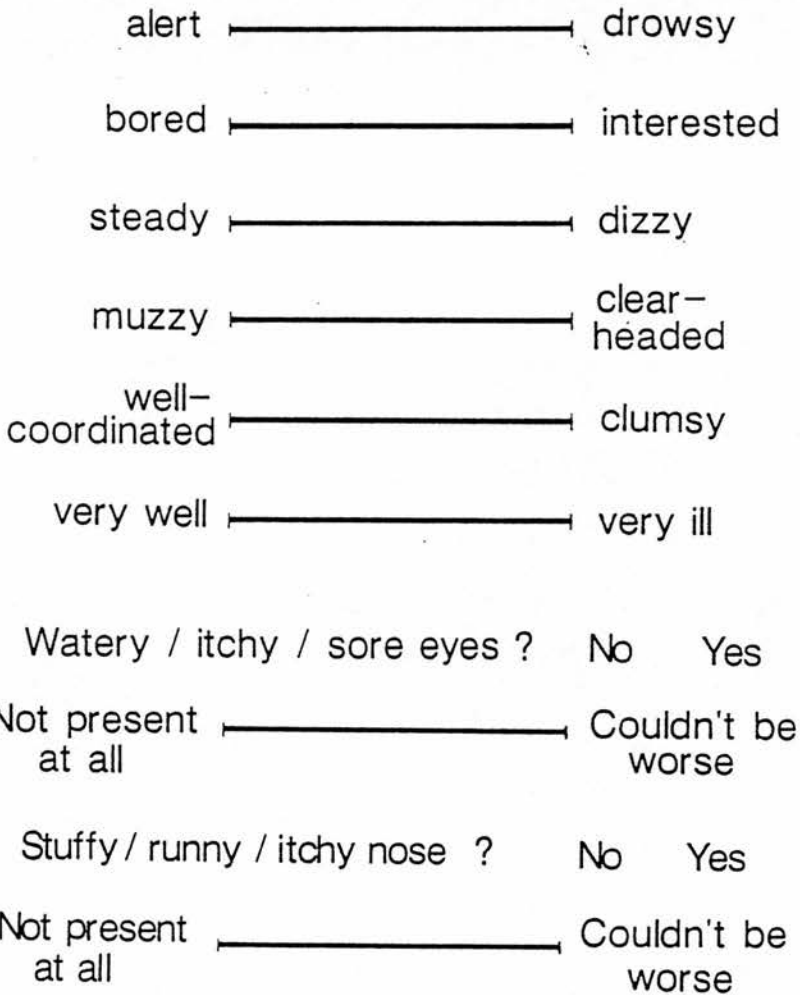


Figure 2.8. Visual analogue scales used in the chlormethiazole study (Chapter 5). The figure above is approximately 0.45X the actual size. The scales were 100 mm long.

'charted' at regular intervals until they disappeared. An example of a side-effects chart is shown in Figure 2.9. This form was used in the chlormethiazole acute tolerance study (Chapter 5) and the nitrous oxide acute tolerance study (Chapter 7).

ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Anaesthetics Sub-Committee of the Lothian Health Board Ethics of Medical Research Committee before each study commenced. Written informed consent was obtained, and all studies were performed in accordance with the Declaration of Helsinki.

EXPERIMENTAL SUBJECTS

Subject recruitment

All subjects were volunteers. Information sheets describing the nature and purpose of the experiments, the likely side-effects and discomforts, a consent form and the instructions for volunteering were circulated amongst staff of several departments at the Royal Infirmary of Edinburgh and at the Astra Clinical Research Unit in Edinburgh. Not all volunteers were, however, staff. In many cases the circulars were passed on to friends and relatives who frequently passed them on again. All subjects were interviewed by the experimenter before they participated in any study.

Several individuals expressed a desire to be circulated with information on a regular basis, and these subjects formed a "pool" of volunteers who took part in more than one investigation. No subject was allowed to take part in more than one experiment at a time, and a gap of at least two weeks was required between participation in different experiments. In general, subjects were not tested on more than one day a week during an experiment.

Subject No: _____

Initials: _____

Date: _____

SEVERITY RATING SCALE

- 0 - absent
- 1 - minimal
- 2 - mild
- 3 - mild to moderate
- 4 - moderate
- 5 - moderate to severe
- 6 - severe
- 7 - very severe

TIME (min)

SYMPTOMS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Numb tongue 2 ¹⁰	0	0	0	2	2	2	2	2	2	2	2	2	2	1	1	1	0									
Tinnitus 2 ⁴⁵			0	2	2	4	4	4	4	4	4	4	4	2	2	2	1	1	1	1	1	1	1	1	1	1
Lightheaded 3 ¹⁵				0	2	4	4	4	4	4	4	4	4	4	4	4	3	1	1	1	1	1	1	1	1	1
"Distant" 6 ⁰⁰						0	2	2	2	2	2	2	2	2	2	2	0									
Warm legs 7 ⁰⁰							0	1	1	1	1	1	1	0	0											
Nystagmus 8 ⁰⁰								0	2	2	2	2	2	0												
Tingling thighs 8 ⁴⁵									0	2	2	2	2	1	0											
Tongue cold 9 ⁰⁰									0	1	1	1	1	0												
Tingling hands 9 ⁴⁵										0	2	2	2	1	0											
Ache in chest 10 ⁰⁰										0	1	1	1	0												
"Twitchy" 11 ⁰⁰											0	2	0													
Dysarthria 11 ⁰⁰											0	1	0													
Legs "cool" 11 ⁰⁰											0	4	3	0												

Figure 2.9. Severity rating scale for signs and symptoms. Once onset of a sign or symptom has been recorded, the subject is questioned at regular intervals to plot the course and severity. The above data was taken from an experiment that we performed with the local anaesthetic drug ropivacaine (Scott et al., 1989).

Subjects were informed that they could withdraw from the study at any time with no "hard feelings". Latterly, the name and telephone number of a doctor who was not involved in the studies was also included on the information sheets, so that the subjects could obtain independent medical advice at any time before, during or after a study.

Although there was no mention of payment on the information sheets in the drug studies, the subjects were paid for taking part. This amount was the same for all participants who completed or attempted to complete a study although payment differed from study to study depending on the time involved. Additional expenses such as taxi fares and lunches were also paid.

Subject selection

All subjects who took part in the drug studies were screened for medical fitness after having a full history taken. Blood samples were drawn for clinical chemistry and haematology, and electrocardiograms were recorded. The results of these tests had to be clinically normal before subjects were allowed to participate. Subjects could only take part if they were in the normal weight range for their height. Heavy drinkers, heavy smokers and individuals who were unable to do without alcohol, cigarettes, tea and coffee for the duration of the experiment were excluded.

Pregnant women, lactating women and women who were taking oral contraceptives were excluded, as were women who were unwilling to take reasonable precautions against pregnancy. Women were asked not to participate during their premenstrual phase.

Subjects who seemed to be unusually anxious about the procedures involved in the experiments were excluded.

Demographic data

Data from 95 healthy volunteers is presented in these experiments. Forty-five of the subjects, 31 males and 14 females aged between 19 and 39, formed the main pool of volunteers who took part in one or more psychomotor experiments involving drugs and placebos. The demographic details for these subjects are shown in Table 2.1. A further fifty healthy male and female subjects aged between 18 and 30 took part in a normative (i.e. control standard) data experiment in which no drugs or placebos were involved (Chapter 3). Demographic data from these subjects is shown in Table 2.2.

Of the 45 subjects in the main pool of volunteers only one was left handed. In the normative data experiment handedness was not recorded.

EXPERIMENTAL CONDITIONS

Test administration

Experiments were carried out in the Anaesthetics Department at the Royal Infirmary of Edinburgh. Subjects were instructed to have an early night before test sessions. They were not allowed to consume caffeine-containing substances or alcohol, or smoke or take cigarettes or other drugs from the evening before till the end of each test day. Food was not permitted for 4 hours before or after drug intake except when the experiment *lasted for more than 4 hours when* light meals were allowed. All venous cannulations were performed before testing started, and the antecubital vein of the non-dominant arm was used for sampling. In the chlormethiazole steady-state experiment a forearm vein of the dominant arm was used for infusing the drug. Subjects were allowed to settle for several minutes before testing began.

Subject	Sex	Age	Height (cm)	Weight (kg)	Smoker	Dominant hand	Chapter 3 Reliability Between Within	Chapt.5 Chlorme-thiazole	Chapt.6 Ethanol Pharma-cokin.	Chapt.6 Ethanol dose ranging	Chapt.6 Ethanol steady state	Chapt.7 N ₂ O steady state	Chap.7 N ₂ O crit. flick.
1	F	32	170	57	N	R	*						
2	M	19	183	76	N	R	*						
3	F	31	152	46	N	R	*						
4	F	29	153	51	N	R	*						
5	F	26	160	56	N	R	*						
6	M	21	180	77	Y	R	*						*
7	F	26	152	47	N	R	*	*					
8	M	28	185	76	Y	R	*						
9	M	39	188	75	N	R	*						
10	M	19	183	76	-	R	*						
11	M	20	175	71	-	R	*	*					
12	F	26	153	54	N	R	*						
13	F	32	168	56	N	R	*						
14	F	28	160	64	N	R	*						
15	F	33	175	65	N	R	*						
16	M	29	178	83	Y	R	*	*	*	*	*	*	*
17	M	33	183	81	Y	R	*	*	*	*	*	*	*
18	M	32	175	84	N	R	*	*	*	*	*	*	*
19	M	27	208	73	Y	R	*	*	*	*	*	*	*
20	M	19	178	76	N	R	*	*	*	*	*	*	*
21	M	23	189	82	Y	R	*	*	*	*	*	*	*
22	M	23	175	69	Y	R	*	*	*	*	*	*	*
23	M	23	177	60	N	R	*	*	*	*	*	*	*
24	M	22	183	82	Y	R	*	*	*	*	*	*	*
25	M	27	183	67	N	R	*	*	*	*	*	*	*
26	M	21	183	109	N	R	*	*	*	*	*	*	*
27	M	22	185	87	N	R	*	*	*	*	*	*	*
28	M	21	183	80	Y	R	*	*	*	*	*	*	*
29	M	21	183	83	N	R	*	*	*	*	*	*	*
30	M	23	179	77	N	R	*	*	*	*	*	*	*

Table 2.1. Demographic data from the pool of healthy volunteers who participated in the experiments in this thesis. The individual studies in which the subjects participated are marked with an asterisk. (-) = unknown. Continued/



/Continued

Subject	Sex	Age	Height (cm)	Weight (kg)	Smoker	Dominant hand	Chapter 3 Reliability Between Within	Chapt.5 Chlorme-thiazole	Chapt.6 Ethanol Pharna-cokin.	Chapt.6 Ethanol dose ranging	Chapt.6 Ethanol steady state	Chapt.7 N ₂ O steady state	Chap.7 N ₂ O crit. flick.
31	M	26	186	69	Y	R	*		*	*	*	*	*
32	M	30	178	73	N	R	*		*	*	*	*	*
33	M	22	180	79	N	R	*		*	*	*	*	*
34	M	20	183	66	Y	R	*	*	*	*	*	*	*
35	M	31	173	69	N	R	*		*	*	*	*	*
36	F	24	165	50	N	R	*		*	*	*	*	*
37	F	27	169	64	N	R	*		*	*	*	*	*
38	M	22	189	74	Y	-	*		*	*	*	*	*
39	F	38	166	62	N	L	*		*	*	*	*	*
40	M	33	188	98	Y	R	*		*	*	*	*	*
41	F	33	158	52	N	R	*		*	*	*	*	*
42	M	31	172	65	N	R	*		*	*	*	*	*
43	M	28	173	67	N	R	*		*	*	*	*	*
44	F	24	160	54	N	R	*		*	*	*	*	*
45	M	30	175	80	N	R	*		*	*	*	*	*

Table 2.1. (Continued). Demographic data from the pool of healthy volunteers who participated in the experiments in this thesis. The individual studies in which the subjects participated are marked with an asterisk. (-) = unknown.

Subject	Sex	Age	Ht. (cm)	Wt. (kg)
1	F	28	167	57
2	F	27	173	54
3	M	22	187	79
4	F	27	157	54
5	F	25	165	40
6	F	20	157	53
7	F	24	170	60
8	F	23	165	47
9	M	19	183	66
10	M	26	178	64
11	M	19	180	60
12	M	23	179	85
13	F	21	170	57
14	F	21	168	54
15	M	26	186	72
16	F	20	175	66
17	F	19	173	51
18	F	23	166	56
19	M	23	173	89
20	F	22	155	51
21	M	29	174	78
22	M	24	173	63
23	F	19	150	53
24	F	21	165	56
25	M	22	176	57
26	M	22	188	76
27	F	22	165	56
28	M	26	175	66
29	M	22	167	67
30	M	30	168	66
31	F	26	160	60
32	F	20	152	51
33	F	19	167	54
34	F	22	158	62
35	M	28	173	67
36	M	23	178	78
37	F	19	169	57
38	F	19	155	60
39	M	23	181	70
40	M	19	186	83
41	M	20	179	82
42	F	28	151	49
43	F	26	163	51
44	M	22	180	83
45	M	30	179	89
46	M	27	175	70
47	F	25	155	54
48	F	23	151	57
49	M	30	176	71
50	M	22	175	82
Mean		23	170	64
s.d.		3	10	12

Table 2.2. Demographic data of the subjects participating in the normative data study. 24 males and 26 females took part.

Instructions were given only during the familiarisation sessions or if the subject forgot how to do a test, which was infrequent. The subject was, however, always told which test he/she was about to do. For convenience, the order of performance of the tests was not counterbalanced except in the nitrous oxide experiment. The test sets were, however, randomised. The presentation of computerised tests decision making time, continuous attention, paired word association and visual vigilance were automatically randomised by the computer. Choice reaction time presentations were randomised by the Leeds psychomotor tester. Digit symbol substitution sets were counterbalanced. Decision making time and paired word sets were administered in the same order except in the normative experiment (Chapter 3) and one of the nitrous oxide experiments (Chapter 7), where the sets were administered in a partially counterbalanced fashion.

Environment

In order to avoid any effects of environment on arousal and performance, the studies were, as far as possible, performed under constant conditions. The same observer, environment, room and equipment were used throughout. The attitude of the observer was pleasant without being overtly friendly. The lighting and temperature were controlled as far as possible and testing was carried out at the same time each day for each individual, usually starting in the morning. Subjects did not leave the testing area except to go the toilet, and were encouraged to read quietly between test runs. Noise and distractions were kept to a minimum. The subjects were not allowed to see their test score results.

CHOICE OF TESTS

The full battery of tests described earlier in this chapter takes a considerable time to perform. Thus it was not possible to use all of the tests at all of the

time intervals in the experiments. The tests were selected for the experiments according to the perceived need for the experiment. In the steady-state experiments (Chapters 5, 6 and 7) all of the tests in the battery were used predrug and after steady-state was achieved.

In order to evaluate drug-induced changes taking place over a short period of time in the experiments described in Chapters 5 and 6, it was found necessary to use short batteries of tests of brief duration to allow frequent repeated testing.

It should be noted that the battery of tests expanded considerably during the course of this work, and so the amount of data collected on the individual tests differs.

STATISTICAL METHODS

The descriptive statistics used in this work were:

- the mean,
- standard deviation,
- median,
- range,
- Z-score,
- % change,
- slope gradient,
- coefficient of variation,
- coefficient of skewness ($\sqrt{B_1}$)

The comparative statistics were as follows:

- reliability
- intraclass correlation coefficients,
- modified Kendall's coefficient of concordance (\bar{F}_2),

Two way analysis of variance with F test,
Friedman non parametric two way analysis of
variance,
Page's L trend test,
Wilcoxon signed rank test,
Spearman rank correlation

Since not all of these statistics are standard, the less common ones will be described below.

Z score

The Z score is a statistic used to standardise scores from different tests to a common scale (see Cronbach, 1970). There are several methods of doing this (Isaac and Michael, 1981). The Z score uses the variability of the baseline score to allow changes to be estimated. Generally speaking, Z scores are used to compare e.g. IQ or neuropsychological subtest scores from individuals with those from the general population. This allows the pattern of individual skills to be assessed. This would seem to be a useful approach for assessing different patterns of impairments after drugs.

The sensitivities of different tests were compared at steady state constant plasma concentrations using this method (Chapters 5, 6 and 7).

The Z score is calculated from:

$$Z = \frac{\text{mean}_2 - \text{mean}_1}{\text{s.d.}_1}$$

where mean_1 = initial mean score for the group,
 mean_2 = final mean score for the group and
 s.d._1 = standard deviation of the first mean.

Reliability statistics

Reliability concerns the accuracy, consistency and stability of measures (Isaac and Michael, 1981).

Statistics appropriate for the study of reliability have been reviewed several times (Bartko and Carpenter, 1976; Kraemer and Korner, 1976; Shrout and Fleiss, 1979 and Lahey et al., 1983). All of these reviewing authors recommended the use of the intraclass correlation coefficient, p (Haggard, 1958), for multiple retests of quantitative data. This can be calculated in a number of ways. Bartko and Carpenter (1976) suggested that the intraclass correlation coefficient be computed via the one-way analysis of variance which provides an expression for the variance between and within subjects. These variance components are used to form the measures of reliability. Shrout and Fleiss (1979) and Lahey et al., (1983) recommended the two-way analysis of variance as a better method of estimating the intraclass correlation coefficient since it also takes the between time (or session) variance into account. However, for nonnormal or nonstandard normal data with multiple retests, Kraemer and Korner (1976) advised the use of either Kendall's coefficient of concordance, W , or \bar{r}_s (a statistic closely related to W) as an estimator of p . They also advised that only \bar{r}_s be used when the number of retests is small.

Since it was likely that the data contained both normally distributed and skewed measures, p was calculated from the 2-way analysis of variance intraclass correlation coefficient as determined using the guidelines of Shrout and Fleiss (1979), and from \bar{r}_s according to Kraemer and Korner (1976). Thus the parametric and nonparametric estimates of reliability could be compared.

Parametric reliability measures

An estimate of ρ was obtained from the intraclass correlation coefficient using the two way analysis of variance. (Shrout and Fleiss, 1979). Model (3,1) was used. This coefficient of reliability also assesses the consistency of the measures, and is the parametric equivalent of \bar{r}_s . This is estimated by

$$ICC (3,1) = \frac{BMS - EMS}{BMS + (k-1)EMS}$$

where ICC = intraclass correlation coefficient,
BMS = between subjects mean square from the analysis of variance,
EMS = residual mean square and
k = number of retests.

The agreement or stability of the measures was also evaluated using the guidelines recommended by Shrout and Fleiss. Model (2,1) was used for this. This model also takes the between times variance into account and is estimated by

$$ICC (2,1) = \frac{BMS - EMS}{BMS + (k-1)EMS + k(JMS - EMS)/n}$$

where ICC = intraclass correlation coefficient,
JMS = between times mean square and
n = number of subjects.

The significance of the F ratio was tested for the 'between measures' variances.

Nonparametric reliability measures

Kendall's coefficient of concordance (Siegel, 1956), W , corrected for multiple (m) retests to the average internal rank correlation, \bar{r}_s , was used to measure the overall consistency of scoring for the repeat measurements (Quade, 1972, cited by Kraemer and Korner, 1976). \bar{r}_s indicates the consistency with which the subjects did the same thing. A score of 1 indicates complete concordance, or consistency, while a score of 0 indicates complete discordance. The statistic \bar{r}_s is related to W by the relationship :

$$\bar{r}_s = \frac{(mW - 1)}{(m - 1)} .$$

The significance of W was tested, the null hypothesis being that rankings of scores from different subjects are independent.

Analyses were performed on each test and visual analogue scale individually. Friedman analysis by ranks (Hollander and Wolfe, 1973) was performed on the repeat measurements in order to determine whether there were differences between any of the sets of repeat measurements obtained.

Page's L trend

Page's L Trend (Page, 1963) was used to test for monotonic order effects in the nitrous oxide dose response study. The L trend was used to test whether the effects significantly increased with dose.

SUMMARY

Psychomotor tests and subjective and objective assessments using rating scales were used as the methods of assessing drug effects. The tests used were drawn from a pool of psychomotor tests consisting of sway, choice reaction time, critical flicker frequency, decision making time, continuous attention, digit symbol substitution, Gibson's spiral maze, paired word association, visual vigilance and tapping. The subjective assessments were made using visual analogue scales and observer ratings using a severity rating scale. Because of the considerable length of time taken to perform the full battery of available tests, smaller batteries were used where necessary.

The experiments were ethically approved, and the experimental subjects were paid, informed, consenting healthy male and healthy non pregnant female volunteers aged 19-39. Recruitment was via information sheets circulated locally. Females did not participate during their premenstrual phase.

The test environment was a pleasant room in which the conditions were kept as constant as possible. The test administration was also standardised.

Standard parametric and nonparametric descriptive and comparative statistics were used to summarise the data in Chapter 3. Parametric measures such as mean and standard deviation were used throughout the body of this work, though the comparative statistics were mainly nonparametric.

CHAPTER3 - RELIABILITY OF THE TESTS IN THE BATTERY,
VARIABILITY AND ASSOCIATION OF THE TEST
SCORES

RELIABILITY, VARIABILITY AND ASSOCIATION OF TEST SCORES

"It is a basic assumption in science that when conditions are constant the experimental results must be the same."

(Festinger and Katz, 1953).

In Chapter 2 the tests used to assess psychomotor performance were considered. This Chapter now deals with the performance of the tests under controlled conditions.

The two critical planning points for dealing with reliability are at the start and the end of an experiment (Sheridan, 1976). Reliability can be assured at the start by anticipating and controlling for the sources of error so that they interfere as little as possible with the use of the measuring instrument. Thus meticulous experimental control should theoretically produce low "noise" levels. The means by which experimental control was attempted have been described in Chapter 2 (Experimental subjects and Experimental conditions). It is, however, impossible to ensure that all conditions are held constant on different occasions so that the experimental results are the same. The extent to which reliability of results has been achieved can be estimated at the end of the experiment by correlating scores from repeated performance of the tests by the same individuals. This can most appropriately be estimated using coefficients of reliability which are correlation coefficients for multiple retests (see Chapter 2 - Statistical Methods).

Test-retest reliability coefficients, if quoted at all, are usually calculated for only two sets of data recorded under less stressful conditions than those commonly encountered in drug studies. These reliabilities may therefore be overestimated.

The reliability of the psychomotor tests and the visual analogue scales, and the magnitude of their variability were therefore assessed between and within sessions using data collected under the full experimental conditions outlined in Chapter 2. The reliabilities were calculated using either 4 or 7 retests.

The digit symbol substitution test and the sway test were chosen for additional study because their short duration and reputed sensitivity made them particularly useful tests when rapid repeated testing was required (Chapters 5 and 6). The effects of practice and the reliabilities of these tests were therefore studied in a larger group of subjects under controlled conditions.

Few criteria need to be satisfied in order to correctly apply nonparametric statistics, and it was planned to use mainly nonparametric statistical methods throughout this work. However, although the criteria for using parametric statistics are more stringent, these reliability methods have the advantage of allowing coefficients of stability to be calculated as well as coefficients of consistency. As discussed in Chapter 2 (reliability statistics), nonparametric reliability methods only calculate consistency. In the event of large practice effects, the data might well demonstrate a high degree of consistency, but low stability. In a crossover design this would add substantially to the error variance. The data type and distributions were therefore assessed to see how well they met the criteria for parametric statistics, and the reliabilities were calculated using parametric and nonparametric methods.

Finally, the baseline scores for the tests were intercorrelated in order to assess the extent to which the tests measured, or were influenced by, the same underlying processes.

Drug studies with crossover designs (e.g. Chapters 5 and 6) often involve subjects being tested on different days. It is important to know the reliability of the 'baseline' results and the magnitude of the daily variations to determine whether large fluctuations in performance are likely to mask any real changes or to produce type I (false positive) errors. The decision can then be made as to whether more practices should be given and/or whether the results would be better analysed in terms of changes from baseline.

The data distributions were assessed, and the day to day variation in baseline test performance investigated.

Methods

Data from several ethically approved psychomotor studies carried out by the author and involving 28 healthy male and 13 healthy female consenting volunteers were pooled. The mean age of the subjects was 26 years (range 19-39 years) and the mean body weight was 71 kg (range 46-109 kg). For individual demographic data on these subjects see Table 2.1.

The studies were all double blind and involved the testing of drugs affecting the central nervous system. The analysis of reliability between sessions was performed on the first set of data obtained on each day from four consecutive experimental sessions after an initial familiarisation session with the test battery. The data used were all pre-drug or pre-placebo test scores obtained under the full experimental conditions described in Chapter 2 (Experimental Subjects and Experimental Conditions). Data distributions were analysed using the scores obtained on the first of the four days. Testing was performed at approximately 09:00 hours at intervals of at least one week.

In the event of there being less than 4 valid values for a particular variable, the subjects were excluded from the reliability analysis for that variable. Study designs varied, and different tests were sometimes used in different experiments. Since the test battery also expanded over the course of this work, the number of individuals valid for the analyses therefore varied for each test. All of the studies involved multiple testing in each session, and the subjects were tested between 22 and 28 times on the test battery between the first and fourth sessions.

The tests analysed were: critical flicker frequency, choice reaction time, continuous attention, body sway, digit symbol substitution, tapping, Gibson's spiral maze, decision making time, paired word association and visual vigilance. The visual analogue scales used were: alert-drowsy, steady-dizzy and bored-interested.

The results were described using the mean, standard deviation, range (maximum and minimum) and the coefficient of variation. The data were analysed nonparametrically using the Friedman two-way analysis of variance over days to test for session effects (Siegel, 1956). The probability values for Kendall's W and the nonparametric coefficient of reliability, \bar{r}_s , over the 4 retests were also calculated to test for concordance and consistency respectively (Siegel, 1956; Kraemer and Korner, 1976. See Chapter 2 for a more detailed discussion of these statistical methods).

The data distributions from session 1 were analysed for skewness using the coefficient of skewness, $\sqrt{b_1}$, according to the method recommended by Snedecor and Cochran (1980). When the number of subjects was sufficiently large (>25), $\sqrt{b_1}$ was tested for statistical significance.

The data from the 4 sessions were analysed parametrically using two way analysis of variance between times, and the parametric intraclass correlation coefficients of reliability for consistency and stability. (The details of these statistical methods are also described in Chapter 2.)

Results

The mean, standard deviation, range and coefficient of variation for each of the variables used in the between session data analysis are shown in Tables 3.1 to 3.3 along with the significance of the Friedman test and the reliability statistic, \bar{r}_s .

Nonparametric analysis

Three of the variables were statistically significant on the Friedman test indicating differences in baseline performance between the sessions. These were digit symbol substitution, tapping and the steady-dizzy visual analogue scale. Digit symbol substitution scores improved over the sessions, whilst tapping rate slowed. The effect on steady-dizzy appeared to be random. Several other variables showed non-significant trends in the direction of improvement; namely, total choice reaction time, choice reaction motor time, Gibson spiral maze time and decision making time. Choice reaction latency and the bored-interested visual analogue scale showed nonsignificant changes in the direction of slowing and boredom respectively over time.

All of the variables were significantly concordant on the Kendall test (P from $P < 0.001$ to $P < 0.05$), indicating that the rank order of subjects remained largely the same irrespective of group improvements or deterioration in performance. This suggests that there was a strong tendency for the performance of the subjects to behave in the same manner.

CHANGES IN SCORE WITH REPEATED TESTING OVER SESSIONS ON 4 DIFFERENT DAYS

TEST	N	SESSION				FRIEDMAN P	\bar{r}_s	
		1	2	3	4			
Critical flicker frequency (Hz)	30	Statistics						
		mean s.d. range CV	31.3 3.6 24.6-39.8 12	31.7 3.8 24.6-41.0 12	31.4 4.3 25.0-42.3 14	31.4 3.9 24.2-39.6 12	0.339	0.82
Total choice reaction time (msec)	30	mean s.d. range CV	478 68 329-638 14	466 64 323-634 14	470 65 352-639 14	463 74 354-688 16	0.330	0.76
		mean s.d. range CV	324 44 236-417 14	320 40 225-412 13	326 40 248-399 12	326 40 241-434 12	0.442	0.72
Choice reaction motor time (msec)	28	mean s.d. range CV	159 45 73-253 28	149 42 66-237 28	146 45 70-257 31	142 46 65-293 32	0.402	0.73
		mean s.d. range CV	11 5 4-25 45	11 5 4-22 45	10 4 4-20 40	11 5 5-25 45	0.804	0.65
Body sway (20' of arc)	30	mean s.d. range CV	75 9 67-100 12	78 9 72-103 12	79 9 73-106 11	80 8 71-99 10	0.023	0.39
		mean s.d. range CV						
Digit Symbol Substitution (no. correct)	11							

Table 3.1. Between session changes in test scores and summary of statistical analysis of test data. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried out on 4 mornings at approximately 09:00 hours. All data were pre-drug, pre-placebo control data.

CHANGES IN SCORE WITH REPEATED TESTING OVER SESSIONS ON 4 DIFFERENT DAYS

TEST	N	SESSION				FRIEDMAN P	\bar{r}_s	
		1	2	3	4			
Continuous attention (number correct)	16	Statistics	38 2 33-40 5	37 2 34-40 5	36 4 26-40 11	4 35 4 24-40 11	0.103	0.62
		mean	1	1	1	1		
		s.d. range CV	1 1 0-4 100	1 1 0-4 100	1 1 0-4 100	1 2 0-6 200		
Continuous attention (number false)	16	Statistics	3 2 0-8 67	4 2 0-8 50	5 4 0-14 80	6 5 0-17 83	0.652	0.31
		mean	573 84 415-685 15	566 88 351-710 16	550 98 343-700 18	548 101 334-700 18		
		s.d. range CV	26 2 22-27 8	26 1 25-27 4	26 1 23-27 4	27 1 25-27 4		
Continuous attention (total errors)	16	Statistics	14 2 10-16 14	14 2 12-17 14	14 2 11-18 14	14 2 11-17 14	0.211	0.55
		mean	19	19	19	19		
		s.d. range CV	12	12	12	12		
Decision making time (msec)	19	Statistics	26 2 22-27 8	26 1 25-27 4	26 1 23-27 4	27 1 25-27 4	0.424	0.86
		mean	12	12	12	12		
		s.d. range CV	11	11	11	11		
Paired word association (number correct)	12	Statistics	14 2 10-16 14	14 2 12-17 14	14 2 11-18 14	14 2 11-17 14	0.783	0.29
		mean	11	11	11	11		
		s.d. range CV	11	11	11	11		
Visual vigilance (number correct)	11	Statistics	14 2 10-16 14	14 2 12-17 14	14 2 11-18 14	14 2 11-17 14	0.839	0.65
		mean	11	11	11	11		
		s.d. range CV	11	11	11	11		

Table 3.2. Between session changes in test scores and summary of statistical analysis of test data. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried out on 4 mornings at approximately 09:00 hours. All data were pre-drug, pre-placebo control data.

CHANGES IN SCORE WITH REPEATED TESTING OVER SESSIONS ON 4 DIFFERENT DAYS

TEST / VISUAL ANALOGUE SCALE	N	SESSION				FRIEDMAN P	\bar{r}_s
		1	2	3	4		
Tapping rate (taps/min)	11	Statistics mean s.d. range CV 399 40 330-465 10	383 50 305-466 13	369 45 294-453 12	364 52 269-452 14	0.002	0.82
Gibson maze time (secs)	12	21.7 4.0 15.5-29.1 18	20.0 3.4 14.8-24.2 17	20.2 3.4 14.7-25.5 17	19.3 3.5 14.6-26.5 18	0.150	0.70
Gibson maze errors (number)	12	15 10 15.5-29.1 67	20 13 14.8-24.2 65	20 13 14.7-25.5 65	20 11 14.6-26.5 55	0.060	0.82
Alert-drowsy (+) (mm)	30	38 27 4-95 71	34 19 6-70 56	31 20 2-77 65	29 23 0-79 79	0.758	0.29
Steady-dizzy (+) (mm)	30	13 12 0-50 92	17 12 0-44 71	21 17 0-56 81	19 17 0-61 89	0.025	0.47
Bored-interested (+) (mm)	30	38 27 23-99 71	34 19 27-99 56	31 20 14-100 65	29 23 22-96 79	0.439	0.67

Table 3.3. Between session changes in test scores, visual analogue scales and summary of statistical analysis of test data. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried out on 4 mornings at approximately 09:00

Data type and skewness

The data obtained from the tests was numerical, continuous (or composed of scales with equal intervals) and by inspection, appeared to be fairly homogeneous.

The coefficients of skewness ranged from -1.649 for paired word association to 1.109 for the steady-dizzy visual analogue scale. Paired word and steady-dizzy were, however, considerably more skewed than the rest of the data. The least skewed distributions were for bored-interested, decision making time and critical flicker frequency being, -0.269, 0.296 and 0.318 respectively. Of those variables which could be tested for significance, total choice reaction time, choice reaction latency, sway, alert-drowsy and steady-dizzy visual analogue scales were significantly skewed to the right (+) and continuous attention was significantly skewed to the left (-, $P < 0.05$). Critical flicker frequency, motor reaction time, decision making time and the bored-interested visual analogue scale were not significantly skewed.

In general, the degree of skewness of the test data was considered to be relatively mild despite the statistical significances. Data transformations were therefore not performed since mildly skewed data may skew in the opposite direction after powerful transformations such as logs are performed. Moreover, the parametric tests used were considered robust enough to cope with the degrees of skewness found in the data. The parametric analyses were therefore performed on untransformed data.

Parametric analysis

The F ratios between times from the analysis of variance were statistically significant for choice reaction motor time, continuous attention correct scores, digit symbol substitution, Gibson spiral maze time, Gibson spiral

maze errors, tapping and steady-dizzy ($P < 0.05$). Choice reaction motor time, digit symbol substitution and Gibson spiral maze time showed changes in the direction of improvement over time, whilst continuous attention, tapping and Gibson spiral maze errors showed changes in the direction of deterioration of performance.

The smallest coefficients of variation were for paired word association (4%-8%), continuous attention correct (5%-11%), tapping rate and digit symbol substitution (both 10%-12%). However, critical flicker frequency, total choice reaction time and choice reaction latency also had coefficients of variation below 15%. The highest coefficients of variation were for continuous attention errors, Gibson maze errors and the visual analogue scales which all had values greater than 50%.

Reliability

The nonparametric reliability scores are shown in Tables 3.1. to 3.3. Decision making time had the highest consistency, the nonparametric reliability coefficient, \bar{r}_s being 0.86. Several other tests also had an \bar{r}_s of greater than 0.8 including critical flicker frequency, Gibson spiral maze errors and tapping. Most of the other reliability scores were > 0.5 . Those with $\bar{r}_s < 0.5$ were continuous attention false, digit symbol substitution, paired word association, alert-drowsy, and steady-dizzy.

The parametric results for consistency were very similar to the nonparametric results and are therefore not shown. In most cases the differences were in the second decimal place. The exception was for the digit symbol substitution test which had a much higher parametric consistency value (0.86 for the intraclass correlation coefficient for consistency, compared to 0.39 for \bar{r}_s).

The parametric reliabilities for consistency and stability were almost identical indicating that changes over time contributed little to unreliability in the

data. The largest difference was for tapping rate, the scores for consistency and stability being 0.75 and 0.69 respectively. Few of the scores showed differences greater than in the second decimal place.

Discussion

Under these experimental conditions several tests showed significant differences across sessions. This is probably attributable to practice for digit symbol substitution since the scores improved. Motor reaction time and Gibson spiral maze times became faster, though the error scores for Gibson spiral maze also increased. The explanation for this latter observation is therefore unlikely to be solely practice, but rather a shift in the speed/accuracy tradeoff with a movement towards speed at the expense of accuracy. (For a discussion of this topic see Fitts and Posner, 1973).

Interestingly, performance on the tapping test slowed over time, and errors of omission on the continuous attention test increased. The mean scores on the bored-interested visual analogue scale also moved towards the bored end of the scale. Eysenck (1967) demonstrated a relationship between arousal, motivation and the number of involuntary rest pauses on a tapping task; the more motivated the subjects the fewer the rest pauses (Eysenck and Eysenck, 1985). Loss of interest and of motivation may therefore explain these decrements and may also explain the lack of practice effects on these tests. Critical flicker frequency, which is said by the Eysencks and others to be a measure of arousal did not, however, diminish over the sessions. It was not clear whether this was because arousal was not involved in the performance decrements, or because the critical flicker frequency test was insufficiently sensitive to detect a relatively small change in arousal.

Some variables which showed trends with the nonparametric Friedman test were statistically significant on the parametric F test (choice reaction motor time, Gibson spiral maze time and decision making time). This indicated that the parametric tests were more powerful, as has often been stated (e.g. Siegel, 1956). However, there was little difference in the reliability scores between the two methods. The reliability coefficients for the day to day tests scores were high for most of the variables and, with the exception of digit symbol substitution, were very similar for both statistical methods. Continuous attention false, paired word association and two of the visual analogue scales, alert-drowsy and steady-dizzy, were the only variables which had reliabilities below 0.5 as assessed both parametrically and nonparametrically.

It was noteworthy that the bored-interested visual analogue scale gave quite high reliability scores. Bond and Lader (1974) stated that test-retest reliabilities were not relevant because the scales were "here and now" ratings of mood. However, this particular scale gave reliable retest scores even when the baseline scores changed significantly. All three visual analogue scales were significantly concordant demonstrating that at the times of testing the subjects' mood had tended to move in the same direction.

The other variables with low reliabilities were also significantly concordant, and had means and group standard deviations which altered little across the sessions. Thus the difference between the parametric reliability coefficients for consistency and stability were also small demonstrating that although there were significant changes over time, these changes contributed little as a source of variability. It therefore appears that analysing results in terms of changes from baseline to overcome large baseline differences in scores between sessions is probably unnecessary for these experiments.

RELIABILITY WITHIN SESSIONS

Many psychomotor studies, including those in Chapters 5 and 6, involve multiple repeated testing at different times during single experimental sessions. It is therefore important to know how reliable the tests are during a single experimental day in order to assess the contribution of time of day effects to drug effects.

Most of these study designs involving centrally acting drugs are also placebo controlled. The placebo effect also offers a large potential source of variation, and if sufficiently large, may affect the ability to detect drug effects. The within session variation in test performance during placebo conditions was therefore investigated.

Methods

The data used in this analysis was pooled from placebo sessions from a number of ethically approved double-blind studies carried out by the author and involving 28 healthy male and 13 healthy female subjects. The mean age of the subjects was 26 years (range 19-39 years) and the mean body weight was 71 kg (range 50-109 kg). Individual demographic data on these subjects can be found in Table 2.1.

The studies all involved the testing of centrally acting drugs.

In all of the experiments, subjects were cannulated for blood sampling at 08:30 hours. A set of control measurements was recorded with the test battery at 09:00am, and the volunteers then took their placebo in the form of tablets capsules or liquids depending on the formulation of the active drug in each experiment.

Testing proceeded hourly throughout the day for 6 to 8 hours. The first seven measurements (0-6 hours) were used to calculate the reliability statistics.

The conditions of testing for each subject and experiment were as described in Chapter 2 (Experimental Subjects and Experimental Conditions). Breakfast was not allowed on the day of testing. However, the subjects were allowed to eat a light lunch after the 3 hour test scores were obtained.

The data for each test is from the first placebo day on which that test was performed by each subject. Analyses were not performed on test sets with missing values. Because differences in study design involved the use of different tests, the number of individuals in the analysis varied for each test. The number of times the subjects were tested in the battery prior to the placebo session varied.

The tests analysed were: critical flicker frequency, choice reaction time, continuous attention, body sway, digit symbol substitution, tapping, Gibson's spiral maze, decision making time and paired word association. The visual analogue scales used were: alert-drowsy, steady-dizzy and bored-interested.

As in the last section (Reliability Between Sessions) the results were described using the mean, standard deviation, range (maximum and minimum) and the coefficient of variation. The data were analysed nonparametrically using the Friedman two-way analysis of variance over hours to test for within session effects (Siegel, 1956). The probability values for Kendall's W and the nonparametric coefficient of reliability, \bar{r}_s , over the 7 retests were also calculated to test for concordance and consistency respectively (Siegel, 1956; Kraemer and Korner, 1976. See Chapter 2 for a more detailed discussion of these statistical methods).

The data were analysed parametrically using two-way analysis of variance between times, and the parametric intraclass correlation coefficients for consistency and stability.

Results

The results of the within session placebo data analyses are summarised in Tables 3.4 to 3.6.

Nonparametric results

Four of the variables, continuous attention correct, continuous attention total errors, alert-drowsy and bored-interested visual analogue scales showed significant differences on the Friedman test over the course of the day. Scores on the components of the continuous attention test were better during the morning, and subjects were also more alert and interested at this time.

All of the measures demonstrated very high concordance as shown by the significance of the Kendall test ($P < 0.001$).

Parametric results

F ratios between times were statistically significant for critical flicker frequency, total choice reaction time, choice reaction latency, continuous attention correct, continuous attention total errors, sway, paired word association and the bored-interested visual analogue scale. Changes were generally in the direction of deterioration of performance over time, with the exception of paired word association which improved. The best scores were usually to be found at some time in the morning.

CHANGES IN SCORE WITH REPEATED TESTING OVER 6 HOURS

TEST	N	TIME (h)							FRIEDMAN P	\bar{r}_s	
		Statistics	0	1	2	3	4	5			6
Critical flicker frequency (Hz)	26	mean s.d. range CV	31.8 4.3 25.2-40.2 14	31.3 3.9 25.7-39.8 12	31.0 4.0 24.7-39.6 13	30.8 4.1 25.0-39.4 13	30.9 3.8 24.3-38.9 12	31.3 3.9 23.9-38.8 12	31.2 4.1 23.9-40.1 13	0.130	0.94
Total choice reaction time (msec)	34	mean s.d. range CV	482 69 343-661 14	497 63 363-649 13	494 62 358-650 13	500 61 379-639 12	495 72 374-689 15	503 72 360-667 14	515 74 400-691 14	0.342	0.76
Choice reaction latency (msec)	34	mean s.d. range CV	329 48 232-464 15	338 45 250-451 13	340 40 247-440 12	343 41 253-443 12	338 47 242-472 14	347 41 254-439 12	347 47 250-446 14	0.246	0.71
Choice reaction motor time (msec)	34	mean s.d. range CV	151 42 73-253 28	158 45 90-266 28	153 45 67-264 29	157 44 77-255 28	155 44 72-242 28	155 49 63-257 32	167 51 63-297 31	0.378	0.76
Body sway (20' of arc)	34	mean s.d. range CV	13 5 4-26 38	12 5 4-22 42	12 5 2-27 42	13 6 2-28 46	13 6 4-28 46	13 6 2-34 46	14 8 4-37 57	0.321	0.73
Digit Symbol Substitution (number correct)	14	mean s.d. range CV	78 11 65-103 14	77 10 61-94 13	78 8 68-99 10	76 9 60-94 12	77 9 62-97 12	77 11 57-104 14	75 10 60-95 13	0.598	0.81

Table 3.4. Within session changes in test scores and summary of statistical analysis of test data. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried over 6 hours starting at approximately 09:00 hours. All 0 hour data were pre-placebo control data.

CHANGES IN SCORE WITH REPEATED TESTING OVER 6 HOURS

TEST	N	TIME (h)							FRIEDMAN		
		Statistics	0	1	2	3	4	5	6	P	\bar{r}_s
Continuous attention (number correct)	25	mean s.d. range CV	36 3 28-40 8	37 3 30-40 8	36 4 27-40 11	36 4 23-40 11	35 5 20-40 14	34 5 21-40 15	34 5 20-40 15	0.018	0.57
Continuous attention (number false)	26	mean s.d. range CV	2 3 0-9 150	1 2 0-6 200	1 2 0-6 200	2 3 0-10 150	2 5 0-23 250	2 3 0-11 150	2 4 0-15 200	0.263	0.49
Continuous attention (total errors)	26	mean s.d. range CV	5 5 0-21 100	5 4 0-14 80	5 4 0-14 80	6 6 0-19 100	7 7 0-26 100	8 6 0-21 75	8 7 0-26 88	0.001	0.61
Decision making time (msec)	11	mean s.d. range CV	456 77 323-571 17	465 85 376-613 18	480 64 402-598 13	477 92 360-688 19	471 84 371-674 18	459 64 364-585 14	455 59 370-556 13	0.726	0.75
Paired word association (number correct)	9	mean s.d. range CV	25 2 22-27 8	26 2 22-27 8	27 1 26-27 4	26 2 23-27 8	26 1 25-27 4	26 1 25-27 4	27 1 25-27 4	0.326	0.47

Table 3.5. Within session changes in test scores and summary of statistical analysis of test data. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried over 6 hours starting at approximately 09:00 hours. All 0 hour data were pre-placebo control data.

CHANGES IN SCORE WITH REPEATED TESTING OVER 6 HOURS

TEST / VISUAL ANALOGUE SCALE	TIME (h)										FRIEDMAN	
	Statistics	0	1	2	3	4	5	6	P	\bar{r}_s		
	N											
Tapping rate (taps min ⁻¹)	15	mean s.d. range CV	398 45 316-476 11	391 52 324-496 13	392 54 311-522 14	394 53 324-525 13	395 57 313-548 14	393 64 311-572 16	394 67 325-594 17	0.923	0.91	
Gibson maze time (sec)	15	mean s.d. range CV	23.6 4.5 16.6-32.1 19	23.6 4.4 17.4-32.4 19	22.9 3.9 17.2-30.5 17	23.4 3.6 17.8-31.0 15	24.0 4.0 17.2-30.7 17	24.2 3.6 17.2-31.1 15	23.8 3.9 17.0-30.5 16	0.216	0.81	
Gibson maze errors (number)	15	mean s.d. range CV	12 8 5-36 67	13 9 4-39 69	12 9 5-39 75	12 9 3-27 75	12 9 0-35 75	11 8 3-33 73	11 8 1-33 73	0.247	0.80	
Alert-drowsy (+) (mm)	33	mean s.d. range CV	28 19 2-79 68	25 18 0-68 72	32 23 0-88 72	26 21 0-82 81	24 18 0-77 75	30 22 0-83 73	31 23 0-94 74	0.021	0.61	
Steady-dizzy (+) (mm)	33	mean s.d. range CV	15 12 0-51 80	20 19 0-82 95	20 19 0-85 95	20 18 0-74 90	17 16 0-62 94	19 18 0-65 95	19 17 0-72 89	0.367	0.85	
Bored-interested (+) (mm)	27	mean s.d. range CV	63 23 13-99 37	59 23 9-95 39	52 24 13-89 46	56 23 9-94 41	54 23 14-93 43	51 23 9-91 45	52 22 8-83 42	0.038	0.76	

Table 3.6. Within session changes in test scores, visual analogue scales and summary of statistical analysis. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried over 6 hours starting at approximately 09:00 hours.

Continuous attention correct, sway, Gibson spiral maze time and paired word association showed a tendency towards improvement mid-morning.

Reliabilities

The reliability coefficient, \bar{r}_s , was high for this within session data. Critical flicker frequency and tapping had reliabilities of 0.94 and 0.91 respectively. Digit symbol substitution, Gibson spiral maze time, Gibson spiral maze errors and the steady-dizzy visual analogue scale all had $\bar{r}_s \geq 0.8$. Only two variables, continuous attention false and paired word association, had nonparametric reliability coefficients < 0.5 .

The parametric intraclass correlation coefficients were similar to those calculated nonparametrically, but tended to be higher. Only the paired word association test had a parametric reliability coefficient < 0.5 . In common with the nonparametric results, the reliability coefficients for the visual analogue scales were quite high, all being ≥ 0.6 . Continuous attention false had a much higher parametric than nonparametric reliability coefficient (0.72 versus 0.49).

Critical flicker frequency and tapping were the most reliable measures having coefficients > 0.9 assessed both parametrically and nonparametrically. Paired word association was the least reliable.

The intraclass correlation coefficients for consistency and stability were almost identical, and differed in the second decimal place at most.

Discussion

These results showed that, for several variables, performance was worse in the afternoon than in the morning. The main exception to this was the memory test, paired word association, which was poorest at baseline.

It was not possible to separate out the placebo effect from the circadian effect and the effects of tiredness and of the early afternoon meal in this data. However, the combined effects were not only sufficient to cause performance decrements in some tasks, but also to offset the expected effects of practice in others. The magnitude of the combined effects may therefore be substantial. Smith and Miles (1986) showed that the overall response times (total choice reaction time) of subjects in a reaction time slowed by about 10% after lunch compared to pre-lunch values, whereas the total response times of subjects deprived of lunch were slowed by only 1%. Circadian variation, on the other hand, produced a 5% slowing of movement time in the afternoon. In the present experiments, none of the tests which showed significant changes over the day were concerned with motor speed. Choice reaction motor time, digit symbol substitution, Gibson spiral maze time, tapping and decision making time showed no significant changes indicating that motor slowing was not a factor. The changes were in the measures of interest and alertness from the visual analogue scales, attention (continuous attention), sensory perception (choice reaction latency) and arousal (critical flicker frequency).

Changes in mood after food consumption was reported by Spring et al., 1982/83. These authors demonstrated differences in feelings of wellbeing in subjects after high protein and high carbohydrate meals with the same calorific value. Subjects tended to be less alert after a high protein meal, but selective attention was unimpaired. Craig et al., (1981), and Smith and Miles

1986 demonstrated, on the other hand, that consumption of lunch impaired performance on paced sensory vigilance and paced cognitive tasks which is more in keeping with our data. It should be noted, however, that in the experiments demonstrating differences in performance due to food consumption the meals were large and often involved three courses. In the experiments from which the present data was collected only light high carbohydrate meals were consumed, and so the effects should have been less. In the drug studies presented in this work food was not allowed in the four hours prior to, or during, testing.

Despite the significant changes, the reliabilities within session were high. The changes in subjects' behaviour were therefore qualitatively similar. The group results were also stable suggesting that the chances of detecting even modest drug effects should have been quite good.

More tests showed statistically significant changes over the day using the parametric analysis of variance compared to the nonparametric analysis of variance. Only one variable was statistically significant with the nonparametric test and not with the parametric, this was the alert-drowsy visual analogue scale.

In the next section, two tests (digit symbol substitution and sway) were evaluated without drugs or placebos, and the effects of the control condition on performance was assessed.

RELIABILITY OF DIGIT SYMBOL SUBSTITUTION AND SWAY UNDER CONTROLLED CONDITIONS

Two of the experiments described (Chapters 5 and 6) involved using short batteries of psychomotor tests in order to assess rapid changes in performance. The tests chosen for these short batteries included digit symbol substitution and sway which take 90 seconds and 2 minutes respectively to complete. Digit symbol substitution was added into the battery at a fairly late stage, and the numbers used to calculate the reliability coefficients between and within sessions were quite small. We therefore decided to look at the reliability characteristics of this test in a greater number of subjects. Since it did not seem to be realistic to design placebo controlled experiments simply for this purpose, the studies were not performed on subjects taking part in drug studies, but on subjects in whom normative data on other tests was being collected. Sway was also measured in these subjects.

Methods

Data were recorded from fifty healthy male and female subjects who were not taking part in drug studies and who had not previously participated in psychomotor studies. The mean age for these subjects was 23 years (range 19 - 30 years) and the mean weight was 64 kg (range 40 - 89 kg). Demographic data for these subjects are shown in Table 2.2. The environmental conditions were as outlined in Chapter 2 except that caffeine containing drinks and breakfast were allowed.

Subjects were tested on digit symbol substitution and sway at hourly intervals for 4 hours starting at 09:00 am after an initial practise the day before.

Results

The mean, standard deviation, range and coefficient of variation are shown in Table 3.7 along with the probabilities from the Friedman test and the nonparametric reliability coefficient, \bar{r}_s .

The results from the mean data showed increases in digit symbol substitution scores indicative of practise effects over time. No such effect was observed with the sway scores which were quite stable.

Nonparametric analysis

The Friedman test was highly statistically significant for digit symbol substitution ($P < 0.001$), but not for sway. Both tests were significantly concordant on the Kendall test ($P < 0.001$).

Parametric analysis

The F statistic from the two way analysis of variance between times was highly significant for digit symbol substitution ($P < 0.001$). Sway was not significant on this test.

Reliabilities

The nonparametric consistency scores, \bar{r}_s , were high, being 0.89 and 0.83 for digit symbol substitution and sway respectively.

The intraclass correlation scores for consistency were also high; 0.90 for digit symbol substitution and 0.74 for sway. The stability coefficient for digit symbol substitution was considerably lower at 0.79, whereas that for sway was identical (0.74).

CHANGES IN NORMATIVE TEST SCORES WITH REPEATED TESTING OVER 4 HOURS

TEST	N	TIME (h)							FRIEDMAN P	\bar{r}_s	
		Statistics	PRAC	0	1	2	3	4			
Digit symbol substitution (number correct)	50	mean	59	63	65	67	67	67	68	0.000	0.89
		s.d. range CV	12 29-85 20	11 36-90 17	11 41-96 17	12 38-97 18	13 39-102 19	13 38-102 19	12 38-100 18		
Body sway (20' of arc)	49	mean	15	15	15	14	15	15	15	0.792	0.83
		s.d. range CV	6 2-30 40	8 1-54 53	6 1-36 40	5 2-31 36	6 1-38 40	6 1-38 40	7 2-44 47		

Table 3.7. Within session changes in digit symbol substitution and sway scores during a single morning after one familiarisation session (PRAC), and summary of of statistical analysis. Results are expressed as mean, standard deviation (s.d.), range (minimum to maximum) and coefficient of variation (CV) for each variable at each time point. N - number of subjects in the analysis for each variable tested, P - probability value of Friedman test, \bar{r}_s - nonparametric reliability coefficient for consistency. Testing was carried over 4 hours starting at approximately 09:00 hours. The familiarisation session was given the day before.

Discussion

There was a pronounced improvement in performance over time for digit symbol substitution, but not for sway. The scores for digit symbol substitution became more or less stable after 3 practices in this normative data experiment, the biggest improvement being between the first and second practice. This large difference between the first and second practice for some tests has been reported previously (McClelland, 1987).

The reliabilities for both tests was high. However, the improvement in digit symbol substitution performance produced a difference between consistency and stability which was the biggest measured throughout this set of experiments (0.90 versus 0.79). Nonetheless, the stability was still good. Thus even when the practice effect was noticeable and statistically significant, the reliability of the data was still high.

These results indicated that, in as far as can be ascertained from control data, digit symbol substitution and sway were probably reliable enough measures to use for the short battery of tests. Although digit symbol substitution scores improved with repeated use, the five practices given to ensure a stable baseline in the drug experiments were probably sufficient.

CORRELATION BETWEEN TESTS

Few, if any, psychological tests measure single skills and most utilise several skills (Lezack, 1983). It is therefore of interest to assess the relationship between different tests in the battery of tests in order to assess the extent to which the tests measure, or are influenced by, the same underlying processes.

Methods

Data from several ethically approved psychomotor studies carried out by the author and involving 41 healthy male and female volunteers were pooled. The mean age of the subjects was 26 years (range 19-39 years) and the mean weight was 71 kg (range 46 to 109 kg). For individual demographic data on these subjects see Table 2.1.

The studies were double-blind and involved the testing of drugs affecting the central nervous system. The intercorrelation analysis was performed on the first set of data obtained from the subjects' first experimental session after an initial familiarisation session with the test battery. The data were all pre-drug or pre-placebo test scores obtained under the full experimental conditions described in Chapter 2 (Experimental Subjects and Experimental Conditions). Testing was performed at approximately 09:00 hours.

The relationship between scores of different variables was assessed using Spearman rank correlations and their significances. The data used were the same as that for the distribution curves, although age was also included in the intercorrelation analysis.

The variables assessed were: age, critical flicker frequency, choice reaction time, continuous attention,

sway, digit symbol substitution, tapping, Gibson's spiral maze, decision making time, paired word association, visual vigilance and the visual analogue scales alert-drowsy, steady-dizzy and bored-interested.

Results

A cross-matrix of Spearman rank correlations and the number of data pairs used in each correlation are shown in Table 3.8. Correlations significant at P equal to or less than 0.05, 0.01 and 0.001 are underlined one, two and three times respectively.

Of 153 correlations performed 35 correlations were statistically significant, and 18 had correlations >0.5 . A number of these statistically significant correlations were therefore <0.5 . Statistical significance was more likely to be achieved with low correlations when the number of data pairs, N, used in the analysis was high. Conversely, when N was small, statistical significance was only achieved when the correlation was relatively high. Consequently, although 8 of these statistical significances would have been predicted by chance, a great many more might have been obtained if larger numbers had been used. This was partly confirmed by the large number of trends in the data.

The highest correlations were generally between component parts of the same tests. Thus total choice reaction time correlated strongly with choice reaction latency (0.712) and choice reaction motor time (0.793), although latency and motor time correlated poorly with each other (0.206).

Similarly, correct scores on the continuous attention test had a strong negative correlation with total error scores on the same test (-0.901). Times to complete the Gibson spiral maze also correlated negatively with their own error scores (-0.743).

Choice reaction - total time	.270 <u>N(41)</u>						
Choice reaction latency time	.220 N(39)	.712 <u><u>N(39)</u></u>					
Choice reaction motor time	.247 N(39)	.793 <u><u>N(39)</u></u>	.206 N(39)				
Sway	-.149 N(41)	.184 N(41)	.276 <u>N(39)</u>	-.006 N(39)			
Alert-drowsy visual analogue	-.407 <u>N(41)</u>	-.215 N(41)	-.266 <u>N(39)</u>	-.208 N(39)	-.167 N(41)		
Steady-dizzy visual analogue	-.369 <u>N(41)</u>	-.284 <u>N(41)</u>	-.068 N(39)	-.366 <u>N(39)</u>	-.008 N(41)	-.463 <u><u>N(41)</u></u>	
Bored-interested visual analogue	.244 N(36)	.380 <u>N(36)</u>	.162 N(34)	.493 <u>N(34)</u>	-.032 N(36)	-.530 <u><u>N(36)</u></u>	-.521 <u><u>N(36)</u></u>
Critical flicker frequency	-.408 <u>N(36)</u>	-.500 <u><u>N(36)</u></u>	-.457 <u>N(34)</u>	-.309 <u>N(34)</u>	-.023 N(36)	.216 N(36)	.307 <u>N(36)</u>
Continuous attention correct	.075 N(28)	-.185 N(28)	-.245 N(28)	-.047 N(28)	-.199 N(28)	.091 N(28)	.054 N(28)
Decision making time	.494 <u>N(27)</u>	.546 <u><u>N(27)</u></u>	.296 N(27)	.519 <u>N(27)</u>	-.118 N(27)	-.253 N(27)	-.271 N(27)
Digit symbol substitution	-.410 N(11)	-.682 <u><u>N(11)</u></u>	-.435 N(11)	-.650 <u><u>N(11)</u></u>	.713 <u><u>N(11)</u></u>	.152 N(11)	-.200 N(11)
Gibson spiral maze time	.210 N(11)	.291 N(11)	.727 <u><u>N(11)</u></u>	-.254 N(11)	.175 N(11)	.032 N(11)	-.306 N(11)
Gibson spiral maze error	-.399 N(11)	-.087 N(11)	-.351 N(11)	.210 N(11)	.166 N(11)	-.380 N(11)	.066 N(11)
Tapping	.352 N(11)	-.173 N(11)	.145 N(11)	-.373 N(11)	.313 N(11)	-.169 N(11)	.064 N(11)
Paired word association	-.338 N(11)	-.373 N(11)	-.074 N(11)	-.373 N(11)	.302 N(11)	.337 N(11)	.374 N(11)
Visual vigilance	.082 N(11)	.190 N(11)	.213 N(11)	.209 N(11)	-.169 N(11)	.098 N(11)	.110 N(11)
Continuous attention total errors	-.753 <u><u>N(11)</u></u>	-.462 N(11)	-.005 N(11)	-.408 N(11)	.334 N(11)	.110 N(11)	-.077 N(11)
	Age	Total reaction time	Choice reaction latency	Motor reaction time	Sway	Alert- drowsy visual analogue	Steady- dizzy visual analogue

Table 3.8. Cross-Matrix of Baseline Control Spearman Rank Correlations collected at 09:00 am approximately. $P < 0.05$, $P < 0.01$ and $P < 0.001$ are

-.142										
N(36)										
-.206	-.147									
N(23)	N(23)									
.284	-.409	-.034								
N(27)	<u>N(27)</u>	N(22)								
-.282	-.247	-.059	-.389							
N(11)	N(11)	N(11)	N(11)							
-.323	-.391	.118	-.164	-.032						
N(11)	N(11)	N(11)	N(11)	N(11)						
.597	-.232	-.517	-.150	-.025	-.743					
<u>N(11)</u>	N(11)	<u>N(11)</u>	N(11)	N(11)	<u>N(11)</u>					
-.023	-.173	.155	-.482	-.169	.600	-.510				
N(11)	N(11)	N(11)	N(11)	N(11)	<u>N(11)</u>	<u>N(11)</u>				
-.187	.149	.193	-.224	.450	.373	-.448	.447			
N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)			
.126	.148	-.130	-.362	-.432	.325	-.293	.422	.228		
N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)		
-.047	.636	-.901	-.060	.035	-.094	.336	-.243	-.000	.000	
N(11)	<u>N(11)</u>	<u>N(11)</u>	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)	N(11)

Bored- Critical Continuous Decision Digit Gibson Gibson Tapping Paired Visual
inter. flicker attention making symbol maze maze maze
visual frequency correct time subst- time errors
analogue intuition association
vigilance

for the tests and visual analogue scales and their significances. Data were underlined once, twice and three times respectively.

A few high correlations were found between different tests. The strongest of these was for choice reaction latency and Gibson's spiral maze time (0.727). However correlations between digit symbol substitution and sway, total choice reaction time and choice reaction motor time were also quite high (0.713, -0.682 and -0.650 respectively).

Total error scores on the continuous attention test had a high negative correlation with age (-0.753) and a high positive correlation with critical flicker frequency (0.636).

Gibson's spiral maze time had a high positive correlation with tapping (0.600).

Age, choice reaction time and critical flicker frequency gave the greatest number of significant correlations with other tests. Sway, tapping and Gibson's spiral maze gave few significant correlations with other tests. The only tests which did not correlate significantly with any others were paired word association and visual vigilance for which the numbers were small. These latter two tests did, however, show several nonsignificant trends.

The visual analogue scales all correlated significantly with each other, and with several of the tests.

Discussion

Correlation coefficients between scores on different tests simultaneously assess the association of processes, the discriminability between individuals and the heterogeneity of the group (Kraemer and Korner, 1976). Thus a high correlation between the tests indicates that all three are present i.e. association, discrimination and heterogeneity. A low score, on the other hand, does not necessarily mean that there is no association between these tests in the population, but may indicate that the group is homogeneous or that the tests are

insensitive to individual differences.

As expected, there were high correlations between total reaction time and its components. Otherwise, the high correlations appeared in the comparisons with low numbers ($N=11$). If, for these low N comparisons, the null hypothesis was rejected at $P<0.01$ instead of $P<0.05$, several of the correlations were still significant, mainly in the expected direction. Thus, there was a high correlation between total choice reaction time and digit symbol substitution which both have a substantial motor component, between choice reaction latency and time to complete the Gibson spiral maze which both have a timed 'observational' component, between continuous attention and its errors and between Gibson spiral maze time and its errors. The latter probably reflects the known trade-off between speed and accuracy (Gibson, 1977; Fitts and Posner, 1973). The reasons for the high correlation between sway and digit symbol substitution are not clear. However, as mentioned previously, for the large number of comparisons made a few significances would have been expected to arise purely by chance, and random effects cannot be ruled out.

The majority of correlations between scores on different tests were fairly low, and of 153 comparisons 135 had $r_s < 0.5$. The low correlations are unlikely to have been caused by lack of sensitivity to individual differences for the majority of tests, since the data distributions were spread out over a range and were therefore not entirely homogeneous. This suggests that the tests were able to discriminate between individuals. Indeed, some of the tests which tended to pile up or skew (e.g. the visual analogue scales and continuous attention correct) produced some relatively high correlation coefficients. However, lack of discriminability between individuals may have been a factor in the low correlations for paired word association and visual vigilance since there was very little spread in the data for these tests.

The visual analogue scales had a high degree of associativity with each other as reported by Bond and Lader (1974). The positive mood aspects of the analogue scales correlated positively with each other, and negatively with the negative aspects, as demonstrated by Persson and Sjoberg (1981) for a self-rating check-list for mood. Two of the visual analogue scales (alert-drowsy and steady-dizzy) also correlated negatively with age.

It has previously been shown that there are age differences in the way subjects rate themselves on visual analogue scales after drugs (e.g. Swift, 1983; Swift et al., 1988; Fagan et al., 1990). Bond and Lader (1974) reported, however, that subjects over a wide age range (16 to 64) used visual analogue scales in the same way when no drug was involved. In contrast, Persson and Sjoberg (1981) demonstrated that younger subjects (<25 years) showed the most negative feelings using their mood adjective checklist. The present results indicate that there may be age differences in the pattern of scoring on baseline visual analogue scales under full experimental conditions even over a small age range. Since the subjects were all cannulated and were expecting to receive either an active drug or a placebo, the results are likely to include a component of anxiety.

Correlations with other commonly used tests are often used in Psychology for indicating the validity of tests (Criterion-related validity; Isaac and Michael, 1981). However, low correlations may also be used to indicate that tests are measuring different aspects of performance (Nagoshi and Wilson, 1987). Sway, which did not correlate with many of the other tests, has been shown to be sensitive to drug effects in many drug studies (Swift, 1984; Starmer and Bird, 1984). Sensitivity of sway to differences in age (over a wider range than in the present studies) and to sex have also been demonstrated (Overstall et al., 1977). The scores on this test are not particularly homogeneous having a

4-fold range (6-26). Thus the evidence indicates that it probably measures a different function compared to the other tests. Although criterion-related validity through correlation with other tests (apart from digit symbol substitution) was not demonstrated for sway, its value in the drug situation has been proved with real use.

GENERAL DISCUSSION

With the single exception of correct scoring on the continuous attention test, the tests which showed changes across single days were different to the ones which showed changes between days. In this respect, continuous attention behaved like a vigilance task since the correct scores declined over time irrespective of any other modifying factors (Buchsbbaum and Sostek, 1980; Parasuraman, 1979).

The other variables which changed significantly between sessions all had motor speed components: choice reaction motor time, digit symbol substitution, Gibson spiral maze (time and errors) and tapping. The tests which changed significantly within sessions were: critical flicker frequency, total choice reaction time, choice reaction latency, continuous attention total errors and sway. Mood was also significantly affected within sessions, and subjects rated themselves as less alert and less interested on the visual analogue scales later in the day. The changes were quite small (7 mm movement towards drowsy, and 12 mm movement towards bored), and approximated to a shift of about 0.5 standard deviations. According to Lezack (1983), however, even slightly reduced alertness can produce inattentiveness and slowing. Other likely contributing factors to the performance changes within sessions may have been circadian variations, tiredness, lunch, anxiety and the placebo effect itself.

The between session improvements on the speeded tasks is hardly surprising since it is well known that performance in skilled tasks continues to improve over long periods of time, the rate of improvement declining as practice continues. However, performance does not inevitably improve with practice, and lack of motivation and lack of knowledge of the results can prevent these improvements (Fitts and Posner, 1973).

The effects of practise on digit symbol substitution were much more pronounced in the normative control study than in the other experimental data. There was no evidence of improvement on this test within sessions after placebo, and only a slight practise effect between sessions. This difference was undoubtedly due to the subjects having at least 5 practises in the familiarisation sessions before entering the drug/placebo studies. The improvement in performance in the normative group was already beginning to tail off by the sixth trial. Thus further improvement in performance in the placebo group would, beyond the sixth trial, have been slight.

The scores for digit symbol substitution were also quite different between the groups, the normative mean scores being considerably lower. Again, lack of practise may have been a factor in this. Another possible reason was the fact that the normative group were a more heterogeneous sample of the population, whilst the subjects from the drug volunteer pool were more academically inclined. Clever subjects would be expected to do better generally (Wechsler, 1944). Interestingly, the first normative score for digit symbol substitution (mean = 59) was higher than the normative scores for a young age group quoted by Lezack (mean = 51). The symbols used were different, however, and this may have been a contributory factor in the higher scoring. Finally, the normative group was allowed breakfast, and the fact that they had had a meal may also have slowed them down (Smith and Miles, 1986).

The parametric and nonparametric reliability coefficients were very similar, and rarely differed beyond the second decimal place. This was despite the nonparametric method being 'less powerful' and the parametric requirement of normality being somewhat violated. It thus appeared that both types of statistics were adequate for this purpose, and seemed to be quite robust over widely differing sets of data. There was little advantage to adding time as a 'mixed effect' in the parametric intraclass correlation

coefficient model to provide a coefficient of stability. Although there was a detectable between session effect for both digit symbol substitution and continuous attention correct, this only produced a small effect on the stability coefficient.

On the whole, the reliabilities for the tests in the battery were satisfactory, and under these experimental conditions, the behaviour of the subjects and environment appeared to be consistent and stable. The least consistent/stable measure was paired word association which had low reliabilities between and within sessions despite small variations in the mean scores. The main reason for this low reliability was that the scores were at a ceiling level for most of the subjects. There were therefore no stable individual differences between the subjects which is a prerequisite for a high value on a score based on correlations (Kraemer and Korner, 1976). For the same reason the paired word test also correlated poorly with the other tests in the intercorrelation analysis.

Some test-retest reliability scores quoted by other workers for the same, or similar, tests are shown in Table 3.9. These scores were calculated by a variety of statistical methods (not always specified), and were usually based on two sets of data recorded under low stress conditions. The "split-half" reliabilities were calculated on only one data set, and involved correlating alternate items (all the odd with all the even) in the same data set (Lamont and Tiplady unpublished data). The reliabilities presented earlier in this Chapter compare favourably with those in Table 3.9 despite the presumed greater stress of the test-retest conditions in this thesis. This may be interpreted in two ways. Either the tests were robust and the reliabilities were not greatly influenced by the modifier variables discussed in Chapter 1, or the tests were less robust, but the care taken to ensure good experimental control paid off in terms of the high

RELIABILITY COEFFICIENTS QUOTED

TEST	r	Method	Authors
<u>Psychomotor tests</u>			
Critical flicker frequency (Hz)	0.88	?	Baker et al., '85
	0.9	?	Levander 1982*
	0.94	Split/H	Parrot 1982
	0.98	?	Simonson, Brozeck '52*
Complex reaction time	0.65	?	Franks et al., '76
Reaction time	0.85	mean r	Kennedy et al., '85
Four choice reaction time	0.80	Spear/B	Bittner et al., '86
Choice reaction movement time	0.86	Spear/B	Bittner et al., '86
Continuous attention correct	0.56	Kendall	Lamont, Tiplady (PC)
	0.74	Split/H	Lamont, Tiplady (PC)
Continuous attention false	0.74	Split/H	Lamont, Tiplady (PC)
Continuous attention total err	0.67	Split/H	Lamont, Tiplady (PC)
Continuous performance	0.71	?	Echeverria et al., '89
Body sway (eyes open)	0.88	?	Franks et al., '76
Body sway (eyes closed)	0.89	?	Franks et al., '76
Digit symbol substitution	0.78	mean r	Kennedy et al., '85
	0.84	Spear/B	Bittner et al., '86
Gibson maze time	0.73	?	Pickles 1966
Gibson maze errors	0.77	?	Pickles 1966
Tapping rate	0.55	am ?	Echeverria et al., '89
	0.77	am/pm ?	Echeverria et al., '89
	0.89	mean r	Kennedy et al., '85
Decision making time	0.67	Kendall	Lamont, Tiplady (PC)
	0.89	Split/H	Lamont, Tiplady (PC)
Paired word association	0.33	Kendall	Lamont, Tiplady (PC)
	0.72	Split/H	Lamont, Tiplady (PC)

Table 3.9. Coefficients of reliability quoted by other authors. Kendall - Kendall coefficient of concordance, Split/H - split half reliability, mean r - mean correlation after several trials, Spear/B - Spearman-Brown reliability-efficiency calculated over 3 minutes, ? - method not specified, * - cited by Curran, (1990); PC - personal communication of unpublished control data on 50 healthy young subjects (18-30 years)

reliabilities obtained. It was not possible to distinguish between the two. For the experiments in this work experimental control was considered important, and care was taken to maintain it throughout.

Results of the intercorrelation analysis suggested that, on the whole, the test battery measured different aspects of psychomotor performance. However, since some of the low correlations were statistically significant it was evident that associations were not totally absent.

Bittner et al., (1986) have used the results of baseline 3 minute reliability scores as a basis for selecting tests for repeated measures applications. Our view was that although the analyses carried out in this Chapter were useful for determining the behaviour of the test scores, it was not useful to speculate, from baseline results, which might be the best tests. The most important characteristic of a good test are validity for the required purpose and sensitivity to change after drugs. It is only possible to assess these characteristics with real usage.

SUMMARY

It was the purpose of this Chapter to examine the reliability of the battery of tests under the conditions of testing usually found in drug studies in healthy human volunteers. In particular, day to day and within day reliabilities and variabilities were assessed. The extent to which the tests measured the same underlying processes was also assessed.

The results showed clearly that the reliabilities of the majority of the tests were good between and within session in the pre-drug and placebo situations. Thus, despite the stress of the experimental situation, performance on the tests appeared to be stable.

Intercorrelations between the tests in the battery suggested that the on the whole, different aspects of performance were assessed, although there was a considerable degree of communality between some test scores.

These analyses imply that the tests in the battery form a reliable set of measuring instruments when used under conditions designed to ensure adequate experimental control.

CHAPTER4 - RATIONALE FOR THE DRUG STUDIES CARRIED OUT
IN THIS THESIS

THE DRUG STUDIES

The reliabilities of the tests in the battery were considered in Chapter 3. In the next three Chapters the test battery was used to explore the issue of acute tolerance (or tachyphylaxis) to drug effects on the central nervous system. The degree to which performance on the various tests was affected by different centrally depressant drugs was also examined.

The methodologies used in Chapters 5, 6 and 7 were different, although the rationale behind the studies was basically the same. The aims were to study plasma drug concentration/effect relationships over a relatively short period of time in the absence of the distribution artifact (Goldstein, 1983), and to avoid rapid changes in plasma concentration during testing. This was done by quickly achieving and maintaining "steady-state" constant plasma concentrations of the drug (Wagner, 1974). The hypothesis was that under these conditions, if tolerance occurred, the functions would recover while the plasma concentrations remained the same. Advantage was also taken of the stable plasma concentration situation to look at the pattern of impairments produced by the drugs and the sensitivities of the tests in the battery.

Chapter 5 deals with the effects of chlormethiazole on performance, and the following two Chapters deal with the effects of ethanol and nitrous oxide respectively. Chlormethiazole was chosen because we had some experience of using this hypnotic drug in our laboratory, and felt that it would be a good candidate for study. The drug can be given by infusion (unlike most benzodiazepines), and does not have active metabolites which might also affect performance. Constant plasma concentrations were achieved rapidly using loading and maintenance infusions of the drug. The target concentration was chosen beforehand, and the amount infused determined individually on the basis of pharmacokinetic variables

already known for the subjects.

Ethanol was selected for study (Chapter 6) because there have been many accounts of acute tolerance with this drug (e.g. Mellanby, 1919, Golberg, 1943) and because it is used as a standard reference drug when tests or test batteries are evaluated. The impairment profile for ethanol has been gradually built up from a huge literature on the drug. It was therefore of interest to discover whether we would find the same profile using a much simpler methodology. The ethanol was administered orally using a large volume initially to quickly achieve "high" concentrations which were then maintained using frequent small "top-up" doses. The volume of the main dose was decided upon after some initial dose ranging experiments. The amounts supplied in the top-up doses were calculated from a single dose pharmacokinetic study performed with the same subjects. Venous and expired concentrations of ethanol were measured allowing the arterial/venous differences to be assessed.

Based on the studies with chlormethiazole and ethanol, a dose response study with each dose at steady-state was considered the next logical step. Nitrous oxide was picked for this because of the ease with which steady-state concentrations could be rapidly and accurately obtained over a wide range of concentrations. Since acute tolerance to the effects of N_2O on performance were previously shown not to occur (Korttila et al., 1981), the aspects of tolerance that we concentrated on were the subjective effects of the drug. In the nitrous oxide studies (Chapter 6) the drug was inhaled. Steady-state constant plasma concentrations were achieved very quickly, and there was no need for a loading dose. The inspired concentrations were titrated to produce the same series of expired concentrations for each individual, and the effects on the test battery were evaluated at each concentration. This allowed dose response relationships for each test to be constructed.

Finally, the effects of nitrous oxide on a single test, critical flicker frequency, were studied in greater detail in order to assess the effects of the drug on arousal.

CHAPTER5 - PSYCHOMOTOR EFFECTS OF CHLORMETHIAZOLE UNDER
CONDITIONS OF CONSTANT PLASMA CONCENTRATIONS

CHLORMETHIAZOLE STUDY

Chlormethiazole is a potent anticonvulsant with sedative-hypnotic properties. The drug has been used extensively in the treatment of alcohol withdrawal symptoms, and for anxiety and agitation in the elderly.

Chlormethiazole is a drug with high hepatic clearance, and the oral bioavailability and plasma concentrations vary considerably between individuals (Pentikainen et al., 1980; Jostell, 1987). Several studies have shown that recovery from the central nervous system effects is rapid (Briggs et al., 1980; Stevenson et al., 1982).

In a previous acute oral single-dose study that we performed, plasma concentrations of chlormethiazole were down to less than a quarter of their peak values by 3 hours post-drug, at which time the sedative effects of the drug were no longer detectable (Fagan et al., 1990). Figure 5.1 shows the onset and offset of central nervous system signs and symptoms in relation to the plasma concentrations for a single subject in that study.

Some of the subjective symptoms of the drug disappeared very quickly (<1h), and at a higher venous plasma concentration than they first appeared. As no arterial samples were taken, it was not possible to say whether this lack of correlation between concentration and effect was due to acute tachyphylaxis, or was an artifact due to a difference between sampling site and effector site concentrations. Since there was a 10-fold difference in plasma chlormethiazole concentration during the first two hours after the drug, it was also difficult to determine which tests (or functions) were most affected by the drug.

Kalant and Khanna (1986) found that larger doses of chlormethiazole given to naive rats gave longer sleep times, but higher blood chlormethiazole concentrations on awakening. The authors interpreted these data as

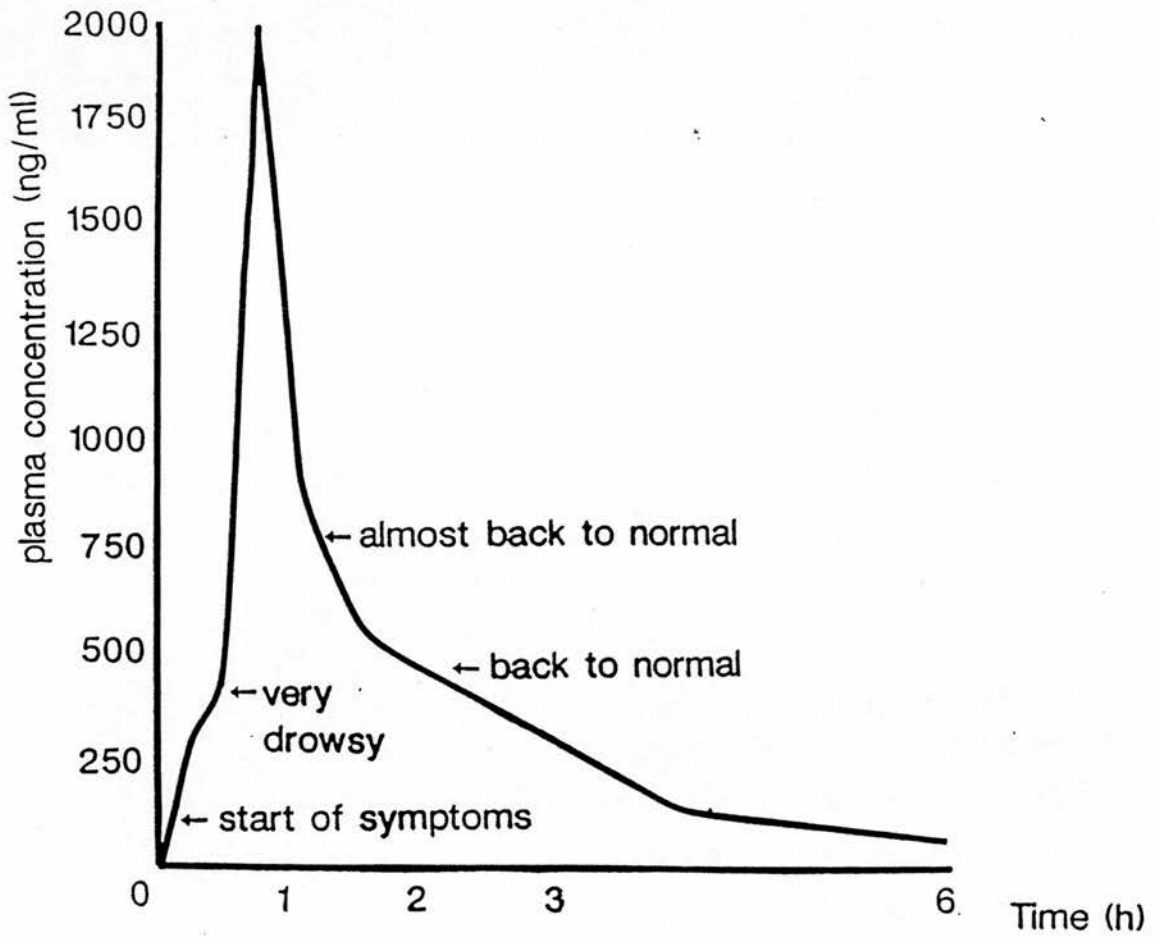


Figure 4.1. Timing of onset and offset of symptoms in relation to plasma concentrations of chlormethiazole for a single subject after 452 mg chlormethiazole.

indicating that the drug produced acute (within session) tolerance.

A constant plasma concentration of chlormethiazole was therefore used in conjunction with a small battery of tests, used repeatedly, in order to determine whether there was acute tolerance to the drug in man. The pattern of impairment was further assessed under these conditions with a single use of a larger battery of tests.

Methods

Six young healthy male and female volunteers (4 male, 2 female) from the volunteer pool participated. The mean age of the subjects was 27 years (range 20-33 years) and the mean weight was 68 kg (range 47-81 kg). (For individual demographic details on these subjects see Table 2.1). The subjects received loading and maintenance infusions of chlormethiazole calculated to achieve a target plasma concentration of $1.5 \mu\text{g}\cdot\text{ml}^{-1}$, which was intended to produce drowsiness without unconsciousness. Subjective and objective symptoms were recorded. The procedures described in Chapter 2 were followed as regarding informed consent, ethics, medical examinations and dietary restrictions. The study schedule is outlined in Figure 5.2.

All of the subjects were right-handed. A blood sampling cannula (18G Venflon) was inserted in the antecubital fossa of the left arm. A smaller cannula (21G Venflon) was inserted into a vein on the forearm of the right arm, and was connected via nylon connecting tubes (Portex) to the infusor system (Harvard pump driving a 50 ml glass Rocket syringe). The cannula and tubing were arranged so as to allow freedom of movement in the arm and hand. A 3-way tap (Viggo) was linked into the lines behind the right shoulder, and was set to the "off" position until the infusion started. This prevented the drug syphoning

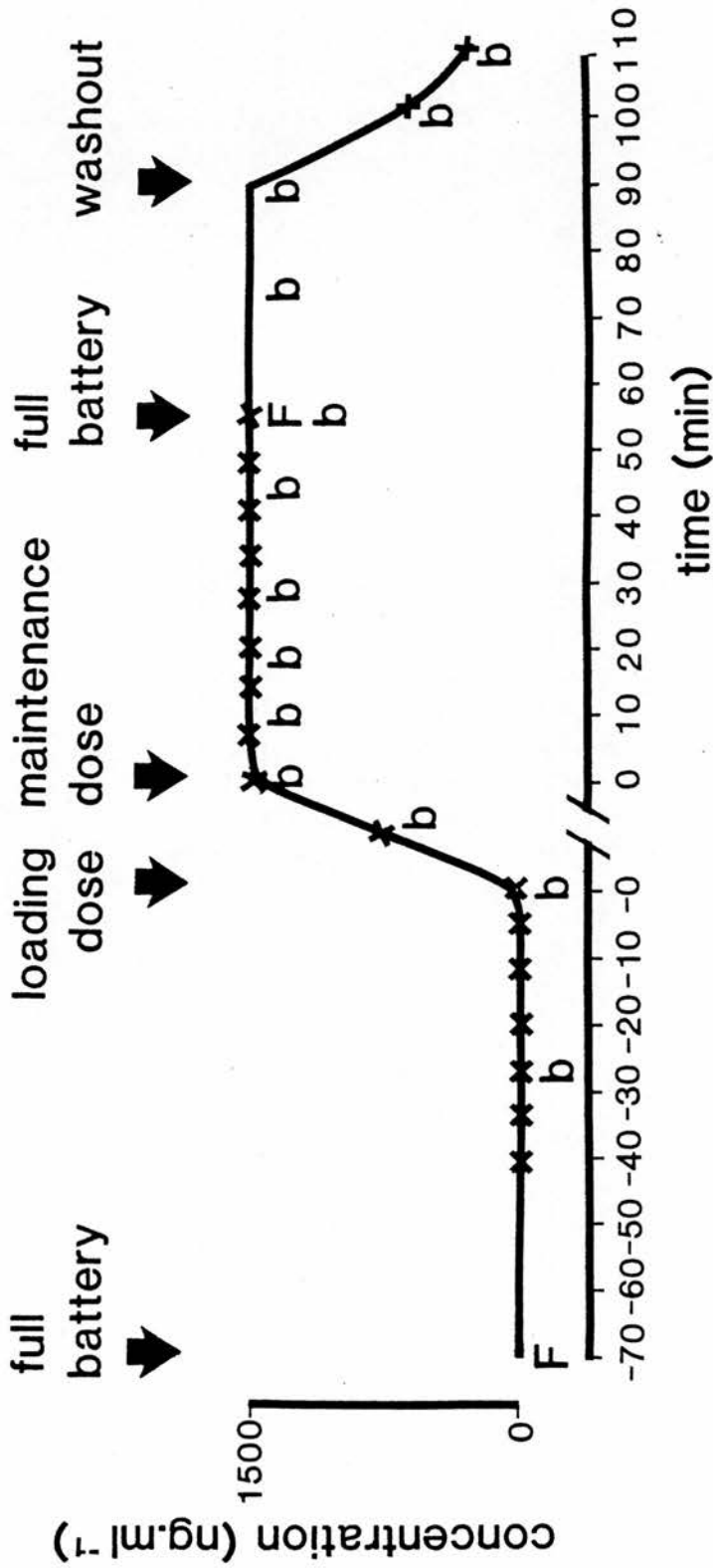


Figure 5.2. Study schedule. Stylised target plasma concentration-time profile with associated testing and sampling times overlaid. X - short battery of tests i.e. visual analogue scales, digit symbol substitution time and body sway; F - full battery of tests i.e. visual analogue scales, digit symbol substitution time, body sway, choice reaction time, choice reaction latency, motor time, critical flicker frequency, continuous attention task, decision making time and Gibson spiral maze; b - blood sample. Loading and maintenance doses and loading times varied between individuals. The target maintenance concentration was 1.5 ug.ml⁻¹.

into the subject, and also minimised backflow of blood from the subject's arm into the connecting tube. The subjects' sleeve was pulled down to cover the cannula and tubing during the experiment. The pump and its connections were behind the subject, and so were out of sight. This enabled the volunteer to be kept blind to the starting time of the infusion (the noise of the pump running was continuous whether or not the pump was delivering fluid). The tap at the shoulder was also adjusted slightly from time to time so that there was no accurate cue to the start of the infusion. The tap was switched on and the infusion started after 6 control runs of the small test battery described below.

The infusion solution contained 8 mg.ml^{-1} (0.8%) chlormethiazole edisylate in a solution of dextrose (Astra Pharmaceuticals Ltd). 5 ml venous blood samples for determination of plasma chlormethiazole were taken into 10 ml lithium heparin blood tubes (Sterilin) before the infusion started, at 10 minute intervals for the first hour, at 15 minute intervals for the next half hour, at the end of infusion and 10 and 20 minutes later. After centrifugation, the plasma was transferred to 5 ml teflon plasma tubes (Cryotube) and deep frozen at -20°C to await analysis. The plasma chlormethiazole concentrations were measured by gas-liquid chromatography in the Bioanalytical Laboratories at ASTRA Alab AB, Sodertalje, Sweden. The volume of blood taken from each individual, including blood for laboratory screening, was 80 ml.

Pharmacokinetic calculations of loading and maintenance doses

The program INFTWO (DEC VAX, Astra) was used to simulate the plasma concentration time profile expected from two constant rate infusions, one fast (L) and one slow (M), designed to rapidly achieve and maintain a plateau. The model variables for each individual were obtained from an acute pharmacokinetic study previously carried out with

these subjects (Fagan et al., 1990). The program was based on the equations given by Wagner (1974) assuming that the drug behaved according to an open two compartment model in each individual (Jostell, 1987).

Values for ten parameters were required in order to run the program. Six of these were pharmacokinetic variables calculated using data from the acute chlormethiazole study mentioned earlier. These were; the volume of the central compartment (V_c), the distribution and elimination phase rate constants (α and β), the rate constants from the central to the peripheral compartment (k_{12}) and from the peripheral to the central compartment (k_{21}), and the rate constant for the elimination from the central compartment (k_{e1}). In this way the infusion rates were individually determined.

The last 4 variables required to run the program were the rate of the initial rapid loading infusion (L), the rate of the second slower infusion used to maintain the chosen steady-state concentration (M), the time for the loading infusion (T_{load}) and the time for the maintenance infusion ($T_{maintenance}$). By adjusting these four variables in the program, different plasma concentration time profiles could be simulated and the peak concentration and time to reach a plateau read off the simulated curve. The best infusion regimes could then be selected.

Small test battery

Subjective and objective measures of performance were assessed before, during and after the infusions.

The small battery of objective tests used comprised visual analogue scales, digit symbol substitution and body sway. These tests were chosen because of their reported sensitivity, their short duration, the fact

that they take a fixed time to perform, and because they require the integration of several different skills for their performance.

The 100 mm visual analogue scales used to assess the predicted subjective symptoms and mood changes were; alert-drowsy, bored-interested, steady-dizzy, muzzy-clearheaded, well-coordinated-clumsy and very well-very ill. There were also 2 scales relating to eye discomfort and nose discomfort where the scales ranged from "not present at all" to "couldn't be worse". The form containing the visual analogue scales is shown in Figure 2.8.

Between performance tests, the subjects were asked to spontaneously report any symptoms experienced, and to grade them on the 8 point severity rating scale described in Chapter 2. Additionally, signs not necessarily reported by the subject, e.g. unsteadiness, but observed by the investigator were recorded and rated on the eight point scale.

The small battery was performed 6 times before the loading dose was started, 2 to 4 times during loading depending on the loading time, and a further 9 times during maintenance.

Full test battery

The tests administered at steady state were choice reaction time (total time, latency and motor time), critical flicker frequency, continuous attention (correct, false positive and total errors), decision making time, Gibson's spiral maze time, Gibson spiral maze errors and the two tests (digit symbol substitution and sway) from the small battery. Paired word association was not performed because the subjects were already familiar with all of the word sets from the young/elderly study. The order of administration was as follows: digit symbol substitution, Gibson's spiral

maze, sway, decision making time, continuous attention, choice reaction time (total choice reaction time, choice reaction latency and choice reaction motor time) and critical flicker frequency. The full battery was performed once at the beginning of the control period, and again 60 minutes after the commencement of the maintenance infusion.

STATISTICAL ANALYSIS

The main (drug) effect

In order to assess whether chlormethiazole produced psychomotor impairments of the measures in the small battery, a comparison was made between the mean score of the pre-drug controls, and the mean score on chlormethiazole maintenance using Wilcoxon Signed Ranks. For the full battery, each pre-drug control value was compared with the value during steady state using Wilcoxon Signed Ranks.

Stable drug effect versus acute tolerance

For constant plasma concentrations, in the absence of acute tolerance, the gradient of drug effect over time should be zero. The gradients of the plasma concentration-time and effect-time plots were calculated for the maintenance period. The actual gradients were then compared with zero gradient to assess whether there were any overall changes in the direction of improvement or impairment. These comparisons were also made using Wilcoxon Signed Ranks.

Sensitivities of tests to chlormethiazole

Z scores (see Chapter 2 - Statistical Methods) and percentage changes from control scores were calculated for each variable in order to assess the pattern of change.

RESULTS

Plasma concentrations

The mean plasma concentrations from the six subjects during the infusions are shown in Figure 5.3. Individual data, means, and standard deviations are shown in Table 5.1. Four of the 6 subjects were within $0.2 \mu\text{g}\cdot\text{ml}^{-1}$ (13%) of the $1.5 \mu\text{g}\cdot\text{ml}^{-1}$ target at the end of the loading infusion. The other 2 subjects had much higher peak levels, $4.11 \mu\text{g}\cdot\text{ml}^{-1}$ for subject 1 and $3.47 \mu\text{g}\cdot\text{ml}^{-1}$ for subject 2. The mean overall plateau concentration from 10 minutes maintenance (10M) to 90 minutes maintenance (90M) was $1.33 \mu\text{g}\cdot\text{ml}^{-1}$ which was within 11% of the target concentration.

Subjective effects

The subjective effects of chlormethiazole (means and standard deviations) from the visual analogue scales are shown in Figure 5.4. Subjects felt significantly more drowsy, dizzy, bored, muzzy, clumsy and ill after chlormethiazole ($P < 0.05$). Subjective eye and nasal symptoms were also greater during the drug infusion than during the control period ($P < 0.05$).

Adverse symptoms and observed drowsiness

All subjects showed signs of drowsiness, the maximum observed scores ranging from 1 to 6 on the severity scale. All subjects also experienced nasal symptoms such as itchiness, secretion and sneezing, and 4 had effects on the eyes such as lacrimation and redness. Headache, clumsiness, dizziness, stumbling, blurred vision, incoherence and talkativeness were also reported or observed in 5, 4, 2, 2, 2 and 2 subjects respectively. The overall severity of adverse symptoms was assessed by summing the scores for each subject at each timepoint.

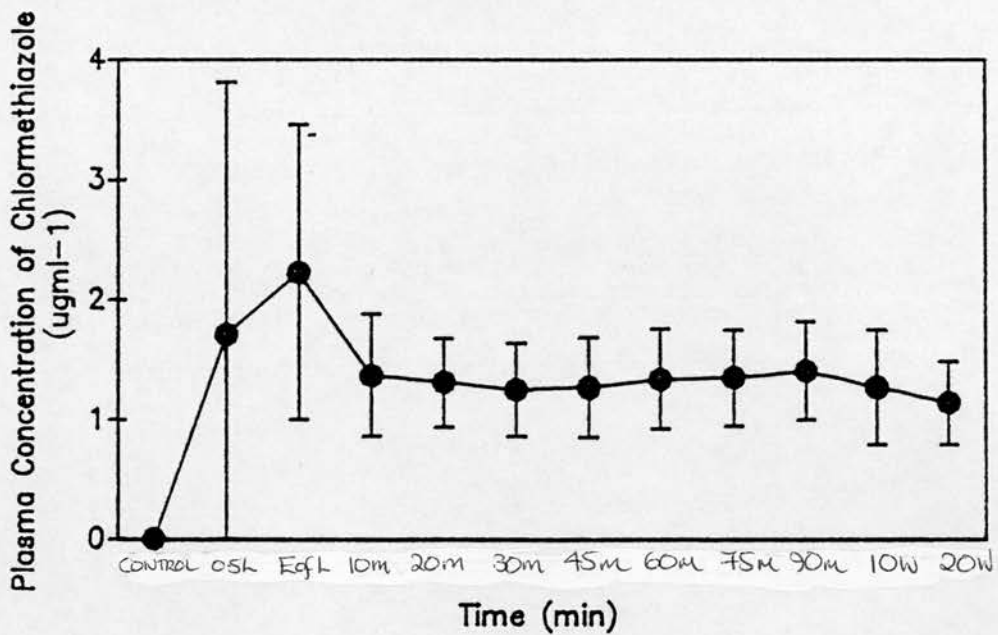


Figure 5.3. Plasma concentrations of chlormethiazole. Means and standard deviations for the 6 subjects during loading and maintenance infusions and during washout. Loading times varied. L - loading, M - maintenance, W - washout i.e. after end of infusion. 0.5L - half loading time, EoL - end of loading infusion, 10M to 90M - 10 minutes to 90 minutes of maintenance infusion, 10W - 10 minutes washout, 20W - 20 minutes washout.

Time	SUBJECT						Mean	s.d.
	1	2	3	4	5	6		
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5 L	5.79	2.20	0.57	0.67	0.52	0.54	1.71	2.10
E of L	4.11	3.47	1.45	1.59	1.45	1.30	2.23	1.23
10min M	2.28	1.50	1.01	1.37	0.82	1.24	1.37	0.51
20min M	1.96	1.14	1.17	1.45	0.86	1.27	1.31	0.37
30min M	1.84	0.98	1.03	1.61	0.86	1.18	1.25	0.39
45min M	1.79	1.04	1.15	1.73	0.68	1.25	1.27	0.42
60min M	1.76	0.97	1.31	1.83	0.78	1.38	1.34	0.42
75min M	1.89	0.99	1.40	-	0.93	1.14	1.35	0.40
90min M	1.97	1.04	1.46	1.71	0.87	1.39	1.41	0.41
10min W	1.94	0.78	1.27	1.70	0.77	1.16	1.27	0.48
20min W	1.60	0.80	1.11	1.42	0.71	1.22	1.14	0.35

Table 5.1. Individual plasma concentrations ($\mu\text{g}\cdot\text{ml}^{-1}$), means and standard deviations (s.d.) of chlormethiazole for subjects during and after loading and maintenance infusions to a target plateau concentration of $1.5 \mu\text{g}\cdot\text{ml}^{-1}$. L- loading dose, E of L - end of loading dose, M - maintenance dose, W - washout. N.B. Loading times varied. One sample (-) was broken in transport.

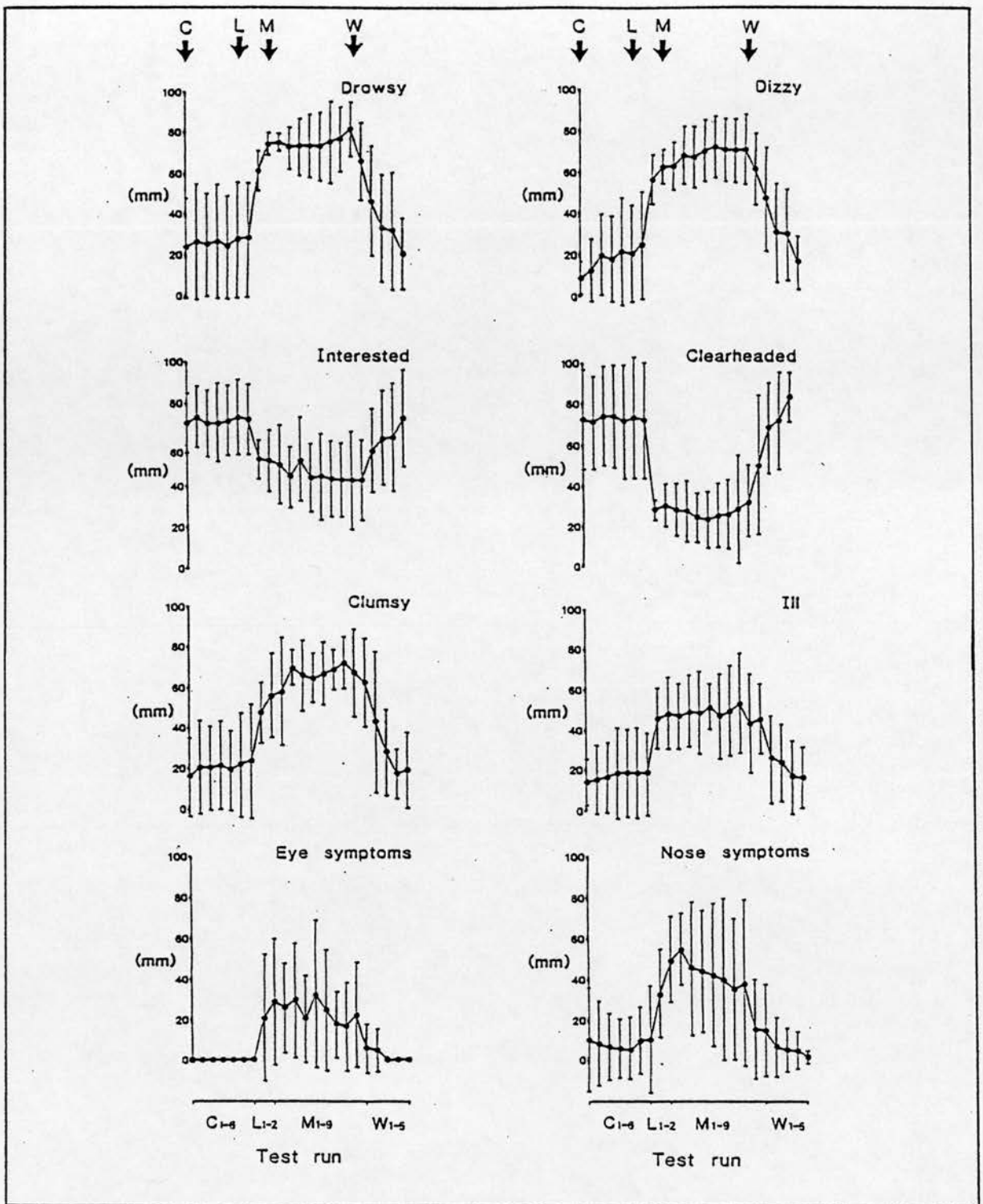


Figure 5.4. Subjective symptoms from the visual analogue scales after chlormethiazole. Figures show means and standard deviations for the six subjects. The scales used were alert-drowsy (+), steady-dizzy (+), bored-interested (+), mu^zzy-clearheaded (+), well-coordinated-clumsy (+), well-ill (+), eye symptoms - not present at all-couldn't be worse (+), nose symptoms - not present at all-couldn't be worse (+). C - control, L - loading, M - maintenance, W - washout (after end of infusion). C1-6 control runs of short battery, L1-2 loading runs of short battery, M1-9 maintenance runs of short battery, W1-2 washout runs of short battery. Test runs started at 7 minute intervals. There was a gap of approximately 30 minutes between the last maintenance run on the short battery and the first washout run. The total time from the start of C1 to the end of W5 was approximately 3 hours.

The mean severity scores for the adverse symptoms excluding drowsiness and those for drowsiness are shown in Figures 5.5 a and 5.5 b.

Small battery

Sway scores and correct digit symbol substitution scores (means and standard deviations) are shown in Figure 5.6. The subjects swayed more, and scored less on digit symbol substitution during the drug infusion ($P < 0.05$). The subjects tended to be more impaired during the loading dose than immediately after, but then became more impaired again as time went on.

Stable drug effects versus acute tolerance

The gradients for the plasma concentration-time profile during the maintenance infusion are shown in Table 5.2 along with the gradients of the slopes for the visual analogue scales, the adverse symptoms and the objective tests. The level of statistical significance for these gradients compared to zero are also shown.

The slopes were significantly different from zero for bored-interested, steady-dizzy, the nasal symptoms from the visual analogue scales, the adverse symptoms and digit symbol substitution ($P < 0.05$). The changes were in the direction of deterioration for boredom, steadiness and digit symbol substitution. However, the nasal symptoms from the visual analogue scales, and the overall adverse symptoms improved significantly during maintenance. There was also a trend towards improvement in the visual analogue scale for eye symptoms. There was no evidence of acute tolerance to drowsiness as assessed by the subjects' visual analogue scores, and by scores from the observer rating scale.

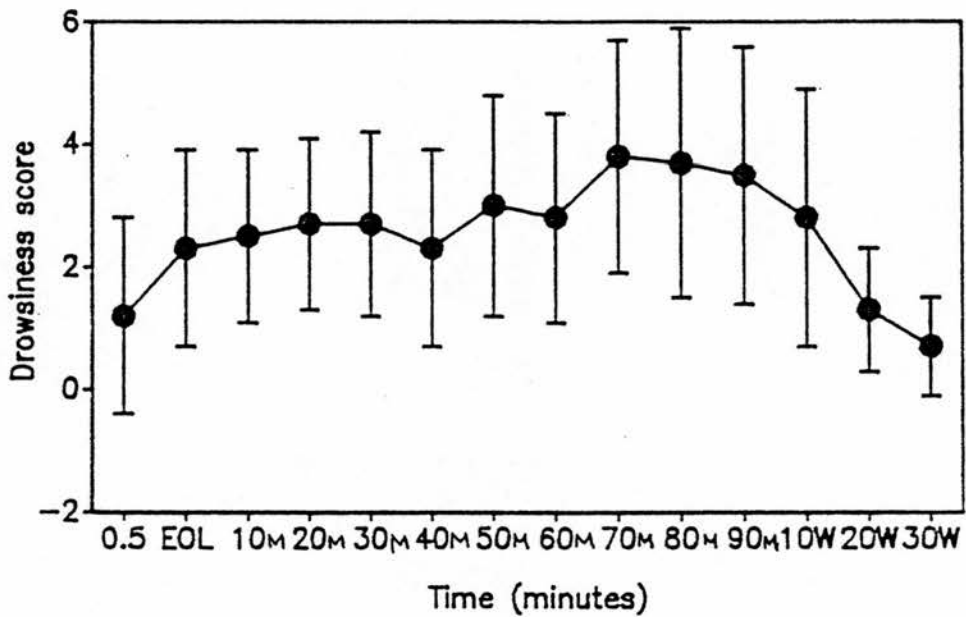
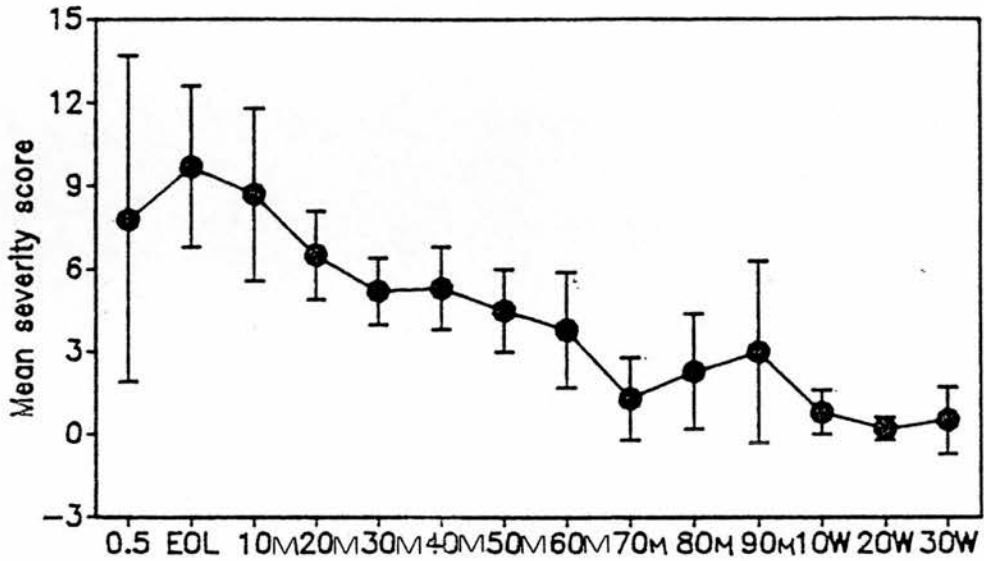


Figure 5.5. Scores from adverse signs and symptoms from the eight point severity scale. Results are presented as means and standard deviations for the group. The upper graph shows the mean of the summed severity scores for the individuals. Drowsiness was not regarded as an adverse symptom, but was recorded on the scale and analysed separately. Mean scores for drowsiness are shown in the bottom graph.

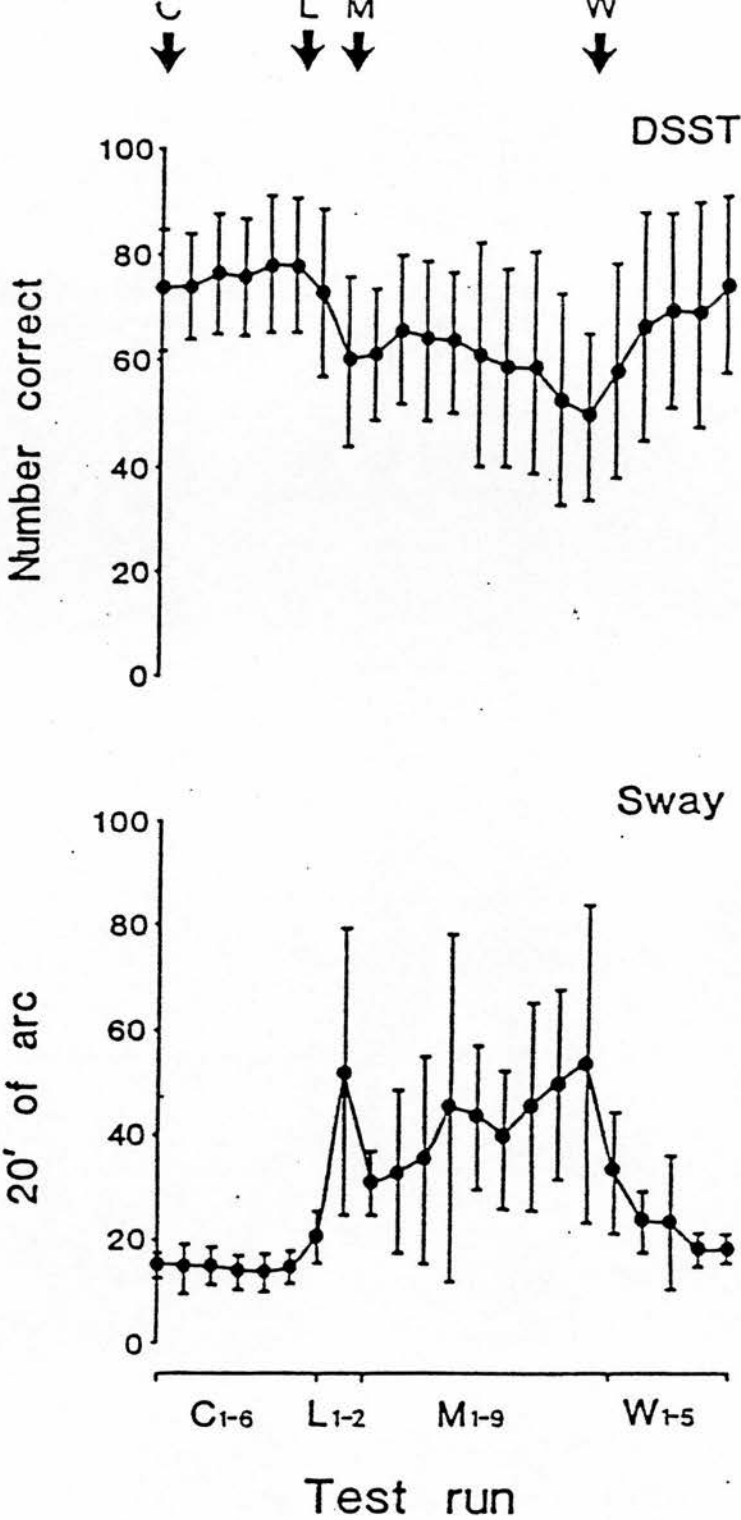


Figure 5.6. Scores from digit symbol substitution and sway after chlormethiazole. Figures show means and standard deviations for the six subjects. L - loading, M - maintenance, W - washout (after end of infusion). C1-6 control runs of short battery, L1-2 loading runs of short battery, M1-9 maintenance runs of short battery, W1-2 washout runs of short battery. Test runs started at 7 minute intervals. There was a gap of approximately 30 minutes between the last maintenance run on the short battery and the first washout run. The total time from the start of C1 to the end of W5 was approximately 3 hours.

Subject	Plasma Concentration µg ml ⁻¹ min ⁻¹	alert-drowsy (+) mm. min ⁻¹	bored-interested(+) mm. min ⁻¹	steady-dizzy (+) mm. min ⁻¹	muzzy-clearheaded (+) mm. min ⁻¹	wellcoordinated-clumsy(+) mm. min ⁻¹
1	-0.003	-0.105	-0.193	-0.040	0.383	-0.174
2	-0.004	0.369	-0.150	0.324	-0.205	0.364
3	0.006	0.207	-0.276	0.214	-0.212	0.110
4	0.005	-0.217	0.060	0.112	0.217	0.040
5	0.001	0.350	-0.369	0.317	-0.362	-0.221
6	0.001	-0.010	-0.063	0.043	-0.058	1.031

Level of significance of Wilcoxon Signed Ranks

n.s.	n.s.	<0.05 (worse)	<0.05 (worse)	n.s.	n.s.
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Subject	very well-very ill (+) mm. min ⁻¹	Eye symptoms mm. min ⁻¹	Nose symptoms mm. min ⁻¹	Adverse Symptoms score. min ⁻¹	digit symbol substitution score. min ⁻¹	sway 20' of arc. min ⁻¹
1	-0.114	-0.348	-0.788	-0.025	-0.019	-0.544
2	0.417	-0.598	-0.612	-0.102	-0.200	0.551
3	0.286	0.957	-0.193	-0.115	-0.400	0.468
4	-0.071	-0.410	-0.933	-0.093	-0.088	0.070
5	-0.038	-0.479	0.102	-0.057	-0.743	1.565
6	-0.597	-0.080	-0.560	-0.099	-0.017	0.143

Level of significance of Wilcoxon Signed Ranks

n.s.	n.s.	<0.05 (improved)	<0.05 (improved)	<0.05 (worse)	n.s.
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Table 5.2. Gradients of plasma concentration, visual analogue scores, adverse symptoms, digit symbol substitution and sway over time during the maintenance infusion, and level of significance of from comparisons of gradients with 0 (i.e. true plateau condition) using Wilcoxon Signed Ranks test. The plasma concentrations used were from 10 minutes of maintenance until the end of the infusion. Adverse symptoms were from the adverse symptoms chart using the 8 point scale. (Improved) - effects lessened over time, (worse) - effects increased over time, n.s. - not significant.

Full battery

The mean and standard deviation of the tests before and during the infusions are shown in Table 5.3, along with the percentage change, the number of shifts in standard deviation and a summary of the statistical significances.

All of the tests except choice reaction motor time (median), critical flicker frequency (fusion to flicker) and digit symbol substitution errors showed significant changes in the direction of impairment ($P < 0.05$). There were too few values on the decision making time test to be able to assess statistical significance.

On a percentage basis, the biggest changes were in error scoring on continuous attention (false positive and total errors), error scoring on Gibson's spiral maze, and on sway. Scores were increased by 325, 255, 215 and 260 % respectively. When changes were expressed in terms of the number of *multiples of the* standard deviation, a different pattern emerged. The biggest changes in this case were on the reaction time measures (total choice reaction time, choice reaction latency and decision making time) and sway. *Changes in* ^{*multiples of*} the standard deviation of the median for total choice reaction time, choice reaction latency and decision making time were 9.3, 12.8 and 9.8 - fold respectively. There was a 12.6 x increase in sway.

TEST	Control Mean (s.d.)	Chlormethiazole Mean (s.d.)	% Change	Z Score	Significance of Wilcoxon Signed Ranks
Total mean choice reaction time (msec)	489 (46)	1232 (1000)	152	16.0	<0.05
Mean choice reaction latency (msec)	347 (15)	948 (895)	173	40.0	<0.05
Mean choice reaction motor time (msec)	143 (41)	293 (143)	105	3.7	<0.05
Total median choice reaction time (msec)	476 (33)	782 (342)	64	9.3	<0.05
Mean choice reaction latency (msec)	336 (14)	515 (191)	53	12.8	<0.05
Motor choice reaction motor time (msec)	156 (64)	267 (156)	71	1.7	n.s.
Critical flicker to fusion frequency (Hz)	33.6 (5.0)	29.6 (4.7)	12	0.8	<0.05
Critical fusion to flicker frequency (Hz)	30.3 (6.0)	26.9 (6.7)	11	0.6	n.s.
Mean critical flicker frequency (Hz)	32.0 (5.4)	28.0 (4.4)	13	0.7	<0.05
Continuous attention (number correct)	33 (6)	16 (6)	52	2.8	<0.05
Continuous attention (false positive)	4 (3)	17 (9)	325	4.3	<0.05
Continuous attention (total errors)	11 (9)	39 (10)	255	3.1	<0.05
Mean decision making time (msec)	566 (101)	>1808 (>1685)	>219	>12.3	-
Median decision making time (msec)	551 (111)	1642 (1828)	198	9.8	-
Gibson spiral maze time (sec)	22.0 (3.2)	31.7 (8.4)	44	3.0	<0.05
Gibson spiral maze errors	11.7 (3.4)	36.8 (22.6)	215	7.4	<0.05
Sway (20min arc)	15 (3)	54 (30)	260	12.6	<0.05
Digit symbol substitution (number correct)	77 (13)	49 (16)	36	2.2	<0.05
Digit symbol substitution errors	0 (0)	1 (2)	ND	ND	n.s.

Table 5.3. Mean, standard deviation (s.d.), % Change, Z scores and statistical significances on the Wilcoxon Signed Ranks test for psychomotor test scores at control and after 90 minutes of maintenance on chlormethiazole for the 6 subjects. Target plateau - 1.5 ug.ml^{-1} . % Change and Z scores were calculated on the group data. Subject 5 scored several decision making time values greater than 10 seconds after chlormethiazole. The decision making time program will not record values greater than this, and so the mean decision making time for subject 5 is unspecified, as is the group mean. Only 5 sets of data were available for decision making time, and so a two-tailed test could not be performed. ND - not defined (division by zero).

DISCUSSION

Pronounced sedation was seen in all subjects after chlormethiazole, and all of the objective and subjective measures demonstrated statistically significant effects of the drug as we have noted previously (Fagan et al., 1990). Recovery was also rapid after cessation of the infusion reflecting the short half-life of the drug. This rapid recovery has been reported previously after oral doses of chlormethiazole (Briggs et al., 1980; Stevenson et al., 1982; Fagan et al., 1990).

There was no evidence of tolerance to the sedative or objective effects during the maintenance infusion. Indeed the effect on digit symbol substitution performance actually increased significantly over time although the plasma concentrations remained constant. Sway scores increased in five of the six subjects during the course of the maintenance infusion, though this was not statistically significant. Additionally, two of the visual analogue scales (bored-interested and steady-dizzy) demonstrated an increased rather than a decreased effect with time.

The detrimental influence of fatigue and boredom on performance is well known (e.g. Mann, 1985), and this may have been the reason for the worsening performance. However, it is also possible that true steady-state was not reached in all parts of the brain during the experiment, and that a pharmacokinetic "deep compartment" (see Paalzow, 1981) continued to fill up with drug. The rapid recovery after the infusion ceased argues against this hypothesis.

Nasal and eye symptoms were the only visual analogue scales changes that decreased during the maintenance infusion, and this was reflected in the results from the severity rating scale for the symptoms which also showed considerable improvement during maintenance. These results suggest that there was no acute tolerance to the

hypnotic effect of the drug during the period of the infusion, but that acute tolerance to the side-effects (nasal irritation in particular) did occur.

The tests which demonstrated the largest effects (in terms of changes in standard deviation) were those involving a latency component of reaction time (total choice reaction time, choice reaction latency, decision making time) and sway. The least affected measure appeared to be critical flicker frequency. Continuous attention total errors, Gibson spiral maze errors and sway were most affected in terms of percentage change.

The effect of chlormethiazole on critical flicker frequency, though statistically significant, was small. This is surprising in view of the high degree of sedation of the subjects in the study and the reported sensitivity of this test (Smith and Misiak, 1976; Hindmarch, 1980). Arousal is believed to be one of the major determinants of critical flicker frequency (Eysenck and Eysenck, 1985), and it would thus be expected to be greatly affected by a substantial dose of a hypnotic. However, the subjects had considerably more difficulty remembering how to do the test when they were drowsy. This may have substantially decreased its sensitivity.

Several authors have shown Gibson's spiral maze to be more affected (in terms of statistical significance) by drug effects (particularly benzodiazepines) than reaction time or digit symbol substitution (Tansella et al., 1974; Bond and Lader, 1972; Salkind and Silverstone, 1975; Zimmerman Tansella et al., 1979). Gibson's spiral maze also appeared to be more affected after chlormethiazole in an elderly group of healthy subjects than in a young group (Fagan et al., 1990). This may be interpreted as indicating a greater sensitivity of Gibson spiral maze errors to the effects of these drugs.

It was not clear, however, how much of the pattern of effects was due to differences in test sensitivities,

and how much was due to a specific impairment pattern of chlormethiazole. Some authors (e.g. Stone, 1984) use level of statistical significance as the criterion of sensitivity. In the presently described study the numbers were too small to use P values of less than 0.05, and this was therefore not a useful approach. This issue of test sensitivity is extremely important, and will be discussed in some detail in Chapter 8 (General Discussion).

The pattern of reported symptoms was as expected from clinical experience with the drug. Anecdotal reports (e.g. Hodson, personal communication) suggest that the severity of the adverse subjective symptoms of chlormethiazole may be dramatically reduced on repeated dosing. This would also indicate acute tolerance to the side effects. The initial coryza-like symptoms produced by this drug may be avoided altogether if the drug is given rapidly by infusion, and do not reappear when the plasma concentrations fall again (Scott, personal communication).

The probable reason for the nasal and eye symptoms diminishing while the other symptoms stayed the same or increased in severity is that these two symptoms may be peripheral rather than central effects of chlormethiazole. Although increased secretion from the nasopharyngeal and lacrimal glands could be explained by a centrally mediated increase in cholinergic activity, this does not account for the coryza-like symptoms which include sneezing, conjunctival irritation and coughing which are usually responses to external stimuli. Additionally, the effects can not be abolished by pretreatment with intravenous atropine (Seow et al., 1984).

Since chlormethiazole is a high clearance drug, and its elimination therefore highly dependent on liver blood flow, large intra-individual differences in plasma concentrations are to be expected. Jostell (1987) found a 3-fold intra-individual variation in peak plasma levels after two oral administrations of chlormethiazole to healthy young subjects. It was hoped that by individualising the dosages and standardising conditions this variability might be reduced. In the event, while the accuracy of predicting the plasma concentrations was reasonable during steady-state (11%), that at the end of loading was rather disappointing, and two of the subjects had very high plasma concentrations during loading.

A criticism of the study design might be that as the tests were not counterbalanced, the results would be biased because the subjects became more fatigued, bored, or pharmacokinetically "filled up" as the study progressed even though there was no rise in plasma concentration. In fact, the order of the impairments on the tests was unrelated to the order of performance. There was no obvious pattern of size of effect during the performance of the battery, and critical flicker frequency (which was the least affected) was the last test to be performed.

SUMMARY

Steady-state constant plasma concentrations of chlormethiazole were rapidly achieved and maintained in a small group of subjects. Testing was carried out at frequent intervals using a small test battery consisting of digit symbol substitution, sway, visual analogue scales and the adverse symptoms severity rating scale. The sensitivities of the tests in a larger battery were also evaluated at steady-state.

All of the visual analogue scales and the tests in the battery were significantly affected by the drug.

There was evidence of acute tolerance overall to the adverse symptoms, especially the coryza-like effects of the drug. Not all symptoms improved, however, and there was no lessening of drowsiness or improvement in the psychomotor tests.

In terms of percentage changes, the biggest effects were on continuous attention errors, Gibson spiral maze errors and on sway. In terms of Z scores, however, the most sensitive tests were sway and those with latency components of reaction time viz. total choice reaction time, choice reaction latency and decision making time.

**CHAPTER 6 - PSYCHOMOTOR EFFECTS OF ETHANOL AT
STEADY-STATE**

INTRODUCTION

Most adult humans have some experience of the effects of ethanol. Because of this familiarity and its wide usage, the effect of ethanol is often used as a yardstick against which impairments due to other drugs are compared. The abundance of data on ethanol and driving accidents has also tended to encourage the use of ethanol in the validation of test batteries.

Measuring the effects of ethanol is not particularly easy. Several reports in the literature over the years have suggested that the effects of acutely ingested ethanol are greater in the rising than the falling phase of the plasma concentration curve (see for example Mellanby, 1919; Goldberg, 1943, and the discussion of this topic in Chapter 1), suggesting acute tolerance to the drug. The principle of steady-state utilized in the last experiment would seem to be useful for clarifying this effect with ethanol. This may also be the optimal condition for assessing the pattern of impairment produced by the drug.

The first experiment in this Chapter was a preliminary pharmacokinetic experiment which was designed to determine individual rates of metabolism of ethanol for a number of male subjects. These rates of metabolism were used to calculate maintenance doses for the subjects in the main study. In between, some dose ranging work was performed in order to find a suitable loading and maintenance regime that allowed high concentrations of ethanol to be achieved, maintained and tolerated by the subjects. The main experiment was a double-blind crossover study in which the subjective and objective effects of constant venous plasma and expired concentrations of ethanol were observed. The breath concentrations were used to provide a practical estimate of arterial concentration (Goldstein, 1983), allowing arterial-venous differences to be evaluated. The data

was examined to determine whether acute tolerance occurred, and to establish whether, at steady-state, the same tests as in the chlormethiazole study (Chapter 5) were the most affected. The pattern of impairment was also examined.

PHARMACOKINETIC STUDY

Ethanol is a rather unusual drug in that very large quantities (grammes rather than milligrammes) are required to produce measurable effects on central nervous system function. The drug obeys saturated Michaelis-Menten kinetics with zero-order kinetics at blood concentrations of about $15 \text{ mg} \cdot 100 \text{ ml}^{-1}$ and above (Minors and Waterhouse, 1985). The metabolism is such that the plasma concentration of ethanol declines in an approximately linear manner with time, the rate of fall being dependent, within limits, on the original dose.

In order to maintain a steady-state, the input rate must equal the output rate for a drug. The rate of metabolism of ethanol for a number of male volunteers was therefore determined in the following experiment, so that the input rate could be calculated for the main steady-state experiment. Since the same volume of ethanol given in the pharmacokinetic study was planned as the loading dose in the steady-state experiment, the peak plasma concentrations obtained were also of interest.

METHODS

Nine healthy male, right handed fasting volunteers participated in the pharmacokinetic study. The mean age of the subjects was 26 years (range 22-31 years) and the mean weight was 73 kg (range 65-82 kg). (For individual demographic details see Table 2.1.) The standard procedures as described in Chapter 2 were followed as regarding informed consent, ethics, medical

examinations and dietary restrictions.

A blood sampling cannula (18G Venflon) was inserted in the antecubital fossa of the left arm and a control blood sample taken. The subjects then drank 70 g ethanol as 237 mls of vodka (37.5% v, Smirnoff) made up to 500 mls with undiluted orange concentrate (Kia-ora) at 09:00 hours after an overnight fast. The subjects were not informed of the exact quantity of alcohol that they were to take, but were informed that it would be "a lot". The drink was at room temperature, and was consumed over a ten minute period in the sitting position. The beverage was always made up on the morning of testing, divided into two and kept in closed containers before drinking. Subjects had to drink the liquid in each container in 5 minutes. 5 ml blood samples were taken into 5 ml fluoride oxalate (Sterilin) tubes at the end of drinking and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 10 hours for estimation of venous plasma ethanol concentrations. The samples were immediately centrifuged and the plasma transferred to 2 ml plastic plasma tubes (Sterilin) and deep frozen at -20°C to await analysis. A light lunch was permitted approximately three hours after the ethanol was consumed. The subjects remained sitting throughout the blood sampling period.

Measurement of venous plasma ethanol concentrations

The plasma ethanol concentrations were measured by gas liquid chromatography.

1 ml of freshly made up working internal standard (0.2 g.l^{-1} acetone, BDH Analar Grade) was added to 100 μl of plasma in 2 ml disposable test tubes (Sarstedt). The tubes were capped and mixed well by inversion. 4 μl aliquots were injected on to the gas liquid chromatograph (GLC, Pye Series 104) using a 10 μl glass syringe (Hamilton Scientific Glass) with a 7 cm needle. A flame ionization detector was utilized along with a 3 metre

Chromosorb G glass 0.25 inch diameter column with OV101 liquid-phase and nitrogen carrier gas (flow rate 60 ml min⁻¹). The column, ^{injector} and detector temperatures were 120°C, 200°C and 180°C respectively. Detector flow rates for hydrogen and air were 30 ml min⁻¹ and 300 ml min⁻¹. The retention time for ethanol was 1 minute, and that for acetone was 2 minutes. The limit of detection of the assay was 2 mmol.l⁻¹ (9 mg. 100 ml⁻¹).

Standard samples of known concentration were prepared in the same way with the exception that 100 µl aqueous ethanol standards (BDH Merck) were used instead of plasma. The ethanol standards used were 11, 22, 43 and 65 mmol.l⁻¹ (or 51, 101, 198 and 299 mg. 100 ml⁻¹). A set of standards was run with each set of samples analysed and a calibration curve constructed. The ratio

$$\frac{\text{height of ethanol peak}}{\text{height of acetone peak}}$$

was calculated from the chromatograms, and the unknown sample concentrations read off the calibration curve.

Pharmacokinetic calculations

The metabolism of ethanol was assumed to proceed at a constant rate (Goldstein, 1983). A crude estimate of the hourly rate of metabolism was obtained by dividing the dose (70 g or 237 ml vodka) by the time in hours to reach zero concentration in the plasma. The latter was obtained by extrapolation of a linear plot of plasma ethanol against time. It was assumed that absorption was rapid and complete, and that distribution was also rapid.

RESULTS

Sample chromatograms from a kinetics run and calibration curves for 4 different days are shown in Figure 6.1. Although there was some variation in the slopes of the calibration curves from day to day, the slopes were all linear with correlation ^{coefficients} $r > 0.99$. The interassay variability (cv) between days for the sets of standards was between 4.8% and 12.4% over the range of concentrations.

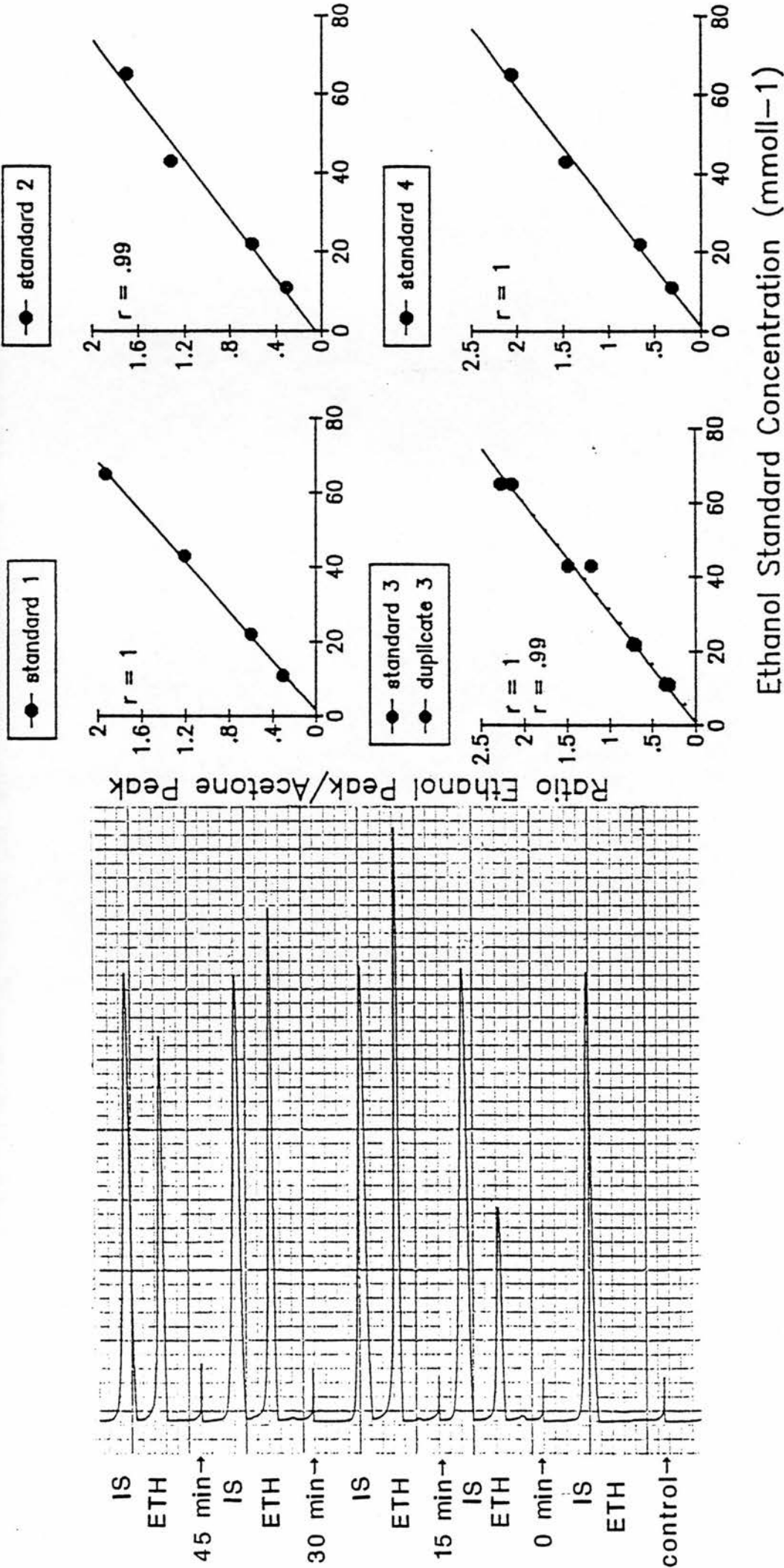


Figure 6.1. Sample chromatograms and 4 ethanol calibration curves recorded on 4 different days. IS - acetone internal standard, ETH - ethanol unknown concentration, → injection point, r = linear correlation coefficient. The 5 ethanol samples shown were the first 5 taken from subject BW in the kinetics study, and were from the control, end of ingestion (0), 15, 30 and 45 minutes post-ingestion samples. The chromatograms were run on the same day as the calibration curve which was run in duplicate.

One subject vomited shortly after consuming the 70 g ethanol, and was excluded from the rest of the study. The plasma concentration-time profiles of the other eight subjects, and the means and standard deviations for the group are shown in Figures 6.2, 6.3 and 6.4. The hourly rate of metabolism, the quantities required for the top-ups, the peak plasma concentrations and the times to peak are shown in Table 6.1.

The mean hourly metabolism rate was $124 \pm 19 \text{ mg.kg}^{-1}.\text{h}^{-1}$, and the extrapolated time to metabolise the ethanol 7.8 ± 0.9 hours. The peak plasma concentrations ranged from 87-183 mg.100 ml^{-1} (mean $120 \text{ mg.100 ml}^{-1}$), and the mean time to peak was 58 minutes.

The mean hourly volumes of vodka calculated to maintain the ethanol at constant concentrations was 30.5 ml (range 27-35 ml).

DISCUSSION

The rates of metabolism, when expressed in terms of body weight were similar, at $124 \text{ mg.kg}^{-1}.\text{h}^{-1}$ (range $105\text{-}156 \text{ mg.kg}^{-1}.\text{h}^{-1}$) to those quoted in standard textbooks ($80\text{-}150 \text{ mg.kg}^{-1}.\text{h}^{-1}$ in Bowman and Rand 1980, and $120 \text{ mg.kg}^{-1}.\text{h}^{-1}$ in Goodman and Gilman 1990 - see Rall, 1990).

When undiluted strong alcoholic drinks are taken on an empty stomach absorption may be slow because of delayed gastric emptying (Goldstein, 1983). Vomiting may also occur due to gastric irritation. In order to avoid these problems, and to make the drink more palatable, the ethanol was diluted to <20%. The absorption should therefore have proceeded fairly quickly with a lowered risk of pylorospasm and gastric irritation. However, one subject did vomit, and it seems that even with the reduced concentration gastric irritation still occurred in this individual.

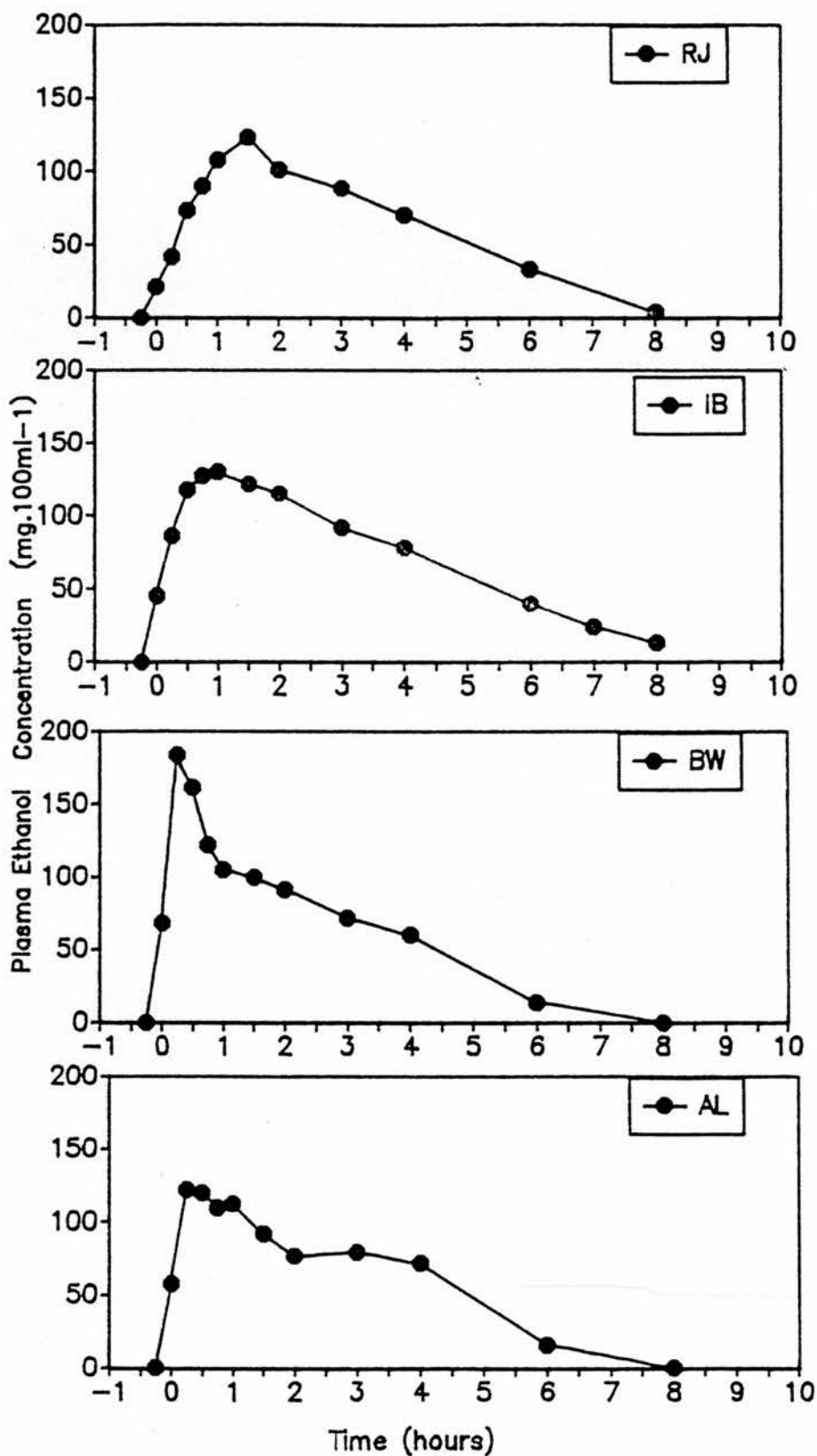


Figure 6.2. Plasma concentration time profiles for 4 subjects, RJ, IB, BW and AL after consumption of 70 g ethanol at 09:00 hours after an overnight fast.

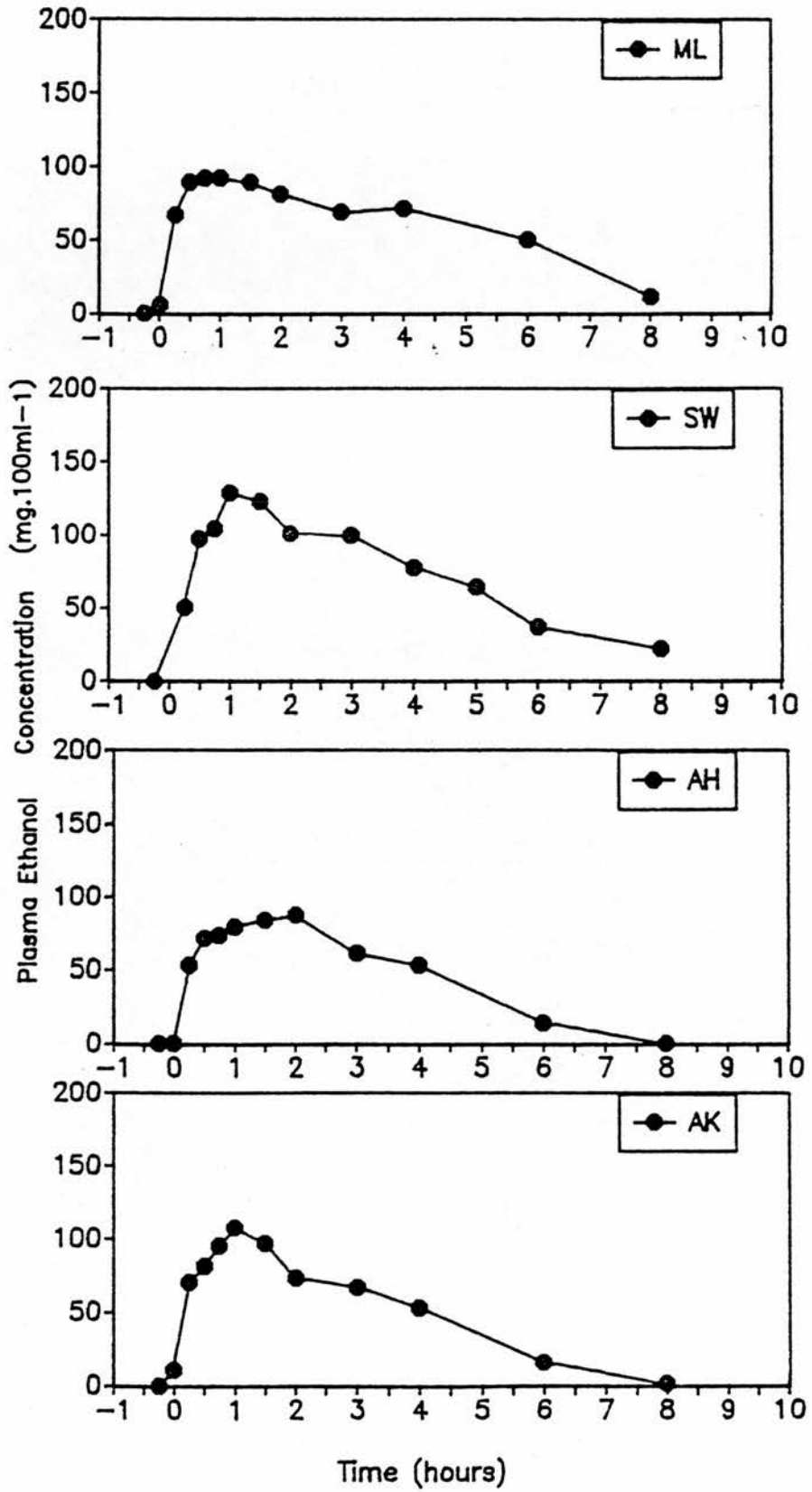


Figure 6.2. Plasma concentration time profiles for 4 subjects, ML, SW, AH and AK after consumption of 70 g ethanol at 09:00 hours after an overnight fast.

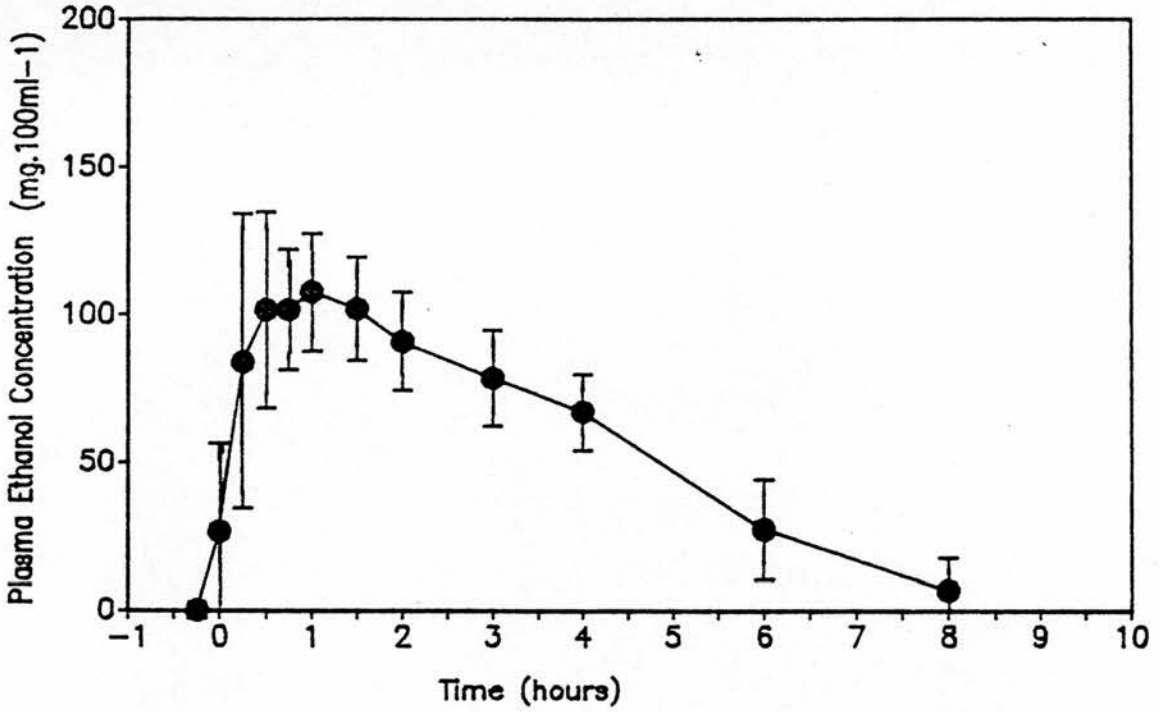


Figure 6.4. Plasma concentration time profiles for 8 subjects after consumption of 70 g ethanol at 09:00 hours after an overnight fast. results are presented as means and standard deviations.

Subject	Time to reach 0 mmoles.l ⁻¹ , by extrapolation	Rate of metabolism mgkg ⁻¹ .h ⁻¹	Volume of vodka for top-up \bar{q} ml.h ⁻¹	Peak plasma concentration mg.100ml ⁻¹	Time to peak min
1	8.2	105	29	109	90
2	8.9	114	27	130	60
3	6.7	121	35	183	15
4	6.7	151	35	121	15
5	8.7	108	27	92	45
6	8.4	114	28	128	60
7	6.8	156	35	87	120
8	8.2	121	29	107	60
mean (s.d.)	7.8 (0.9)	124 (19)	30.5 (4.1)	120 (30)	58 (35)

Table 6.1. Individual, mean and standard deviation (s.d.) of: time to reach 0 mg.100ml⁻¹ ethanol by extrapolation, the rate of metabolism, the top-up dose calculated to maintain the subjects at constant plasma concentrations, the peak plasma concentrations and time to peak ethanol concentrations for the subjects after 70 g ethanol in 10 minutes.

There was very rapid absorption with high initial plasma ethanol concentrations in subject BW. This subject had a much higher peak plasma concentration than the others (Figure 6.2), despite being the largest individual in the study. Although the peak concentrations were quite high (mean = 120 mg.100 ml⁻¹), none of them approached severely toxic concentrations (> 300 mg.100ml⁻¹, Bowman and Rand, 1980). None of the subjects showed overt signs of drunkenness, and most read books or newspapers quietly throughout.

The input rates for steady-state were calculated from the rates of metabolism (Table 6.1). These were used in dose-ranging experiments designed to find a suitable loading and maintenance regime for the main ethanol acute tolerance/ steady-state study. The dose-ranging studies are described next.

DOSE RANGING STUDIES

Having measured the peak plasma concentrations from single large doses of ethanol, the volumes required to maintain subjects at stable steady-state concentrations were calculated.

It was originally planned to use 70 g loading doses for the steady-state experiment, as in the pharmacokinetic experiment. However, when combined with the top-ups, this dose was poorly tolerated by the first subject in the steady-state experiment, although he had tolerated it well in the pharmacokinetic study.

It was necessary, therefore, to find a suitable dosage regime that would give a sufficiently high steady-state concentration to produce measurable impairments of central nervous system functions, but that would be well tolerated by the subjects.

Expired ethanol concentrations can be used to provide a reasonably close estimate of arterial blood concentrations assuming a constant blood/breath partition coefficient (Goldstein, 1983). Since brain concentrations of drugs are more closely reflected by arterial than peripheral venous concentrations, a commercially available alcohol meter (see p. 142) was used to assess arterial ethanol concentrations in three subjects.

METHODS

Four healthy fasted male subjects who had participated in the ethanol pharmacokinetic study took part in these dose ranging experiments. Their ages ranged from 23-33 years and their weights ranged from 69-84 kg. (Individual demographic details for these subjects are shown in Table 2.1.) The experimental conditions were as outlined in Chapter 2 (Experimental Subjects and Experimental Conditions). Blood sampling procedures, storage and

analysis were as described in the ethanol pharmacokinetic study. Food and other beverages were not allowed until the experimental sessions were completed.

Maintenance "Top-ups"

For each participating individual, the calculated volumes of vodka from the crude hourly rate of metabolism (ethanol pharmacokinetic study) was divided into six. This small volume was supplied at 10 minute intervals as a 50:50 vodka:orange mixture using the same brands of vodka and orange (Smirnoff and Kia-ora) as used in the kinetics study. It was hoped that, once absorption was complete, these top-ups would maintain the subjects at the same plasma concentration. The mixture was made up freshly each morning and divided into the appropriate aliquots which were kept in 10 or 12 ml plastic screwtop plasma tubes (Sterilin) depending on the volume of the top-ups.

Blood samples were taken 10 minutes before commencement of the loading dose, at the end of the loading dose, and then at 20 minute intervals for the times specified below.

Measurement of blood ethanol concentrations using expired ethanol concentrations

Expired ethanol concentrations were measured using a hand held alcohol meter (Lion Alcolmeter SD-2) identical to that used at traffic incidents by the United Kingdom Police Force. This apparatus uses an electrochemical fuel cell to detect and measure the concentration of alcohol vapour in expired breath. A built in pressure switch/timer indicates when the subject has provided a suitable sample of breath for analysis. The apparatus uses a blood/breath partition ratio of 2200, and displays the blood ethanol concentration in $\text{mg} \cdot 100 \text{ ml}^{-1}$, with an accuracy of $\pm 5 \text{ mg} \cdot 100 \text{ ml}^{-1}$.

Measurements were made on deep lung air sampled after a minimum volume of 2 litres of breath was expired.

Subjects were required to rinse their mouths out with water before breath samples were taken. Breath measurements were always made before the top-ups were taken. Thus once the top-ups started, the breath measurements were taken at 10 minute intervals.

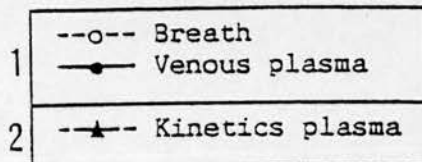
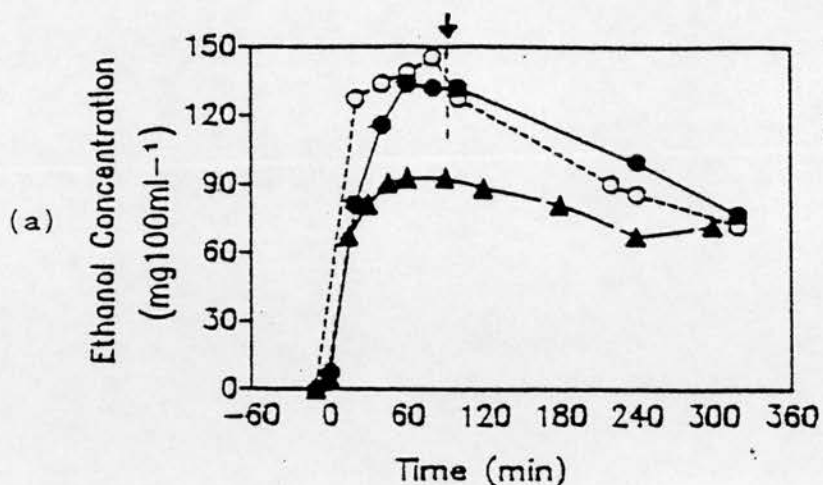
STEADY-STATE REGIMES

Details from the one session in which a subject took 70 g loading dose followed by top-ups are summarised below. Brief details of other arbitrary regimes evaluated are also given.

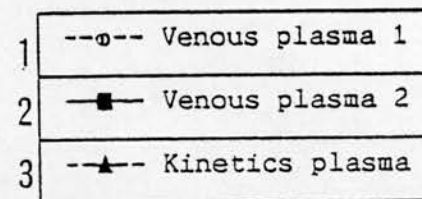
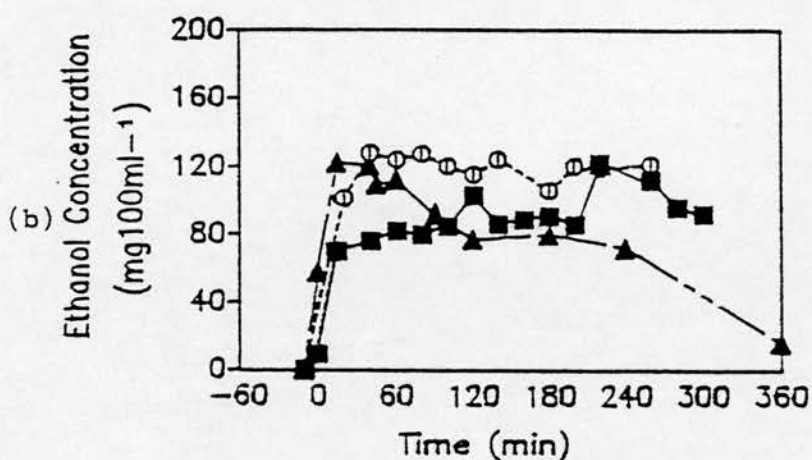
70 g loading dose + top-ups from 10 minutes on.

One subject took this dosage. Venous plasma and expired concentrations were measured, and psychomotor testing performed at 20 minute intervals using a small battery of tests. The venous plasma and breath concentrations for the subject are shown in Figure 6.5a along with the plasma concentrations from the same individual obtained in the kinetics study.

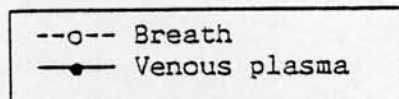
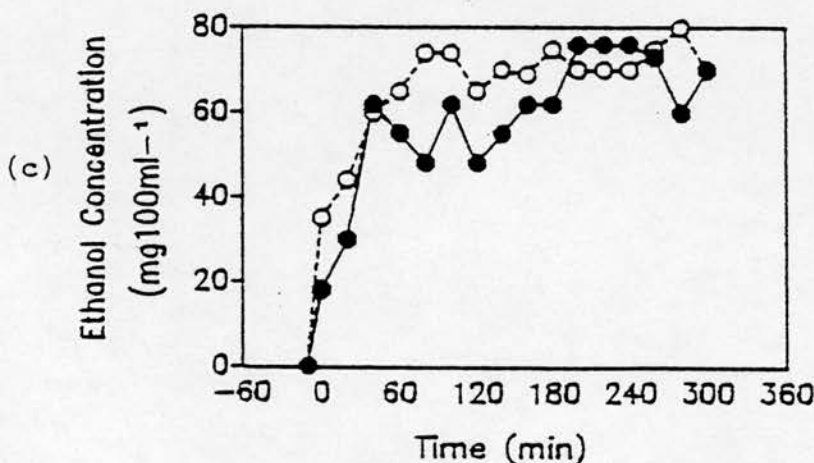
The effect of starting the top-ups at 10 minutes was to considerably raise the peak concentration. The subject coped well until approximately 100 minutes after the loading dose when he suddenly felt nauseated and vomited shortly after. He was unable to continue, and top-ups were stopped. The subject withdrew from the rest of the ethanol experiments. This individual appeared to be approaching a plateau before the top-ups stopped. Breath concentrations of ethanol were higher than venous plasma concentrations until the dosing ended, after which they were slightly lower. The subject showed substantial psychomotor impairments and changes in the visual analogue scales on this dosing regime.



Subject ML. Results from two experiments. 1) 70 g loading dose plus top-up doses from 10-100 min. Subject vomited at 100 min and top-ups ceased. 2) 70 g single dose given in pharmacokinetic study.



Subject AL. Results from three experiments. 1) 50 g loading dose plus top-up doses from 10 min. 2) 60 g loading dose plus top-up doses from 60 min. 3) 70 g single dose given in pharmacokinetic study.



Subject RJ. Results from one experiment. 50 g loading dose plus top-up doses from 60 min.

Figure 6.5. Plasma and expired (breath) concentrations of ethanol from three subjects in the dose ranging experiments. Plasma concentrations for the same subjects in the kinetics study are shown in (a) and (b) for comparison.

Based on the experience with this individual the loading dose was reduced.

50 g + top-ups from 10 minutes on

This regime was followed in one volunteer. Only venous plasma samples were measured. The plasma concentrations were high in this individual when top-ups were started at 10 minutes. Indeed the peak plasma concentration was higher than that obtained for this subject in the pharmacokinetic study where the dose was 70 g (Figure 6.5b). Interestingly, the plasma concentrations in this dose-ranging experiment were also considerably higher than after a 60 g loading dose with top-ups starting at an hour (see p. 152, AL), even though the amount of ethanol in the dose ranging study was less (58.8 g total in an hour). The plasma concentrations were, however, relatively stable indicating that the top-ups provided a good approximation of steady-state.

Because of the unpredictably high concentrations found when the top-ups started early during absorption, the top-ups were started after an interval of one hour.

50 g + top-ups from 60 minutes on

Two subjects were evaluated on this regime. Figures 6.5c and 6.6a show the plasma and expired concentrations from these individuals. Although reasonably good stable breath concentrations were obtained, the venous concentrations tended to vary more. Both subjects had breath concentrations which approached the U.K. legal driving limit, however, psychomotor testing showed that the subjects were hardly affected at these concentrations.

Based on these experiments, the loading dose was raised to 60 g, and the top-ups started at an hour.

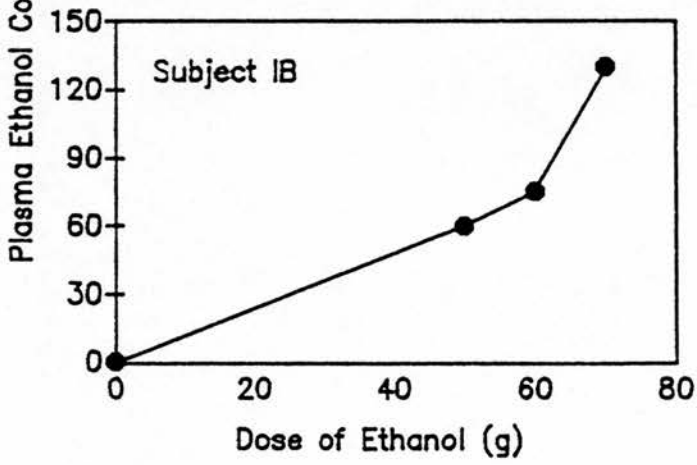
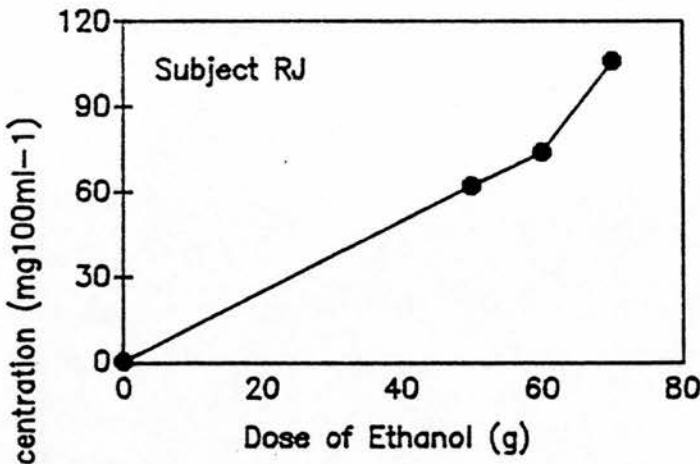
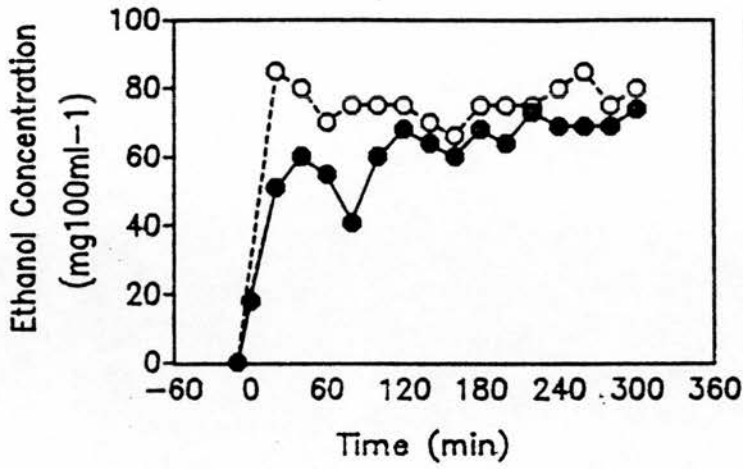


Figure 6.6.(a, top), (b, middle) and (c, bottom). (a) shows plasma and expired (breath) concentrations of ethanol from one subject in a dose ranging experiment. (b) and (c) show plasma ethanol concentrations at 60 minutes for subjects RJ and IB over the dose range 50 - 70 g ethanol.

60 g + top-ups from 60 minutes on

This regimen was used for the main experiment (next section) involving 8 subjects.

Taking the plasma concentration at an hour after loading, venous plasma concentration curves could be constructed for subjects IB and RJ over the dose range 50-70 g using the data obtained in the pharmacokinetic, dose ranging and steady-state experiments (Figures 6.6b and 6.6c). In both cases the change in the plasma concentration was greater between 60 and 70 g ethanol than between 50 and 60 g ethanol.

COMMENT

These experiments indicated that the top-ups produced reasonably stable breath and venous plasma concentrations of ethanol, and that the breath concentrations were more stable than the plasma. However, the ethanol concentrations could be unpredictably high when top-ups were started at 10 minutes. Furthermore, 50 g loading with top-ups started at an hour gave plasma concentrations in a range known to produce only marginal effects, if any, on many psychomotor tests (Wallgren and Barry, 1970; Levine et al., 1975; Mills and Bisgrove, 1983; Wilson and Plomin, 1985; Fagan et al., 1987). From the kinetics experiment, the concentrations reached after 70 g were also considered to be too high in some individuals to be sustained for several hours. A loading dose of 60 g was therefore chosen for the main experiment since the concentrations obtained were sufficient to produce measurable impairments, but were well tolerated by the participating subjects. The top-ups were started at an hour when the peak plasma concentration was reached in most subjects.

ETHANOL ACUTE TOLERANCE/STEADY-STATE STUDY

The purpose of this study was to determine whether, using a constant steady-state methodology, acute tolerance to ethanol could be found. The magnitude and pattern of the impairments at steady-state were also examined.

METHODS

Eight healthy male volunteers participated in this study including seven subjects who had participated in the pharmacokinetics study. Since the rates of metabolism were found to be fairly constant (Table 6.1) another subject was recruited and given standard top-up doses amounting to 30 ml.h⁻¹ vodka. A blood sampling cannula (18G Venflon) was inserted in the antecubital fossa of the left arm at approximately 08.45 hours, and a 5 ml specimen obtained. The subjects were allowed 15 minutes to settle. A visual analogue form was completed and the full battery of tests performed, followed by two runs of a small test battery. At 10:00 hours the subjects drank either 60 g ethanol as a 50:50 mixture of Smirnoff vodka and Kia-ora orange drink over ten minutes or an equivalent volume of water and orange drink. The total volume consumed was 406 ml. A blood sample was taken and testing with the short battery of tests started. Blood sampling, expired breath sampling and testing continued every twenty minutes for the next five hours. Subjects rinsed their mouth out with water before testing with the Alcolmeter in order to remove any residual ethanol. Testing of expired air always took place before each top-up. At 11:00 hours, one hour after the loading dose, the subjects began to drink top-up doses of the mixture individually calculated to maintain their plasma concentrations at a constant level, or a water-orange mixture. The top-ups were given as a 50:50 vodka:orange mix, and were taken every 10 minutes until the end of the experiment. The full battery of tests was performed

again at 15:00 hours. The volume of blood taken from each individual, including blood for laboratory screening was 250 ml.

Small test battery

Subjective and objective measures of performance were assessed before and during consumption of ethanol and placebo.

The 100 mm visual analogue scales used to follow the subjective symptoms and mood changes were interested-bored, alert-drowsy, dizzy-steady, clear-headed-muzzy, sober-drunk and hangover-no hangover.

The objective tests used to assess the effects of ethanol on performance were digit symbol substitution (correct and errors), the Gibson spiral maze (time and errors), choice reaction time, tapping and body sway. The test battery took approximately 10 minutes to complete, and was performed every 20 minutes.

The small battery was performed twice (once as part of the full battery) before the loading dose was started, and a further 13 times during maintenance of steady-state.

Full test battery

The tests compared at steady state were critical flicker frequency, continuous attention (correct, false positive and total errors), decision making time, paired word association, and the tests from the small battery. The order of administration was as follows: digit symbol substitution, Gibson's spiral maze, sway, decision making time, continuous attention, choice reaction time and critical flicker frequency. Although two of the subjects had already participated in a study involving paired word association the test was still used since a

considerable period of time had elapsed and it was, at that time, considered unlikely that previous exposure to the word lists would interfere with the results.

The full test battery was performed at the beginning of the control period of the experiment, and then again after 4 hours during which steady-state plasma ethanol concentrations had been maintained. Each full run took approximately 30 minutes to perform.

STATISTICAL ANALYSIS

The main (drug) effect

In order to test for ethanol effects on psychomotor impairments per se, a comparison was made between the mean scores on maintenance for placebo and ethanol using Wilcoxon Signed Ranks. For the full battery, each control score was compared with score during steady state using Wilcoxon Signed Ranks. Comparisons were also made between baseline scores in the placebo and alcohol sessions to check that the baseline results were similar, and between the baseline and afternoon runs in the placebo session to check for time of day effects (Wilcoxon Signed Ranks).

Stable drug effects versus acute tolerance

The gradients of the plasma concentration-time and effect-time plots were calculated for the maintenance period. The gradients on ethanol were then compared with the gradients on placebo. These comparisons were also made using Wilcoxon Signed Ranks.

Z scores and mean % changes were calculated for each variable as outlined in Chapter 2 "Statistical Methods".

RESULTS

Plasma concentrations

The individual and mean plasma ethanol concentrations from the main study and the individual and mean expired ethanol concentrations for seven of the subjects are shown in Figure 6.7, 6.8 and 6.9. The alcohol meter was not available for use with the remaining subject. The mean \pm s.d. overall plateau concentrations were 94 ± 5 and 99 ± 3 mg.100 ml⁻¹ for plasma and expired concentrations respectively. The concentration gradient did not differ from zero for either venous plasma or expired concentrations. For the first 100 minutes after the end of the loading dose the mean expired concentration of ethanol was higher than the mean venous plasma concentration. This difference was statistically significant at 20, 40 and 100 minutes ($P < 0.05$). Subject SD was the individual who did not participate in the kinetics study. As can be seen from the graphs for this subject, the standard volume used for the top-ups produced stable plasma and breath concentrations.

Main effect - small battery

The subjective effects of ethanol (means and standard deviations) from the visual analogue scales are shown in Figures 6.10 and 6.11.

The subjects felt dizzier, muzzier and more drunk after ethanol than after placebo ($P < 0.02$). They also tended to feel more hung over ($P < 0.10$). Although drowsiness and boredom also increased, there was no significant difference overall between ethanol and placebo.

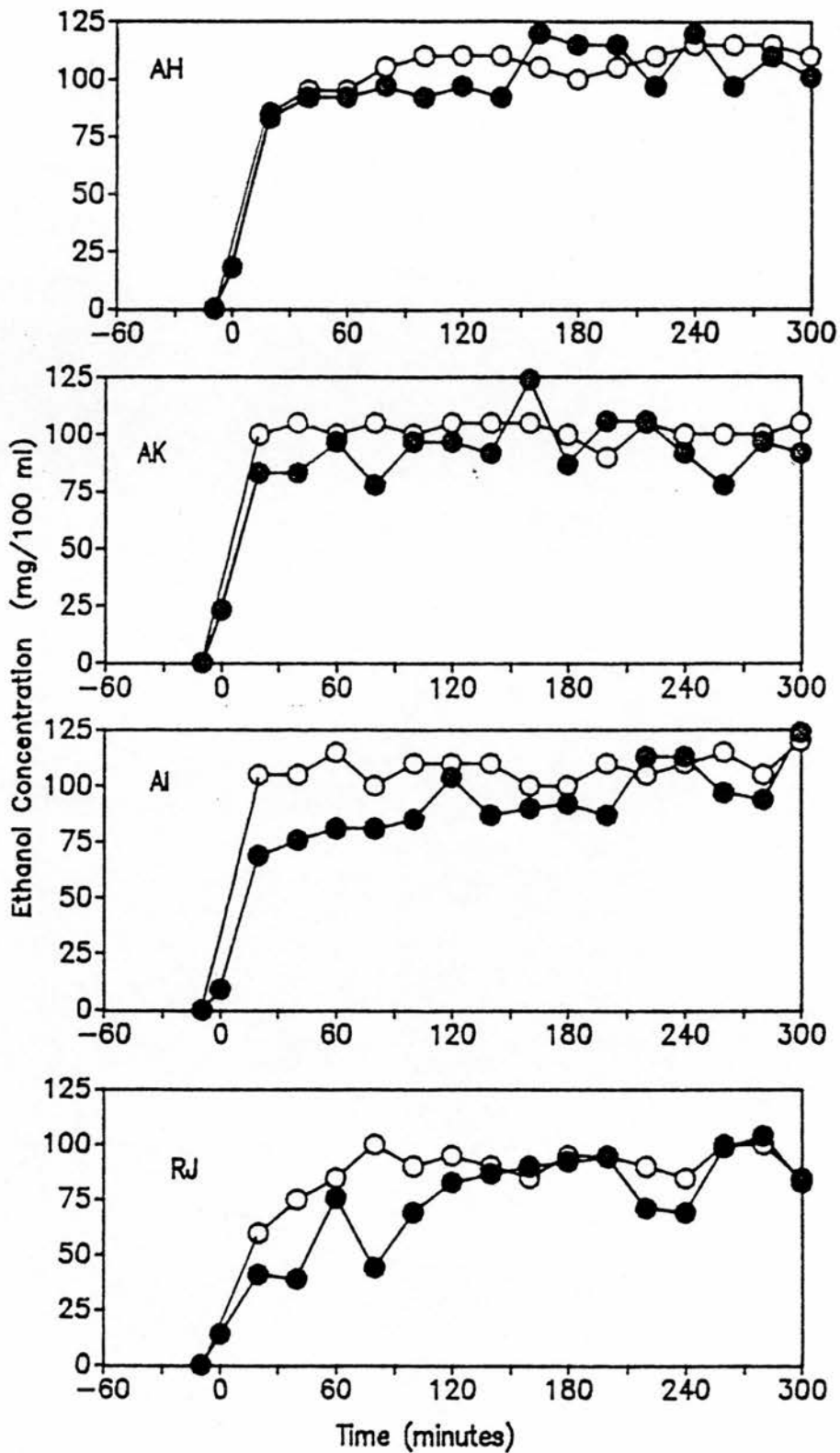


Figure 6.7. Plasma (●) and expired (○) concentrations of ethanol from four subjects, AH, AK, AL and RJ in the ethanol steady state experiment. Subjects took 60 g ethanol over 10 minutes. Top-up doses commenced at 60 minutes.

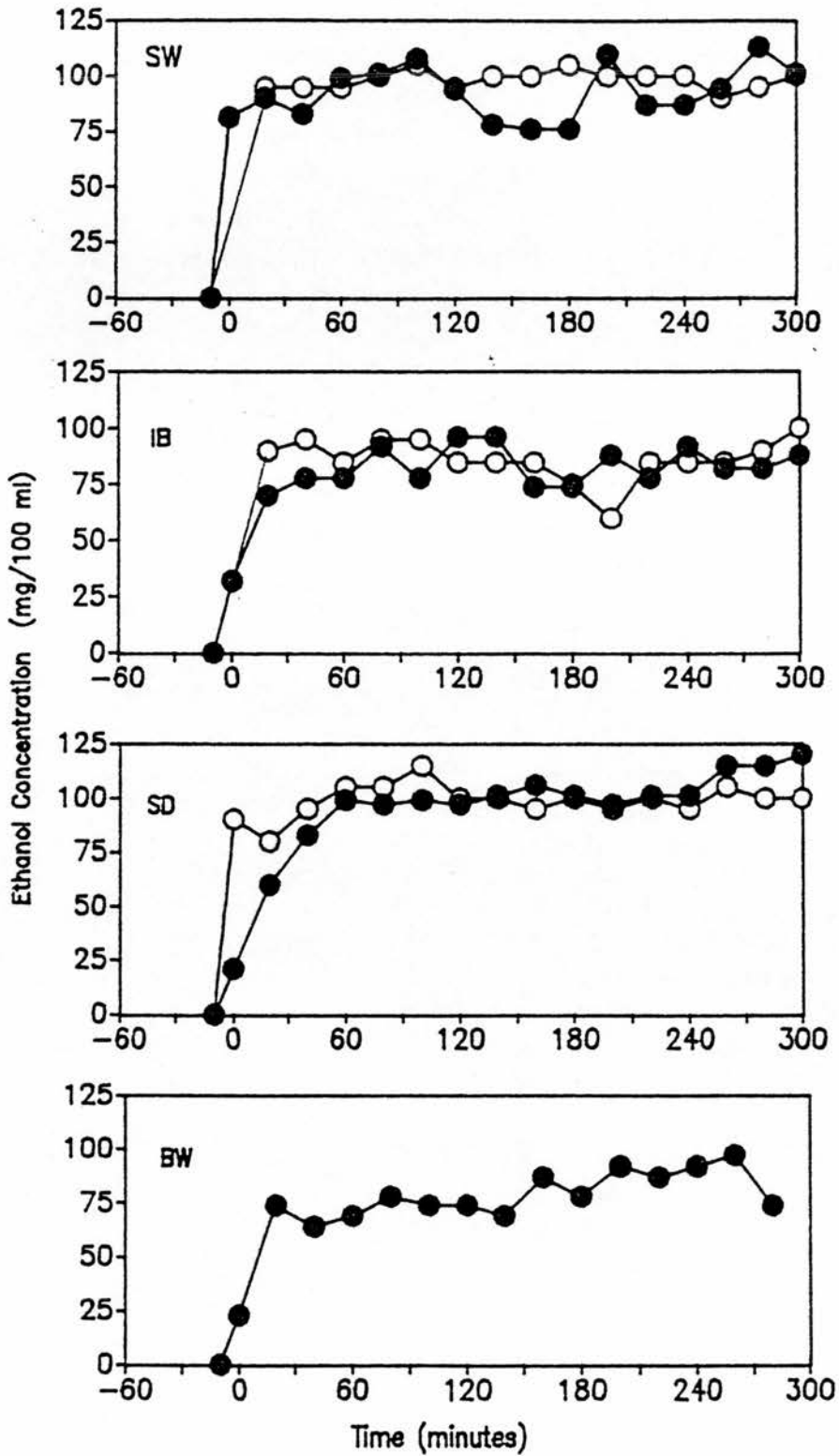


Figure 6.8. Plasma (●) and expired (○) concentrations of ethanol from four subjects, SW, IB, SD and BW in the ethanol steady state experiment. Subjects took 60 g ethanol over 10 minutes. Top-up doses commenced at 60 minutes.

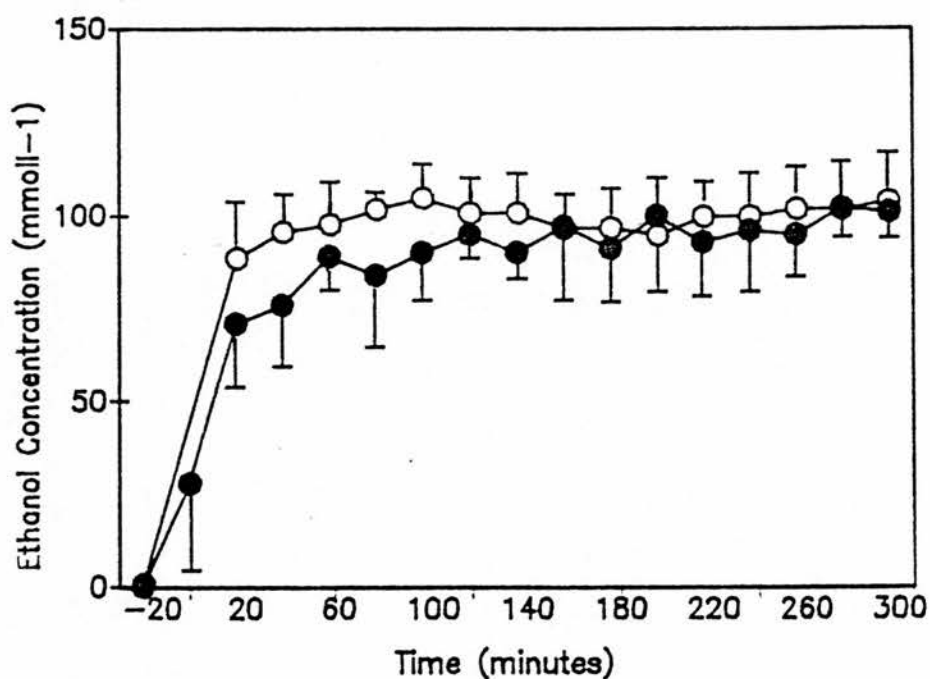


Figure 6.9. Plasma (●) and expired (○) concentrations of ethanol from subjects in the ethanol steady state experiment. Results are presented as means and standard deviations. Subjects took 60 g ethanol over 10 minutes. Top-up doses commenced at 60 minutes. $N = 8$ for plasma concentrations and 7 for expired concentrations.

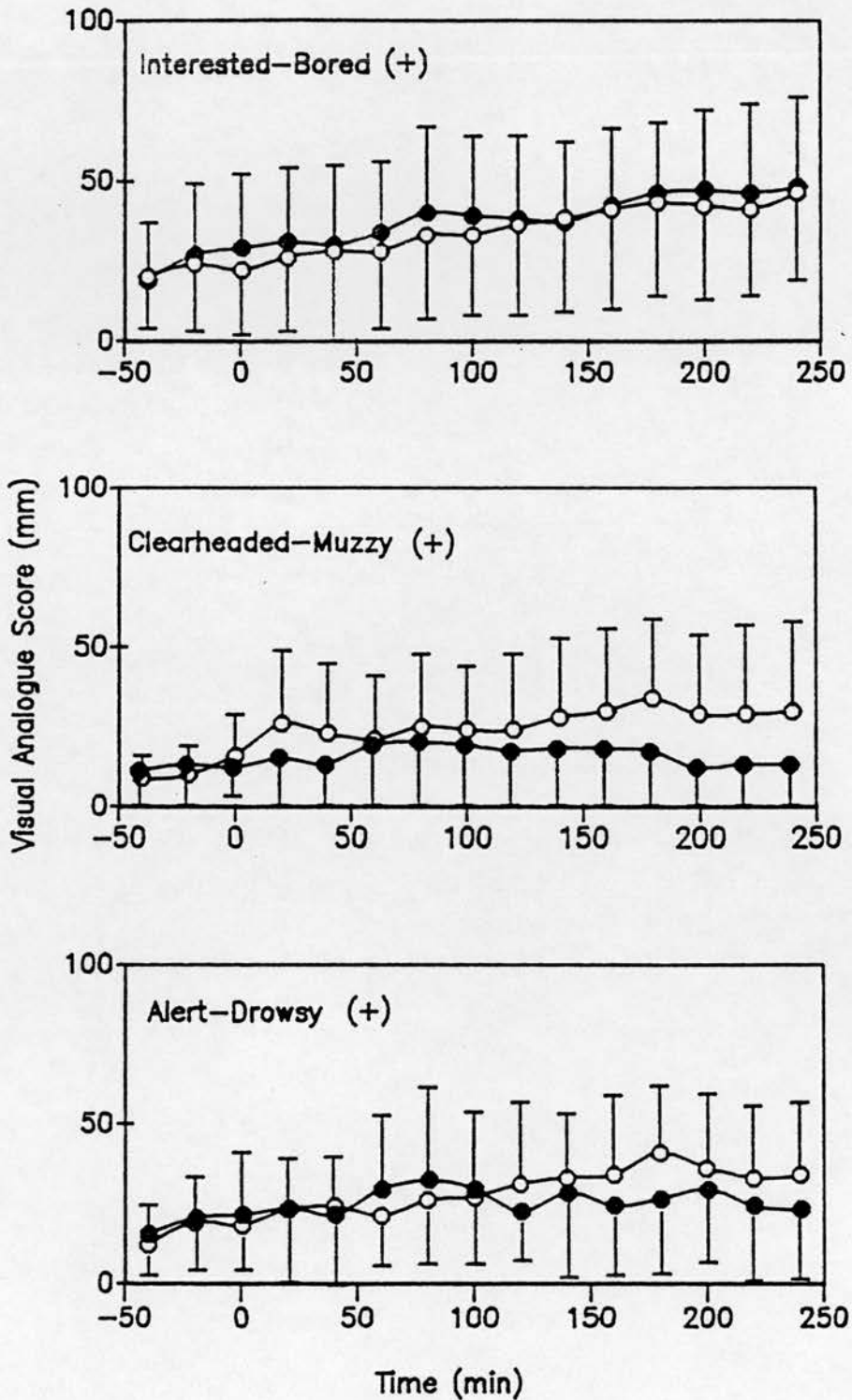


Figure 6.10. Subjective effects of steady-state ethanol (o) and placebo (●) from the visual analogue scales. Results are presented as means and standard deviations. Subjects took 60 g ethanol over 10 minutes. Time 0 - end of ingestion of main dose. Top-up doses commenced at 60 minutes. Visual analogue ratings were carried out at 20 minute intervals.

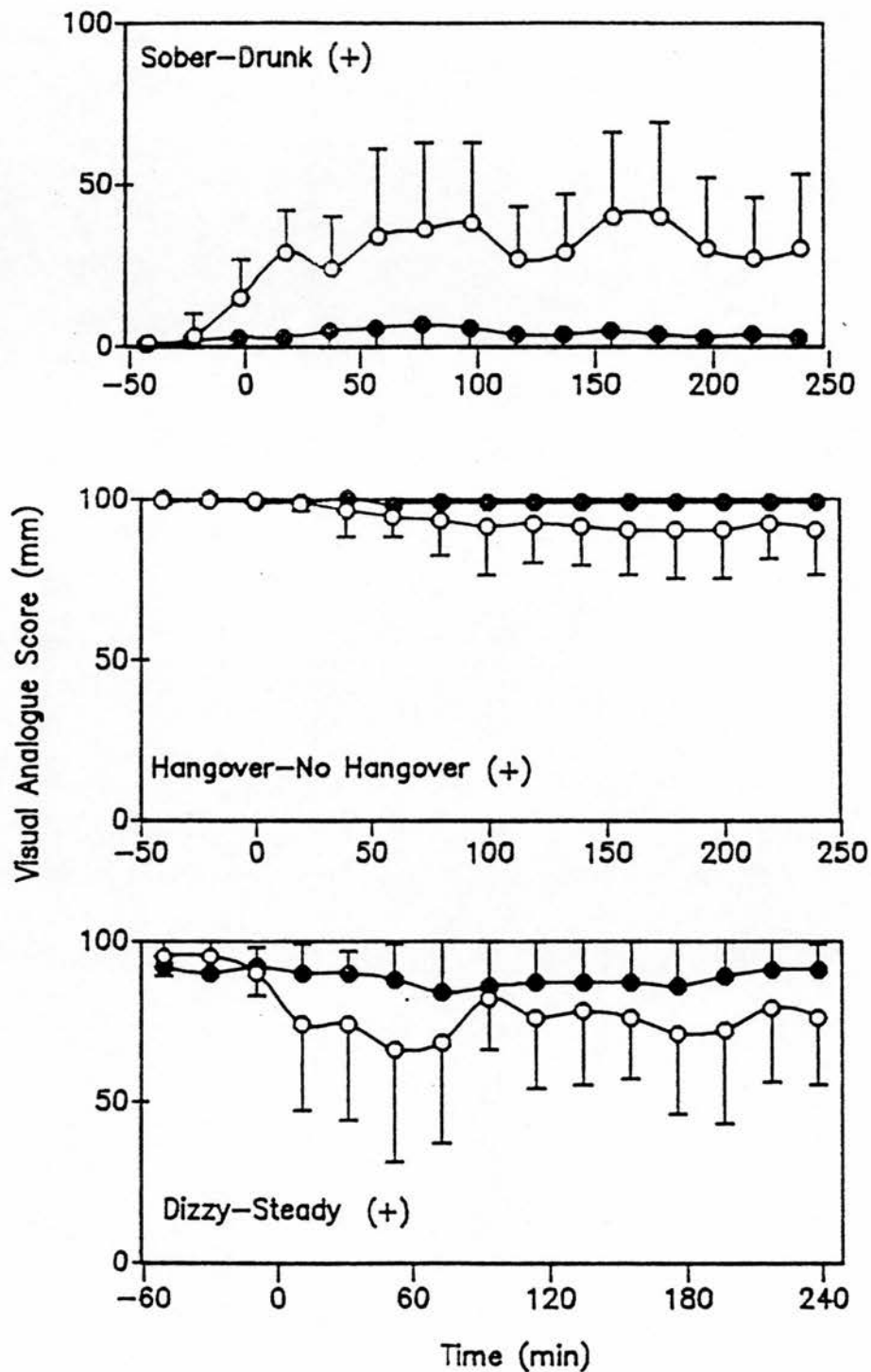


Figure 6.11. Subjective effects of steady-state ethanol (o) and placebo (●) from the visual analogue scales. Results are presented as means and standard deviations. Subjects took 60 g ethanol over 10 minutes. Time 0 - end of ingestion of main dose. Top-up doses commenced at 60 minutes. Visual analogue ratings were carried out at 20 minute intervals.

The results for the psychomotor tests are shown in Figures 6.12 and 6.13. The subjects made significantly fewer substitutions on digit symbol substitution ($P < 0.02$), without making significantly more errors, but conversely, made more errors on the Gibson spiral maze test ($P < 0.02$) without taking longer to perform the test. Sway was also significantly increased ($P < 0.02$). Tapping rate was not significantly altered.

Stable drug effects versus acute tolerance

The gradients of the slopes for the objective tests and the subjective symptoms on maintenance are shown in Table 6.2, along with the level of statistical significance for the alcohol gradients compared to placebo. The slopes were significantly different for clearheaded-muzzy, Gibson spiral maze errors and sway. There was also a trend for drowsiness and hangover. The significant changes were all in the direction of greater deterioration with time after alcohol than after placebo. There was no evidence of tolerance to the effects of ethanol on any of the assessment measures.

Psychomotor tests - full battery

The mean and standard deviation of the tests before the infusions i.e. at baseline and at steady-state placebo and ethanol are shown in Table 6.3. The percentage changes, Z scores, and a summary of statistical significances from the Wilcoxon signed ranks tests are shown in Table 6.4.

There were no significant differences between baseline scores in the ethanol and placebo sessions. There were, however, some significant differences between the morning and afternoon test runs on the placebo day. Digit symbol substitution and decision making time both slowed significantly (by about 7%) on the placebo afternoon compared to the morning, and the visual

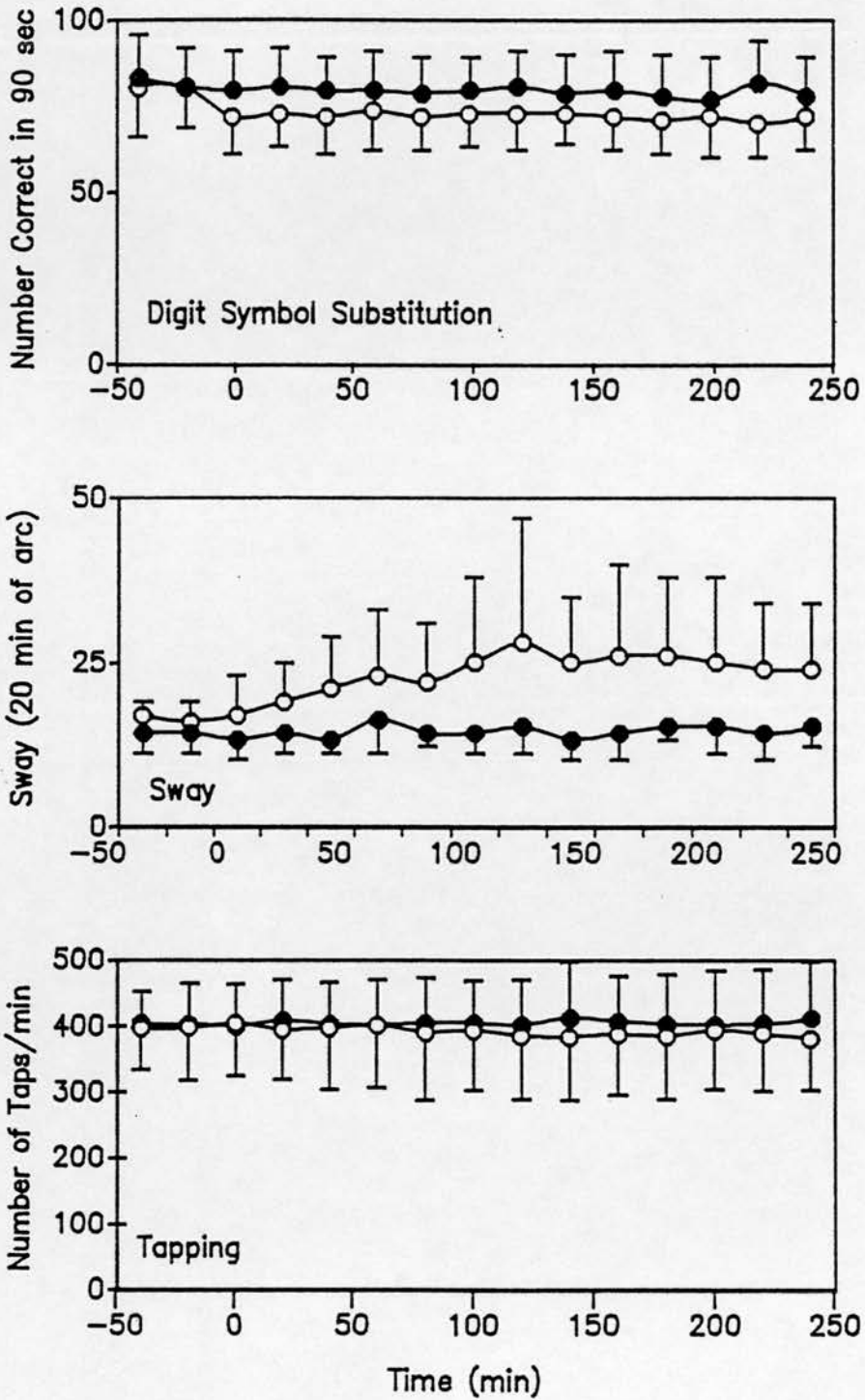


Figure 6.12. Effects of steady-state ethanol (o) and placebo (●) on digit symbol substitution (correct), sway and tapping from the small battery of tests. Results are presented as means and standard deviations. Subjects took 60 g ethanol over 10 minutes. Time 0 - end of ingestion of main dose. Top-up doses commenced at 60 minutes. Testing was carried out at 20 minute intervals.

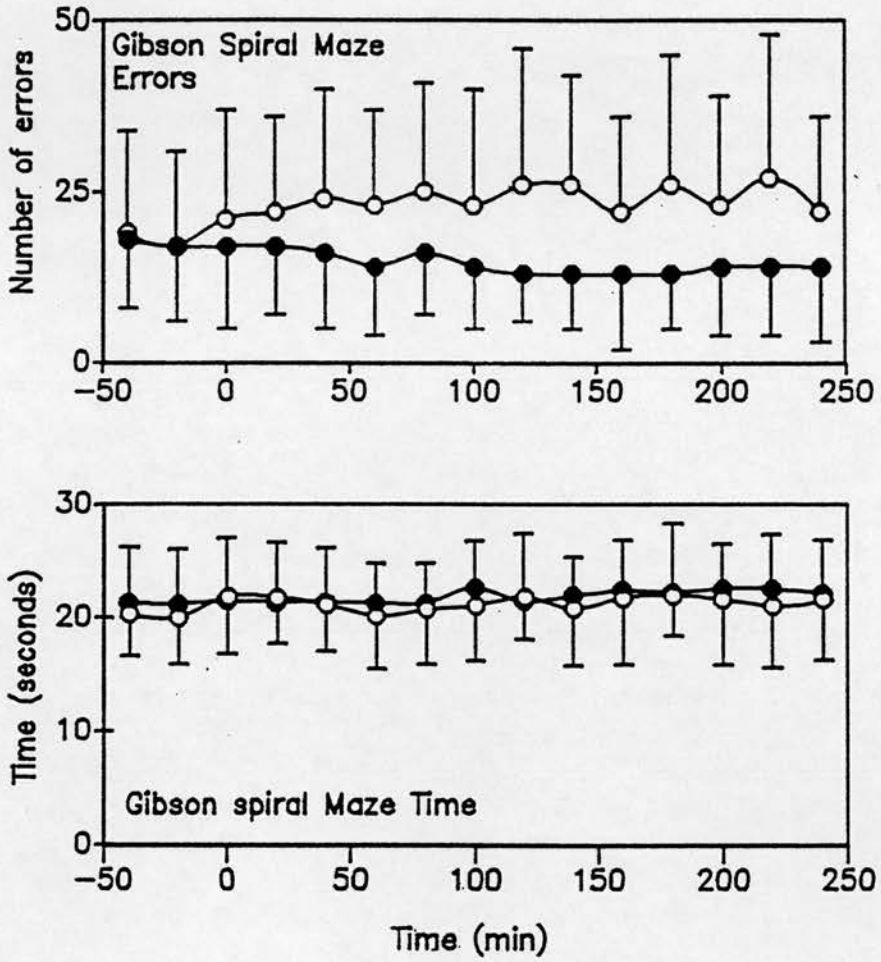


Figure 6.13. Effects of steady-state ethanol (o) and placebo (●) on Gibson spiral maze time and total errors from the small battery of tests. Results are presented as means and standard deviations. Subjects took 60 g ethanol over 10 minutes. Time 0 - end of ingestion of main dose. Top-up doses commenced at 60 minutes. Testing was carried out at 20 minute intervals.

SMALL BATTERY - MEAN SLOPES AND STATISTICAL SIGNIFICANCES

TEST/SCALE	PLACEBO		ETHANOL		WILCOXON SIGNED RANK PROBABILITY
	Mean	s.d.	Mean	s.d.	
Gibson spiral maze time errors	0.34 -0.87	0.46 1.00	0.07 0.52	0.38 1.45	n.s. 0.025
Digit symbol (correct) errors	-0.57 -0.08	1.62 0.20	-0.33 0.08	1.38 0.24	n.s. n.s.
Tapping	0.82	8.40	-4.06	3.61	n.s.
Sway	0.14	0.65	1.55	1.82	0.025
Alert-drowsy	0.34	4.50	4.64	4.95	0.069
Dizzy-steady	-0.05	2.23	-0.53	6.64	n.s.
Interested-bored	4.88	4.06	5.54	4.66	n.s.
Clearheaded-muzzy	-0.34	4.23	2.97	3.96	0.017
Sober-drunk	-0.27	0.70	2.09	6.21	n.s.
Hangover-no hangover	0.04	0.21	-2.14	3.54	0.075

Table 6.2. Gradients of effect-time slopes for the tests and visual analogue scales in the small battery of tests, and the significance of the ethanol/placebo differences using Wilcoxon Signed Ranks. Results are presented as means and standard deviations (s.d.).

TEST / VISUAL ANALOGUE SCALE	Baseline values Placebo day Mean (s.d.)	Steady-state vals. Placebo day Mean (s.d.)	Baseline values Ethanol day Mean (s.d.)	Steady state vals. Ethanol day Mean (s.d.)	$F_{1,WSR}$
Critical flicker frequency (Hz)	32.7 (4.9)	32.6 (5.5)	32.1 (5.3)	30.1 (4.7)	n.s.
Total choice reaction time (msec)	468 (35)	469 (45)	470 (49)	503 (59)	n.s.
Choice reaction latency (msec)	332 (25)	336 (31)	334 (43)	362 (44)	n.s.
Choice reaction motor time (msec)	137 (27)	132 (30)	136 (27)	141 (27)	n.s.
Continuous attention (correct)	35 (8)	35 (7)	37 (3)	30 (9)	0.028
Continuous attention (false)	0 (0)	0 (1)	1 (1)	1 (2)	n.s.
Continuous attention (total errors)	5 (8)	5 (7)	4 (4)	11 (9)	0.028
Sway (20 min of arc)	16 (4)	17 (3)	17 (2)	24 (10)	0.043
Digit symbol substitution (correct)	83 (13)	77 (12)	81 (15)	72 (10)	0.043
Digit symbol substitution (errors)	1 (1)	0 (1)	0 (0)	0 (0)	n.s.
Gibson spiral maze time (sec)	21.2 (4.9)	22.1 (4.7)	20.4 (3.7)	21.6 (5.2)	n.s.
Gibson spiral maze (errors)	18.0 (10.1)	14.2 (10.7)	18.6 (15.4)	21.5 (15.0)	n.s.
Tapping rate min ⁻¹	404 (49)	412 (87)	396 (62)	380 (79)	n.s.
Decision making time (msec)	556 (58)	595 (77)	575 (44)	626 (83)	0.028
Paired word association (correct)	26.9 (0.4)	26.8 (0.5)	26.5 (1.1)	24.8 (1.8)	n.s.
Visual vigilance (correct)	14.5 (1.3)	14.6 (1.3)	13.8 (1.2)	13.5 (1.4)	n.s.
Visual vigilance (errors)	0.25 (0.71)	0.25 (0.46)	0.88 (0.84)	0.25 (0.46)	0.068
alert-drowsy	15 (10)	23 (23)	12 (10)	34 (24)	0.028
dizzy-steady	92 (10)	91 (8)	95 (6)	76 (21)	0.046
interested-bored	19 (18)	48 (29)	20 (16)	46 (27)	0.028
clearheaded-muzzy	11 (11)	13 (22)	9 (7)	30 (28)	0.075
sober-drunk	1 (3)	3 (4)	1 (1)	30 (23)	0.028
hangover-no hangover	100 (0)	99 (7)	99 (1)	89 (14)	0.068

Table 6.3. Mean and standard deviation (s.d.) for test scores and visual analogue scales pre-drug and pre-placebo (baseline) and at "steady-state" after 4 hours dosing with ethanol and placebo. Probability values (P) from statistical analysis of the tests and visual analogue scales using Wilcoxon signed ranks (WSR). n.s. - not statistically significant.

TEST / VISUAL ANALOGUE SCALE	Between day - baseline			Within day - placebo			Steady-state Pl. vs Eth		
	Z score	% change	P WSR	Z score	% change	P WSR	Z score	% change	P WSR
Critical flicker frequency (Hz)	-0.12	-2	0.401	-0.02	-0	0.889	-0.47	-8	0.050
Total choice reaction time (msec)	0.06	0	0.834	0.03	0	1.000	0.76	7	0.208
Choice reaction latency (msec)	0.08	1	0.834	0.16	1	0.529	0.84	8	0.124
Choice reaction motor time (msec)	-0.04	1	1.000	-0.19	-4	0.889	0.30	7	0.554
Continuous attention (correct)	0.22	5	0.753	0.01	0	0.889	-0.73	-14	0.063
Continuous attention (false)	0.83	152	0.273	0.28	52	0.715	1.38	268	0.173
Continuous attention (total errors)	-0.21	-32	0.779	-0.00	-1	0.933	0.88	116	0.021
Sway (20 min of arc)	0.25	6	0.529	0.19	4	0.529	2.26	42	0.036
Digit symbol substitution (correct)	-0.15	-2	0.208	-0.46	-7	0.025	-0.42	-7	0.036
Digit symbol substitution (errors)	-0.44	-100	0.180	-0.18	-40	0.715	-0.24	-33	0.686
Gibson spiral maze time (sec)	-0.02	-4	0.401	0.18	4	0.401	-0.11	-2	0.484
Gibson spiral maze (errors)	0.06	3	0.726	-0.38	-21	0.035	0.68	51	0.012
Tapping rate min^{-1}	-0.02	-2	0.401	0.16	2	0.889	-0.37	-8	0.012
Decision making time (msec)	0.33	3	0.183	0.67	7	0.012	0.40	5	0.161
Paired word association (correct)	-1.14	-2	0.423	-0.29	-0	0.593	-4.00	8	0.028
Visual vigilance (correct)	-0.53	-5	0.094	0.08	1	0.855	-0.85	8	0.128
Visual vigilance (errors)	0.89	252	0.068	ND	ND	1.000	ND	ND	1.000
alert-drowsy	-0.23	-15	0.612	0.90	60	0.263	0.48	46	0.018
dizzy-steady	0.26	3	0.345	-0.07	-1	0.917	-1.89	-17	0.025
interested-bored	0.06	5	0.779	1.59	153	0.012	-0.07	-4	0.834
clearheaded-muzzy	-0.15	-16	0.612	0.21	22	0.933	0.80	136	0.012
sober-drunk	-0.28	-57	0.285	0.38	79	0.593	7.34	1116	0.012
hangover-no hangover	-1.25	-1	0.180	-1.25	-1	0.109	-11.00	-10	0.043

Table 6.4. Z scores, % changes and probability (P) values from the statistical analysis of the tests and visual analogue scales using Wilcoxon Signed Ranks (WSR) in the ethanol steady-state experiment. Baseline values were pre-placebo and pre-ethanol values recorded at 09:00 hours approximately. Pl.- placebo full battery test run at the end of the day's testing at 4 hours; Eth. - ethanol full battery test run at the end of the day's testing at 4 hours. ND - not defined.

analogue scales scale moved in the direction of boredom (all $P < 0.05$). 21% fewer errors were made on Gibson's spiral maze ($P < 0.05$) on the afternoon of the placebo day indicating an improvement on this test.

Of the 10 psychomotor tests used, 7 demonstrated impairment of at least one component after ethanol compared to placebo. The tests and scales which were significantly impaired after ethanol were critical flicker frequency, continuous attention total errors, sway, digit symbol substitution correct, Gibson spiral maze errors, tapping rate, paired word association correct, alert-drowsy, dizzy-steady, clearheaded-muzzy, sober-drunk and hangover-no hangover ($P < 0.05$). The measures of choice reaction time, continuous attention (correct and false positive), digit symbol substitution errors, Gibson spiral maze time and the interested-bored visual analogue scales did not change significantly.

On a percentage basis, between steady-state placebo and alcohol, the biggest changes were in sway and the error scores for continuous attention and Gibson's spiral maze. It may not be useful to use the scores for continuous attention false and digit symbol substitution errors in this way because so few errors were made to begin with. When the changes were expressed in terms of Z scores a different pattern emerged. The biggest changes were on paired word association ($Z=4$) and also on sway ($Z=2.26$).

A number of the tests showed percentage changes of very similar size (7-8% impairment) on at least one measure. These were critical flicker frequency, total choice reaction time, choice reaction latency, choice reaction motor time, digit symbol substitution correct, tapping, decision making time, paired word association and visual vigilance correct.

DISCUSSION

These results clearly demonstrated the effects of ethanol on objective performance and subjective measures. Significant effects were found on critical flicker frequency, digit symbol substitution, tapping rate, paired word association, sway, Gibson's spiral maze errors and continuous attention errors. Choice reaction time was not significantly impaired even with these quite high ethanol concentrations as noted previously (Wallgren and Barry, 1970; Fagan et al., 1987).

Acute tolerance was not found. None of the objective measures used showed diminution of the effects of ethanol with time, although there was a suggestion of a temporary improvement on the steady-dizzy visual analogue scale (Figure 6.11). The results are therefore in agreement with those of Klotz et al., (1986) who found, in an open infusion study, no evidence of acute tolerance to ethanol with similar plasma concentrations under steady-state conditions.

Klotz et al., (1986) used visual analogue scales and choice reaction time to assess acute tolerance. Choice reaction time was significantly impaired in their experiment, and has been shown to be capable of detecting acute tolerance at steady-state with other drugs (see Chapter 1). However, Hurst and Bagley (1972), considered this test to be resistant to ethanol tolerance, and it may therefore not have been an appropriate single test for studying the phenomenon. These latter authors regarded digit symbol substitution and sway as more sensitive measures of acute tolerance, and these were used in the present study. Despite using these more sensitive tests, there was no evidence of acute tolerance at steady-state.

Kaplan et al., (1985) looked at acute tolerance to ethanol at steady-state with similar ethanol concentrations, but used a battery of tests including

visual analogue scales, sway, tracking and a word category recall test. Oral doses were used with topping doses at half hourly intervals. These workers found that performance on word category recall partially recovered during steady-state. However, there was a slight fall in ethanol concentrations during the six hour study period which may also have accounted for the difference. The work of Biersner (1972) and Biersner et al., (1977) also indicates that acute tolerance may not be the explanation for this recovery of memory performance. These authors demonstrated that scores on word association memory tests showed improvements across all experimental conditions, and not just after drugs. Fowler et al., 1985 considered that this improvement may have been due to changes in strategy.

It was interesting, therefore, that at five hours after the start of ethanol consumption in our experiment the size of the impairment on the paired word association test was the same as for the majority of other tests. Intoxicated learning and task practice have been proposed as being facilitators, or even prerequisites of ethanol tolerance on some tests (see discussions of Haubenreisser and Vogel-Sprott, 1983; Le et al., 1989). Our subjects only performed paired word association four times in the whole experiment, and only once after ethanol. In the study of Kaplan et al., (1985), however, the subjects performed the word category test 14 times, and practice may therefore have played some part in the partial recovery on the memory test in their study.

Nagoshi and Wilson, (1987, 1989) have also used oral loading and "topping" doses to assess genetic differences in acute tolerance to ethanol at steady-state. The topping doses in these open experiments were administered hourly, and so were larger than in our study. There were few testing times in order to reduce the effects of intoxicated practice, and the authors also assessed individual sensitivities to the drug. However, testing

was only carried out during ethanol absorption after the large main dose and smaller topping doses, and the brain concentrations must have changed considerably during testing. The results are, therefore, not directly comparable with the other steady-state studies. Additionally, the method of presentation of results using only derived values does not allow for easy interpretation or comparison with the results of other studies.

Klotz et al., (1986) and Kaplan et al., (1985) found greater steady-state impairments on the tests (approximately 45% and 30% change respectively) compared to our experiment (8% change). The age range was greater in the experiment of Klotz and co-workers (25-45 years), and there was no placebo condition which may account for the difference. Other environmental differences such as bed rest and food consumption may also have contributed to their greater impairment since subjects also reported feeling quite sedated (Angus, 1985; Spring et al., 1982/83). Large interindividual and interstudy differences in response to ethanol have previously been reported (Wilson and Plomin, 1985; Levine et al., 1975), and this variability may account for the differences. Another possible reason for these quite large interstudy differences is that the dose response curve for the impairments with ethanol may increase sharply over a relatively small dose range. This will be discussed further in Chapter 8 (General Discussion).

The time to reach a performance plateau varied between the tests. Thus digit symbol substitution performance was impaired at the end of the loading dose and did not appear to worsen appreciably as the breath ethanol concentrations rose by another 10%. Sway, on the other hand, gradually increased until the plasma and breath concentrations peaked, and then reached a stable plateau. It was interesting that there was no rapid initial impairment with sway, and that sway appeared to follow the plasma/breath curves only after a high

concentration was reached. This suggests either a higher threshold for impairment or initial compensation for the effects of alcohol. Hurst and Bagley (1972) noted a reduction in tremor after a moderate dose of ethanol which appeared to offset body sway movements. However, when these authors used a higher dose, a substantial impairment in sway was observed which declined sharply as the blood alcohol concentration fell. Since the sway scores were higher during absorption of the ethanol, these authors concluded that this was evidence of acute tolerance to body sway. However, a combination of the distribution artifact (Goldstein, 1983) and a steep dose response curve with a high threshold could also explain their results.

In this investigation the most "sensitive" tests for demonstration of impairment due to ethanol at steady-state (in terms of changes in standard deviation) appeared to be paired word association and sway. However paired word association had an inflated Z value because of a small standard deviation on control (because of a ceiling effect), and sway was not sensitive to changes in blood ethanol concentration during absorption. Z scores therefore did not appear to be particularly useful in determining the test sensitivities. Nagoshi and Wilson (1987, 1989) have used standardised regression residuals as indices of both acute drug sensitivity and acute tolerance. This method is also of limited value since it relies on stable individual differences being maintained across treatments, and these conditions are not always met after ethanol (Nagoshi and Wilson, 1989). Assessment of test sensitivities will be discussed in detail in Chapter 8 (General Discussion).

The fact that ethanol produced the same percentage change in tests assessing aspects of arousal, reaction time, memory and attention was somewhat surprising. With the exception of mood, sway and Gibson's spiral maze the pattern of impairment appeared to be global. This confirms the relatively nonspecific nature of the

impairments with ethanol, in keeping with its known general anaesthetic properties. However, it contrasts with the more recent notion of ethanol having specific effects on attention and memory (e.g. Levine et al., 1975; Bloom, 1987). The effect of ethanol on continuous attention correct was greater, but not statistically significant.

Levine et al., (1975) reviewed the results of a number of ethanol studies. Using an abilities classification, they categorised the tests into one of three domains; cognitive, perceptual-sensory and psychomotor. The tests in the cognitive domain were mainly divided attention tests, those in the perceptual-sensory were mainly vigilance/ attention tests and those in the psychomotor domain were reaction time tests. Tests which did not fit these classifications were rejected from the analysis. Dose response curves were then constructed for the three domains.

Levine and co-workers reported that the effect of ethanol on the cognitive domain was approximately twice that of ethanol on the psychomotor domain (20% versus 10%) at doses of about 0.8 g.kg^{-1} (1/4 -1/3 of a bottle vodka). The perceptual-sensory scores fell in the middle. Our results appear to confirm the findings of Levine et al., since there was a large significant effect on continuous attention total errors, and the size of effect on reaction time was comparable to that on their psychomotor domain. However, other error scores also increased in our study suggesting that the change in continuous attention may not have been a specific effect. The use of error scores in the analysis of Levine et al., (1975), the more frequent use of divided attention tests in the studies reviewed (and in subsequent studies), and the narrow range of abilities assessed makes it difficult to draw firm conclusions about the specific attentional effects of ethanol.

Gibson spiral maze time actually improved. However, there was a large increase in errors on this test indicating a possible shift in speed/accuracy tradeoff as proposed for ethanol by Rundell and Williams (1979) and confirmed by Landauer and Howat (1982). This improvement was not seen with the reaction time tests which demonstrated the same size of effect as the other tests (although these changes were not statistically significant).

More comprehensive and reliable information on the pattern of effects was produced in this study than in a previous conventional dose-response study that we performed (Fagan et al., 1987). There is some evidence, however, that the pattern of effect of drugs may change with dose. Digit symbol substitution appeared to be more impaired than sway at fairly low concentrations of ethanol, but the reverse was true at higher concentrations. There was also an impression in the chlormethiazole experiment (Chapter 5) that of the short battery of tests, digit symbol substitution was more sensitive at detecting effects of the drug at the threshold level of impairment, while sway was relatively more affected at high concentrations. This suggests a difference in the shape of the dose-response curve with these tests. This idea will be explored further in the next Chapter.

SUMMARY

A pharmacokinetic study was performed in order to determine individual rates of metabolism of ethanol for eight male subjects. These rates of metabolism were then used to calculate the quantities of ethanol required to maintain ethanol at steady-state constant plasma concentrations.

After some dose ranging experiments were carried out, a double-blind crossover study was performed in which the effects of constant plasma and breath concentrations of ethanol were observed. Plateau concentrations were achieved using a 60 g loading dose of ethanol followed by "top-ups" at 10 minute intervals throughout. The top-up volumes were calculated from rates of metabolism obtained in the kinetics study.

There was no evidence of acute tolerance to the subjective or objective effects of ethanol in this experiment as assessed using a short battery of tests consisting of sway, tapping, digit symbol substitution, Gibson spiral maze and visual analogue scales.

Comparing the results between ethanol and placebo at 4-5 hours of steady-state, most of the tests demonstrated statistically significant impairments in at least one component of the task. The exceptions were the reaction time tasks (choice reaction time and decision making time) and visual vigilance. The similarity in the magnitude of impairment, *whether statistically significant or not (see table 6.3)* on the majority of the tests viz. critical flicker frequency, choice reaction time (total choice reaction time, choice reaction latency and choice reaction motor time), digit symbol substitution correct, tapping, decision making time, paired word association correct and visual vigilance correct appear to confirm the general nonspecific nature of the impairment. The exceptions were sway and the components of continuous attention and Gibson's spiral maze.

CHAPTER 7 - PSYCHOMOTOR EFFECTS OF NITROUS OXIDE
AT STEADY-STATE

It was observed during the loading infusions in the chlormethiazole steady-state study (Chapter 5) that the digit symbol substitution scores were usually reduced before sway was impaired, though sway was much more impaired during steady-state. Sway also tended to recover before digit symbol substitution during the washout period. Sway therefore seemed to be less affected than digit symbol substitution at lower plasma concentrations, but more affected at higher plasma concentrations.

There were also some qualitative differences in impairment pattern with these two tests during the ethanol steady-state study (Chapter 6). For example, digit symbol substitution was impaired at the end of the ethanol loading dose when the plasma concentrations were low, whereas sway became gradually more impaired as plasma concentrations increased. At steady-state, however, there was a greater impairment of sway.

One possible explanation for these findings is that the tests, or the functions they represent, have different dose response curves with different sensitivities at various stages of the dose response curves. The fact that these phenomena have been observed with different drugs also suggests that the phenomenon may be a general one.

In order to test whether this was the case a dose response study was performed with another central depressant drug with each dose at steady-state. The main experiment in this Chapter was thus designed to observe the subjective and objective effects of different steady state concentrations of a general anaesthetic, nitrous oxide (N_2O), in order to evaluate and compare dose response curves for each of the tests. N_2O was chosen because of its presumed non-specific acute actions on the central nervous system, and

because of the ease with which steady-state concentrations could rapidly be achieved and maintained. A second experiment concentrated on the effects of N_2O on critical flicker frequency alone.

METHODS

Twelve healthy volunteers (eight males and four females) participated in this five way crossover study. The mean age of the subjects was 29 years (range 20-35 years) and the mean weight was 69 kg (range 50-98 kg). All but one of the volunteers, a female, was right handed. (For individual demographic details on these subjects see Table 2.1). The experiment was carried out in the Main Laboratory of the Anaesthetics Department of the Royal Infirmary of Edinburgh.

Subjects received in a partially counterbalanced order either 0%, 5%, 10%, 20% or 40% N_2O (BOC) in oxygen (O_2 , BOC) delivered from a Boyle's machine (Ohmeda) through a non-rebreathing circuit fitted with a 12 litre reservoir (2 X 6 litre Leymed bags), one-way low-resistance valve (Ambu) and close fitting mask (Ambu). The mask was held in place with a Klausen harness and the fresh gas flow adjusted to slightly exceed the subject's minute volume as evidenced by the fullness of the reservoir bags. Any excess gas flow vented automatically through the Ambu valve.

Two holes were drilled in the mask. The first was attached via 1.5 metres of 3 mm propylene tubing (Argyleth) to stethoscope earpieces to allow the observer to hear the subject's comments. The other hole allowed a sampling probe from an Airspec 2,000 Mass Spectrometer to be placed directly in front of the subject's mouth. The inspired and expired concentrations of N_2O were sampled breath by breath, and the output from the mass spectrometer displayed on a servoscribe paper and ink recorder. Using this apparatus it was possible to observe the anaesthetic gas

concentrations and any nitrogen spikes indicating air entry into the mask. The mask could then be tightened if necessary.

The gases were administered for approximately 60 minutes by an attending anaesthetist who also monitored the subjects vital signs continuously. Subjects were fasted for at least 4 hours before each experimental session commenced, and remained in the laboratory until they reported feeling "back to normal". In order to prevent hypoxia, subjects breathed 100% oxygen for a few seconds at the end of each session.

The mass spectrometer was not working during 4 of the 60 sessions (two 20% N₂O, and two 40% N₂O). For these sessions an O₂ analyser (Servomex) was used to determine the O₂ percentage in the inspired gases. The O₂ analyser was accurate to within 0.5% (manufacturers specifications).

Subjective assessments and psychomotor tests

Subjects completed a set of visual analogue scales before the mask was strapped on, and 15 minutes after the start of inhalation. During this 15 minute period subjective reports were recorded and rated on the 8 point severity scale (see Figure 2.9) at the time of onset of any spontaneously reported symptoms and from non-specific questioning at 5 minute intervals timed from the start of inhalation. Testing started at 15 minutes, and the test battery took approximately 45 minutes to complete. The full test battery was administered in a partially balanced randomised order so as to compensate for any effects of time. The subjects completed another set of visual analogue scales at the end of testing.

The 100 mm visual analogue scales used to chart the subjective symptoms and mood changes were interested-bored, alert-drowsy, dizzy-steady, clear-headed-muzzy, unpleasant-pleasant and ill-well.

Testing was carried out at the same time of day for each individual at least one day apart.

STATISTICAL ANALYSIS

The coefficient of variation was used to determine the variability of the expired and inspired concentrations of N_2O .

Friedman nonparametric two way analysis of variance was used to determine whether there were differences in impairment on the variables between any of the concentrations, and Page's L test for Trend was used to test for monotonic dose-related effects.

Our hypothesis was that, being a non-specific compound, N_2O might affect performance on all of the tests though possibly to different extents. In order to assess which measures were capable of detecting changes at the various concentrations of N_2O , comparisons were made between the scores on 0% N_2O (100% O_2) and the scores at each concentration of N_2O using Wilcoxon Signed Ranks. The P values from this analysis were therefore viewed essentially as variables (Sinclair, 1988), and we did not consider that non-significance necessarily meant no effect.

RESULTS

Nitrous oxide concentrations.

The individual mean inspired and expired concentrations of N_2O along with group means, standard deviations and coefficients of variation are shown in Table 7.1. None of the coefficients of variation were greater than 6% for any of the expired concentrations, and the coefficients of variation decreased as the N_2O concentration increased. The coefficients of variations for the inspired concentrations were higher at the lower N_2O concentrations, the greatest value being 5%

NITROUS OXIDE CONCENTRATIONS

SUBJECT	0%		5%		10%		20%		40%	
	EXP.	INSP.	EXP.	INSP.	EXP.	INSP.	EXP.	INSP.	EXP.	INSP.
1	0	0	4.6	5.2	10.0	10.4	-	20±0.5	39.6	40.2
2	0	0	4.4	5.4	9.8	10.4	19.6	20.0	38.8	40.6
3	0	0	5.0	5.6	9.6	10.4	20.0	20.2	38.6	40.2
4	0	0	5.4	6.0	9.8	10.2	19.6	20.4	40.0	40.8
5	0	0	4.8	5.2	9.8	10.4	20.4	21.4	*	*
6	0	0	5.2	5.4	10.0	10.4	20.0	20.2	39.8	40.4
7	0	0	4.8	5.6	9.4	9.7	19.2	20.2	-	40±0.5
8	0	0	4.8	5.2	9.6	10.2	19.6	20.0	-	40±0.5
9	0	0	4.8	5.4	10.2	10.4	19.8	20.4	40.0	40.8
10	0	0	4.8	5.2	9.6	10.4	-	20±0.5	39.4	40.2
11	0	0	4.6	5.2	10.2	10.8	20.0	20.4	39.8	40.2
12	0	0	5.2	5.6	10.0	10.4	20.0	20.4	39.2	40.0
Mean	0	0	4.9	5.4	9.8	10.3	19.8	20.3	39.5	40.3
s.d.	0	0	0.29	0.25	0.25	0.25	0.33	0.31	0.51	0.30
CV(%)	0	0	5.9	4.6	2.6	2.4	1.7	1.5	1.3	0.7

Table 7.1. Individual, mean, standard deviation (s.d.) and coefficient of variation (CV) for inspired and expired concentrations of 0, 5, 10, 20 and 40% nitrous oxide. INSP. - inspired concentration; EXP - expired concentration; On four occasions the mass spectrometer was not working and the inspired concentration was measured using a Servomex Oxygen Analyser. There were no values (-) for the expired concentrations on these occasions. * - subject 5 was unconscious on 40% nitrous oxide, and so this run was abandoned.

variation for 5% N₂O. The concentrations of N₂O delivered had to be increased by approximately 0.5% for the expired concentrations to reach the desired values.

Acute tolerance - symptom rating scale

The subjective effects of N₂O from the 15 minute run-in period are shown in Table 7.2 along with the numbers of subjects experiencing each symptom. The total severity scores for each individual, and the group means and standard deviations are shown in Table 7.3 and graphed in Figure 7.1 (a and b). The most commonly reported symptoms were central nervous system related. These included ^aparesthesia, dizziness, lightheadedness, euphoria and tinnitus. The number of symptoms and the number of subjects reporting them increased as the dose increased. The total severities also increased linearly with dose from what appeared to be a threshold of 10%, although there were symptoms reported at 0% and 5% N₂O.

The total scores for the side-effects lightheaded/dizzy and ^aparesthesia are shown in Figure 7.2 (a and b). The severity scores for these symptoms were greater during the first five minutes than subsequently after 40% N₂O. ^aParesthesia also appeared to lessen after 5 minutes on 20% N₂O.

Visual analogue scales

The subjective effects of N₂O (mean and standard deviation) from the visual analogue scales are shown in Figure 7.3, and a summary of the statistics is shown in Table 7.4.

SYMPTOM	NITROUS OXIDE CONCENTRATION				
	0%	5%	10%	20%	40%
	N = 12	N = 12	N = 12	N = 11	N = 11
Par ^o esthesia	0	5	1	7	10
Dizziness	1	3	6	8	9
Drowsiness	3	4	1	2	4
Euphoria	1	2	2	5	8
Dysphoria	0	0	0	0	3
Tinnitus	0	1	0	1	6
Hyperacusis	0	0	0	1	4
Dulled hearing	0	0	0	2	0
Nausea	0	0	0	0	3
Taste/smell	0	1	1	4	2
Dry mouth	2	3	1	0	2
Warm	0	2	0	1	2
Dysarthria	0	0	0	0	1
Desire to hyperventilate	0	1	1	1	0
Altered awareness of time	0	0	1	0	2
Cardiovascular awareness	0	0	0	0	1
Difficulty focussing	0	0	0	0	1
Watery eyes	0	0	0	0	1
Feeling of deja vu	0	0	0	0	1
Darkness "closing in"	0	1	0	0	0
Distractable	0	1	0	0	0
Headache	0	0	1	0	0

Table 7.2. Summary of symptoms reported during run-in period with N₂O and numbers of subjects reporting each symptom. The symptoms reported at the end of the experiment are also included. N - number of subjects. Symptoms were not recorded for one subject on 20%, and another on 40% (subject was unconscious).

SUBJECT		NITROUS OXIDE CONCENTRATION																			
		0%			5%			10%			20%			40%							
		(minutes after start)			(minutes after start)			(minutes after start)			(minutes after start)			(minutes after start)							
	0	0-5	10	15	0	0-5	10	15	0	0-5	10	15	0	0-5	10	15	0	0-5	10	15	
1	0	2	1	1	0	1	0	0	0	1	0	0	0	0	5	4	5	0	18	28	20
2	0	0	1	1	0	0	2	0	0	0	0	0	0	0	6	5	7	0	14	7	7
3	0	0	2	2	0	0	0	1	0	4	4	2	2	-	-	-	-	0	10	8	8
4	0	0	0	0	0	0	3	3	0	2	2	2	0	0	8	7	7	0	25	29	26
5	0	0	0	0	0	4	5	9	0	0	0	0	0	0	8	7	6	0	*	*	*
6	0	0	0	0	0	1	0	0	0	1	2	2	2	0	10	9	9	0	14	17	17
7	0	0	0	0	0	4	4	4	0	0	0	0	0	0	6	6	8	0	15	20	19
8	0	0	0	0	0	0	0	1	0	2	2	2	2	0	8	2	2	0	2	2	0
9	0	3	3	4	0	2	2	2	0	1	2	2	2	0	7	6	9	0	26	25	25
10	0	0	0	0	0	1	1	2	0	0	1	0	0	0	2	2	3	2	10	9	10
11	0	0	0	0	0	3	13	14	0	0	0	0	0	0	4	6	10	0	26	30	38
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	14	15	0	11	3	10
Mean	0	0.4	0.6	0.7	0	1.3	2.6	3.2	0	0.9	1.1	0.8	0	6.7	6.2	7.4	0.2	15.5	16.2	16.4	
s.d.	0	1.0	1.0	1.0	0	1.6	3.7	4.2	0	1.2	1.3	1.0	0	2.5	3.3	3.6	0.6	7.6	10.8	10.8	

Table 7.3. Summary of total severity scores, means and standard deviations (s.d.) from the eight point severity scale for each subject during the 15 minute run-in period with nitrous oxide.

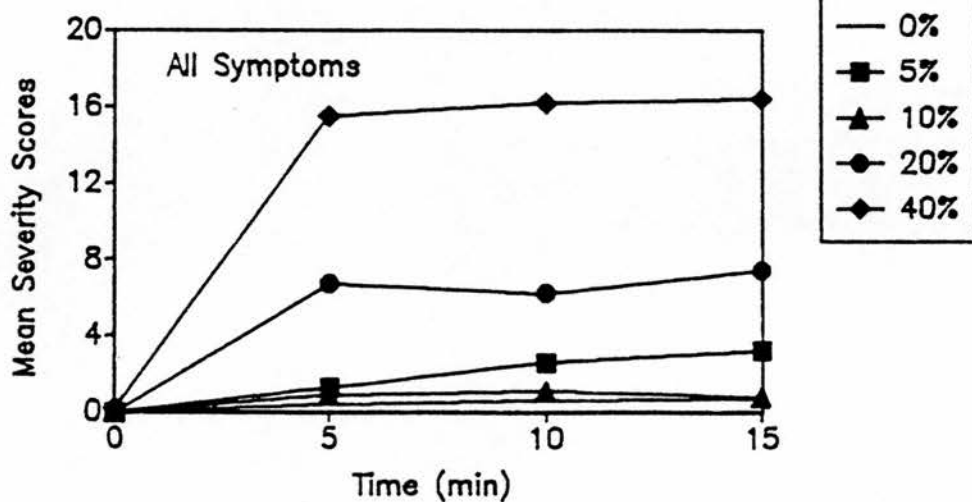
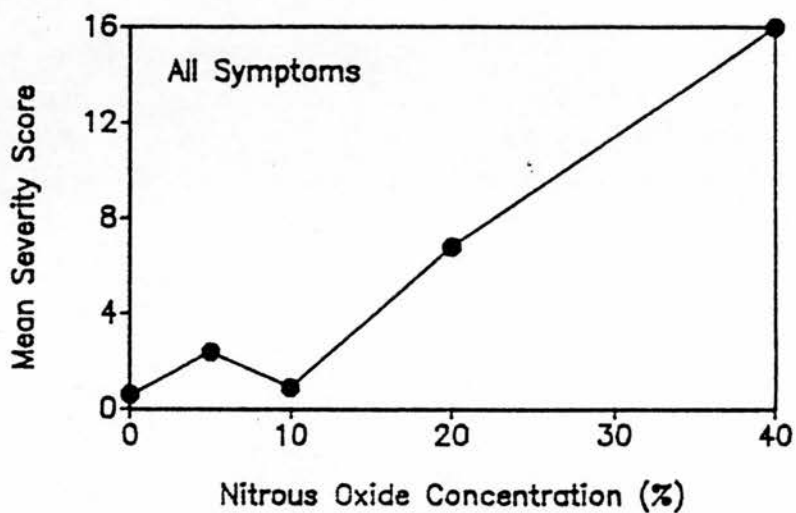


Figure 7.1. (a, top) and (b, bottom). Dose response effect of nitrous oxide on the summed severity scores on the eight point adverse signs and symptoms scale. Results are presented as group mean scores for all symptoms. (a) shows the maximum effect during the first 15 minutes of inhalation. (b) shows the change in effect with time.

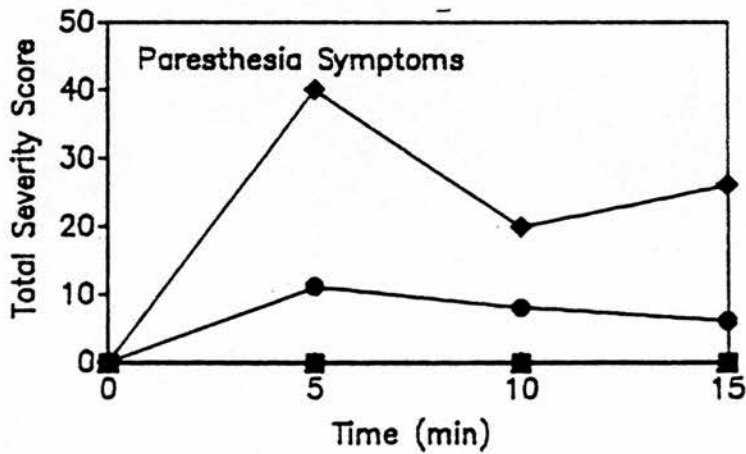
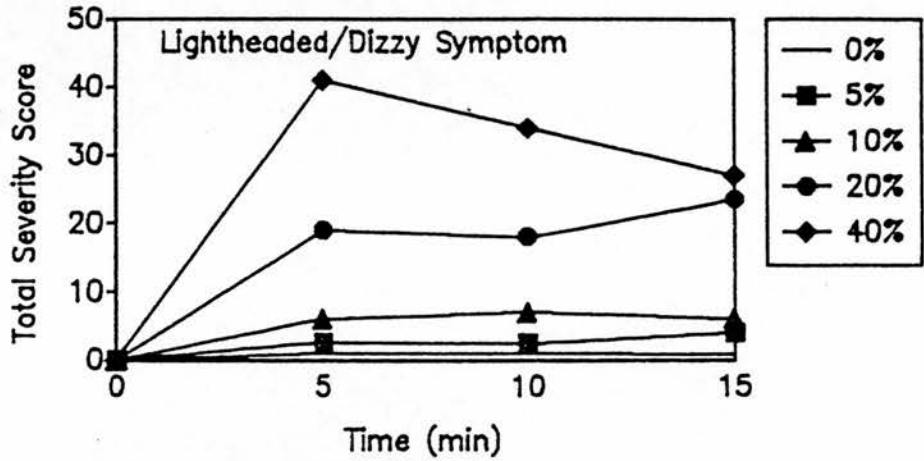


Figure 7.2.(a, top) and (b, bottom). Dose response effect of nitrous oxide on the summed severity scores for lightheaded/dizzy and paraesthesia on the eight point adverse signs and symptoms scale. Results are expressed as group mean scores for these symptoms. (a) shows the change in lightheaded/dizzy with time. (b) shows the change in paraesthesia with time.

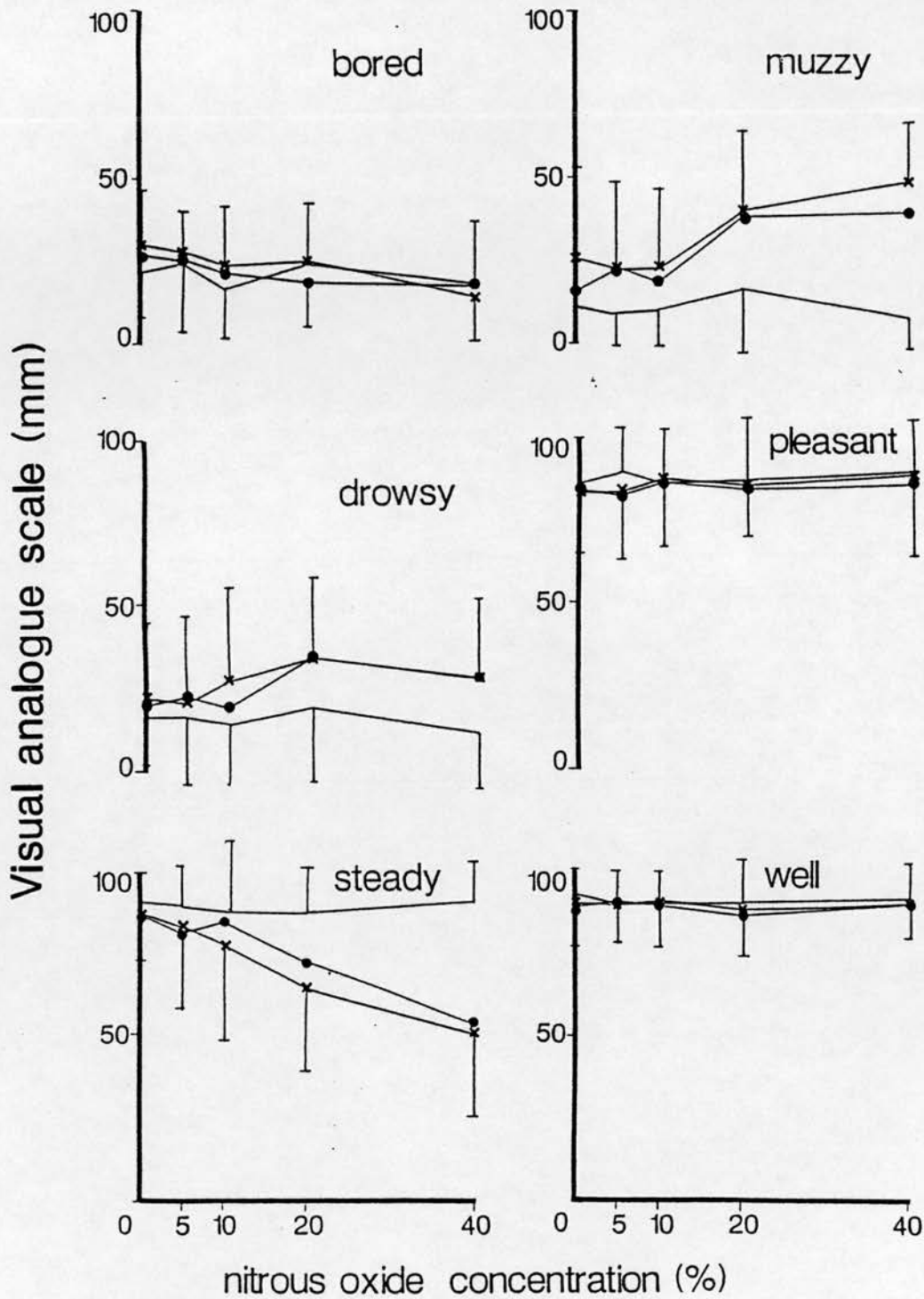


Figure 7.3. Effects of nitrous oxide on the visual analogue scales at control (—), after 15 minutes inhalation (●-●) and at the end of testing after approximately 45 minutes inhalation (X-X). Results are expressed as means and standard deviations. Bored (+) - interested-bored, muzzy (+) - clearheaded-muzzy, drowsy (+) - alert-drowsy, pleasant (+) - unpleasant-pleasant, steady (+) - dizzy-steady and well (+) - ill-well.

VISUAL ANALOGUE SCALE	CONTROL				15 minutes				End of Testing					
	P of Wilcoxon Signed Ranks 0/5 0/10 0/20 0/40		P of F L		P of Wilcoxon Signed Ranks 0/5 0/10 0/20 0/40		P of F L		P of Wilcoxon Signed Ranks 0/5 0/10 0/20 0/40		P of F L			
Interested-bored	ns	ns	ns	0.07	ns	ns	ns	ns	ns	0.05	ns	0.08	ns	0.01
Alert-drowsy	ns	ns	0.06	0.04	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.05
Dizzy-steady	ns	ns	ns	ns	ns	ns	0.00	0.00	0.00	ns	ns	0.00	0.01	0.00
Clearheaded-muzzy	ns	ns	ns	ns	ns	ns	0.00	0.08	0.00	ns	ns	0.06	0.03	0.00
Unpleasant-pleasant	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ill-well	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 7.4. Summary of statistical results and probability (P) values for visual analogue data from nitrous oxide study. F - Friedman nonparametric analysis of variance; L - Page's L Trend test. 0/5 - comparison between 0% and 5% N₂O; 0/10 - comparison between 0% and 10% N₂O; 0/20 - comparison between 0% and 20% N₂O; 0/40 - comparison between 0% and 40% N₂O. ns - not statistically significant. Probability values below 0.1 are given numerically. P less than 0.001 is shown as 0.00 above. The changes were in the direction of greater interest, drowsiness, dizziness and muzziness.

There were no significant subjective effects of N_2O on the visual analogue scale below 20%, apart from one, possibly spurious, isolated increase in interest at the end of testing on 10% N_2O . The subjects felt significantly dizzier and muzzier after 20% and 40% N_2O , and both of these tests showed significant increases with dose on the L-trend test. The scores on drowsiness were inconsistent, and there was a slight non-significant trend with this symptom.

Full test battery

The effects of N_2O on the objective measures of performance are shown in Figures 7.4 - 7.7 which are the dose-response curves for the Z scores and percentage changes in responses. The mean data and percentage changes from placebo are also tabulated in Tables 7.5 and 7.6. A summary of the statistics is shown in Table 7.7.

All of the objective tests apart from critical flicker frequency showed statistically significant effects on the Friedman test, and there were dose-related impairments (L-Trend) on the main measures of all of the tests except critical flicker frequency.

With the exception of critical flicker frequency, all tests showed significant impairment at 40% N_2O . In terms of percentage change, the largest effects were on sway, continuous attention errors and Gibson's spiral maze errors.

No measure was significantly affected by 5% N_2O , although for some measures (continuous attention correct and errors, choice reaction time) there was a trend towards slight improvement at this concentration.

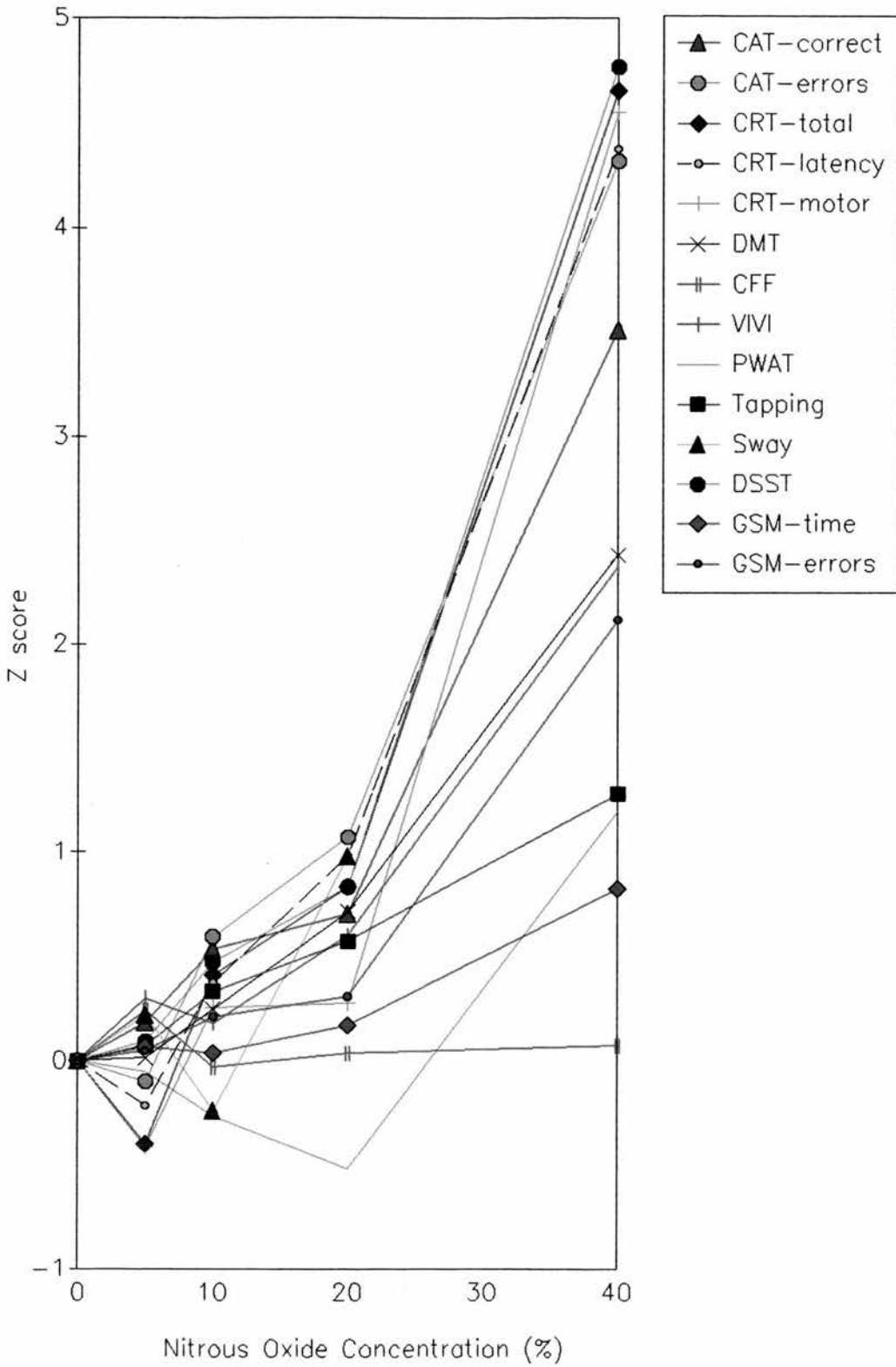


Figure 7.4. Dose response effects of nitrous oxide on Z scores (i.e. changes in multiples of standard deviation from placebo scores) for each variable. 1 Z score = 1 standard deviation. CAT = continuous attention test, CRT = choice reaction time, DMT = decision making time, CFF = critical flicker frequency, VIVI = visual vigilance, PWAT= paired word association test, DSST = digit symbol substitution test, GSM = Gibson's spiral maze.

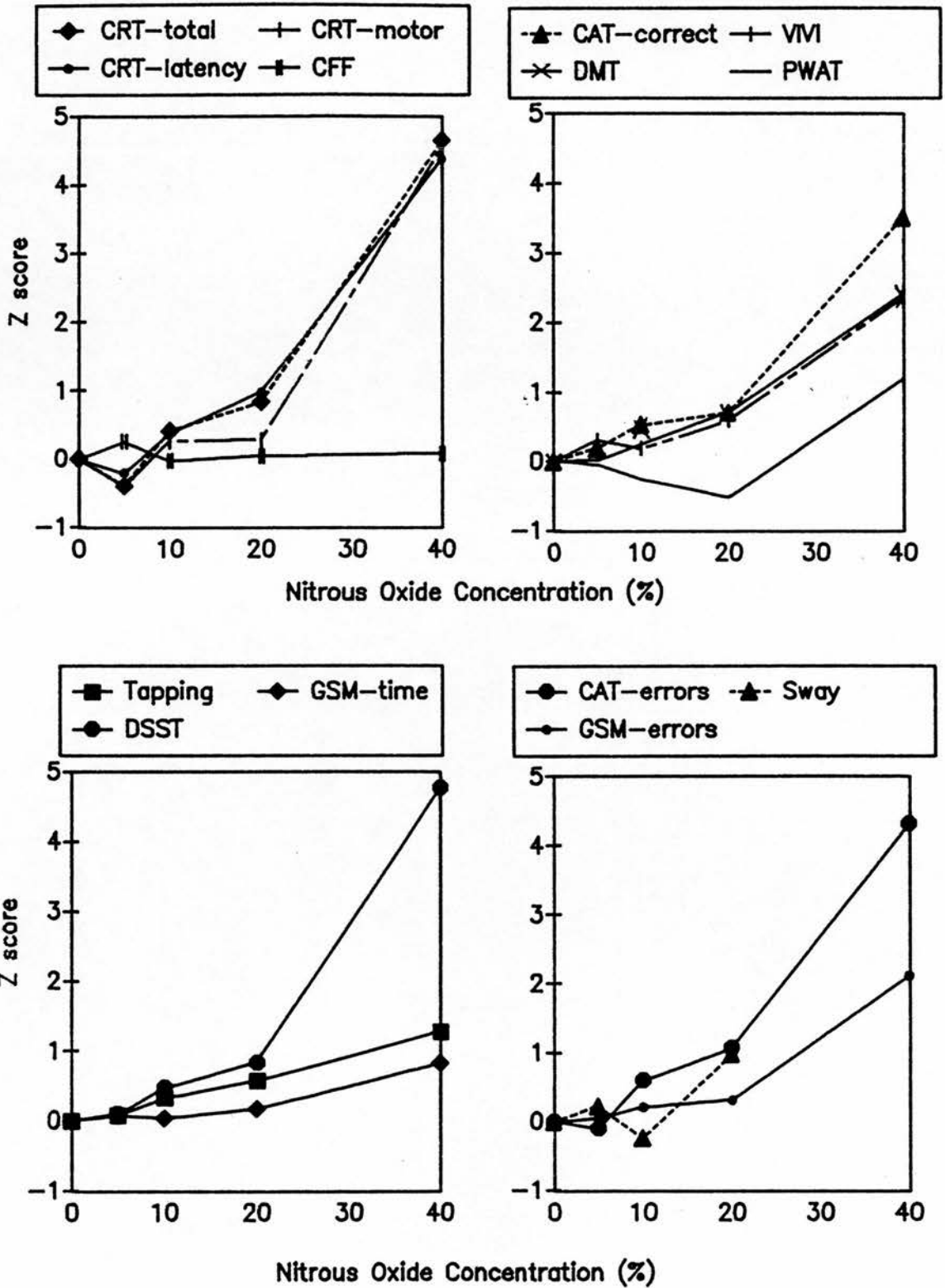


Figure 7.5. Dose response effects of nitrous oxide on Z scores (i.e. changes in multiples of standard deviation from placebo scores) for each variable. 1 Z score = 1 standard deviation. CAT = continuous attention test, CRT = choice reaction time, DMT = decision making time, CFF = critical flicker frequency, VVI = visual vigilance, PWAT = paired word association test, DSST = digit symbol substitution test, GSM = Gibson's spiral maze. The data used is the same as that in Figure 7.4, but is split for greater clarity.

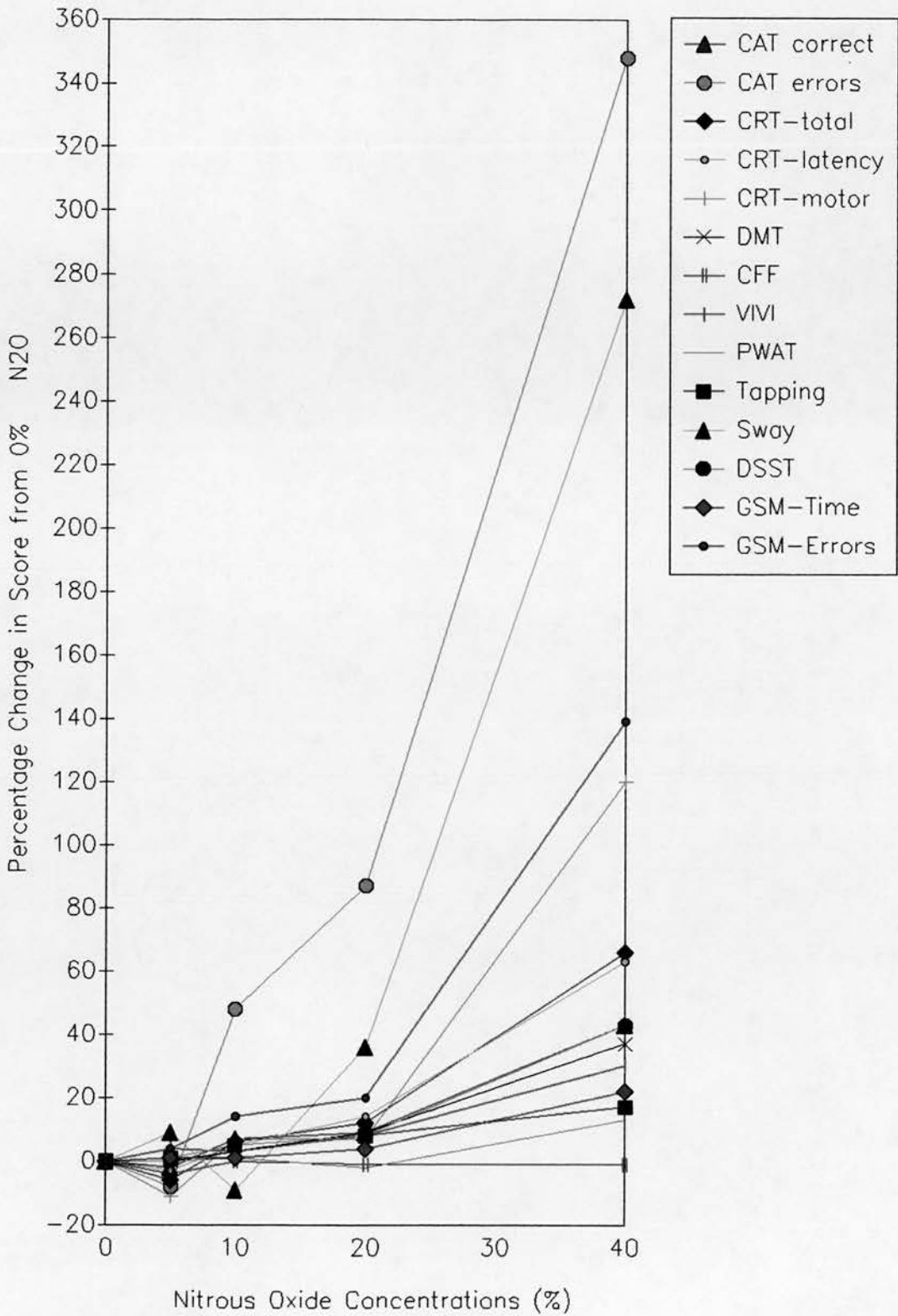


Figure 7.6. Percentage change in test scores for increasing nitrous oxide concentrations. Positive percentage changes indicate impairments, negative percentage changes indicate improvements. CAT = continuous attention test, CRT = choice reaction time, DMT = decision making time, CFF = critical flicker frequency, VIVI = visual vigilance, PWAT = paired word association test, DSST = digit symbol substitution test, GSM = Gibson's spiral maze.

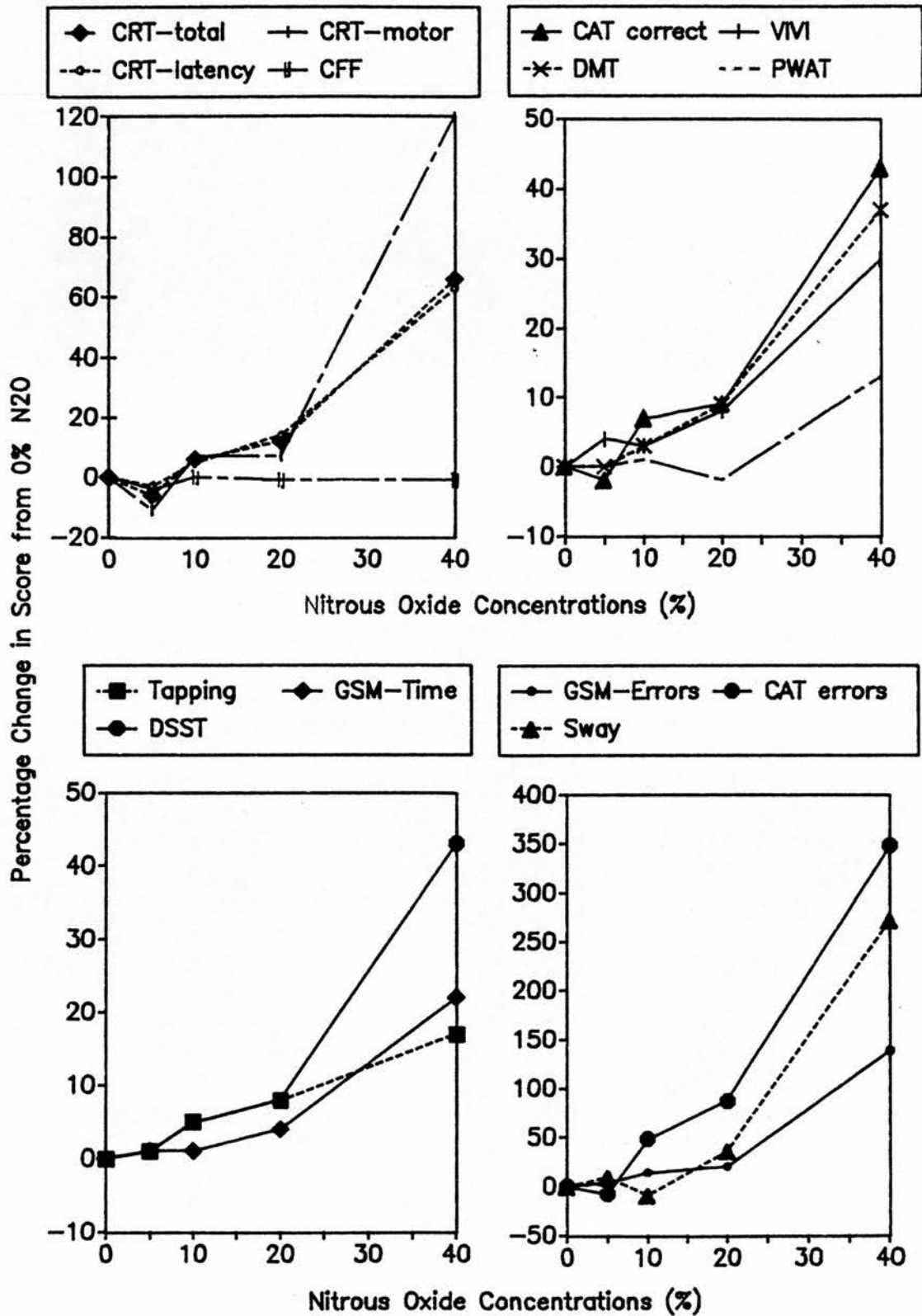


Figure 7.7. Percentage change in test scores for increasing nitrous oxide concentrations. Positive percentage changes indicate impairments, negative percentage changes indicate improvements. CAT = continuous attention test, CRT = choice reaction time, DMT = decision making time, CFF = critical flicker frequency, VIVI = visual vigilance, PWAT = paired word association test, DSST = digit symbol substitution test, GSM = Gibson's spiral maze. The data is the same as that in Figure 7.6, but is split for greater clarity.

TEST	N ₂ O concentration					
	Statistics	0%	5%	10%	20%	40%
Critical flicker frequency (Hz)	mean (s.d.)	27.6 (4.3)	28.7 (3.4)	27.5 (3.2)	27.8 (3.6)	27.9 (3.9)
Total choice reaction time (msec)	mean (s.d.)	586 (83)	553 (84)	620 (86)	655 (177)	972 (682)
Choice reaction latency (msec)	mean (s.d.)	392 (56)	380 (44)	413 (40)	447 (118)	637 (427)
Choice reaction motor time (msec)	mean (s.d.)	194 (51)	173 (58)	207 (82)	208 (86)	426 (566)
Body sway (20' of arc)	mean (s.d.)	11 (4)	12 (3)	10 (4)	15 (4)	41 (26)
Digit Symbol Substitution (no. correct)	mean (s.d.)	80 (7)	79 (10)	76 (7)	74 (8)	46 (15)
Tapping rate (taps/min)	mean (s.d.)	400 (54)	396 (72)	382 (49)	369 (51)	381 (52)
Gibson maze time (secs)	mean (s.d.)	21.3 (5.6)	21.6 (5.5)	21.5 (5.4)	22.2 (7.0)	25.9 (10.3)
Gibson maze errors (number)	mean (s.d.)	19.6 (12.9)	20.3 (12.9)	22.3 (15.0)	23.6 (15.4)	46.9 (21.8)
Continuous attention (number correct)	mean (s.d.)	35.2 (4.3)	36.0 (4.1)	32.9 (6.0)	32.2 (5.4)	20.1 (11.7)
Continuous attention (total errors)	mean (s.d.)	5.2 (4.2)	4.8 4.6)	7.7 (6.1)	9.7 (6.2)	23.3 (11.3)
Decision making time (msec)	mean (s.d.)	582 (77)	583 (77)	601 (77)	637 (116)	769 (195)
Paired word association (number correct)	mean (s.d.)	26.0 (1.6)	26.1 (2.1)	26.4 (0.7)	26.6 (0.7)	22.5 (2.9)
Visual vigilance (number correct)	mean (s.d.)	15.1 (1.9)	14.5 (1.9)	14.7 (1.7)	13.9 (1.9)	10.5 (2.5)

Table 7.5. Changes in test scores with N₂O. Results are expressed as mean and standard deviation (s.d.) for each variable at each concentration.

TEST	N ₂ O concentration				
	0%	5%	10%	20%	40%
Critical flicker frequency (Hz)	0	-4	0	-1	-1
Total choice reaction time (msec)	0	-6	6	12	66
Choice reaction latency (msec)	0	-3	5	14	63
Choice reaction motor time (msec)	0	-11	7	7	120
Body sway (20' of arc)	0	9	-9	36	272
Digit Symbol Substitution (no. correct)	0	1	5	8	43
Tapping rate (taps/min)	0	1	5	8	17
Gibson maze time (secs)	0	1	1	4	22
Gibson maze errors (number)	0	4	14	20	139
Continuous attention (number correct)	0	-2	7	9	43
Continuous attention (total errors)	0	-8	48	87	348
Decision making time (msec)	0	0	3	9	37
Paired word association (number correct)	0	0	1	-2	13
Visual vigilance (number correct)	0	4	3	8	30

Table 7.6. Percentage changes in test scores from 0% with N₂O.

VARIABLE	P of Wilcoxon Signed Ranks			P of F	P of L
	0/5	0/10	0/20		
Digit symbol correct errors	ns	0.00	0.01	0.00	0.00
Choice reaction time:total	ns	ns	0.02	0.00	0.00
mean	ns	0.05	0.01	0.00	0.00
latency	ns	ns	ns	0.00	0.00
motor	ns	ns	0.01	0.00	0.00
total	ns	ns	0.01	0.00	0.00
latency	ns	ns	0.01	0.00	0.00
motor	ns	ns	ns	0.01	0.00
Tapping	ns	0.04	0.01	0.01	0.00
Continuous attention correct	ns	0.01	0.01	0.01	0.00
false positive	ns	ns	0.01	0.01	0.00
total errors	ns	0.01	0.00	0.01	0.00
Sway	ns	ns	0.02	0.00	0.00
Visual vigilance correct errors	0.06	ns	0.04	0.01	0.00
Gibson Spiral Maze time errors	ns	ns	ns	0.01	ns
	ns	ns	ns	0.00	0.05
	ns	ns	ns	0.00	0.00

VARIABLE	P of Wilcoxon Signed Ranks			P of F	P of L
	0/5	0/10	0/20		
Decision making time total	ns	0.09	0.02	0.01	0.00
latency	ns	ns	0.02	0.01	0.00
motor	ns	ns	ns	0.01	0.00
errors	ns	ns	ns	0.03	0.05
Paired word:correct	ns	ns	ns	ns	ns
easy	ns	ns	ns	ns	ns
incorrect	ns	ns	ns	ns	ns
no answer	ns	ns	ns	ns	ns
total errors	ns	ns	ns	ns	ns
correct	ns	ns	ns	0.02	0.05
incorrect	ns	ns	ns	0.01	0.05
no answer	ns	ns	ns	ns	ns
total errors	ns	ns	ns	0.02	0.05
correct	ns	ns	ns	0.01	0.05
incorrect	ns	ns	ns	ns	ns
no answer	ns	ns	ns	0.07	ns
total errors	ns	ns	ns	0.04	ns
correct	ns	ns	ns	0.02	0.01
incorrect	ns	ns	ns	0.01	0.05
no answer	ns	ns	ns	ns	ns
total errors	ns	ns	ns	0.02	0.05
correct	ns	ns	ns	0.01	0.00
incorrect	ns	ns	ns	0.01	0.01
no answer	ns	ns	ns	0.04	ns
total errors	ns	ns	ns	0.01	0.00
Critical flicker frequency	ns	ns	ns	ns	ns

Table 7.7. Summary of statistical results and probability (P) values for variables measured in the nitrous oxide study. F - Friedman analysis of variance; L - Page's L trend test. 0/5 - comparison between 0% and 5% N₂O; 0/10 - comparison between 0% and 10% N₂O; 0/20 - comparison between 0% and 20% N₂O; 0/40 - comparison between 0% and 40% N₂O. ns - not statistically significant. Probability values below 0.1 are given numerically. P less than 0.001 is shown as 0.00. All significant changes were in the direction of impairment.

With respect to the Wilcoxon Signed Ranks tests, the detectable impairments began at different concentrations. The most sensitive tests appeared to be digit symbol substitution, choice reaction latency, tapping and continuous attention (correct and errors) which showed significant effects at 10% N₂O.

Several of the tests were unable to detect the effects of 20% N₂O. These were; choice reaction motor time, digit symbol substitution errors, critical flicker frequency, decision making motor time and errors, paired word association and visual vigilance errors.

Using Z scores as an index of sensitivity of the tests, the comparative sensitivities of the tests appeared to change as the dose increased. Choice reaction time, for example, had one of the greatest shifts in standard deviation at 5%, but the change was in the direction of improvement. Choice reaction time also had one of the biggest Z scores at 40% N₂O, but not at 10% N₂O. Sway was greatly affected at 40% N₂O and had a high Z value, but was not significantly affected below 20% N₂O. Digit symbol substitution and tapping had only modest Z scores throughout, but detected effects at 10% N₂O, suggesting that they were two of the most sensitive tests.

Most of the subjects were affected by N₂O, and several subjects likened the effects of the highest dose to that of being slightly to moderately drunk. One subject vomited near the end of the testing session, and the experiment was stopped. He was given oxygen and lay down for several minutes until he no longer felt ill or "strange", and was eventually driven home. Another subject became unconscious shortly after she began to inhale 40% N₂O and was therefore withdrawn from that session. The anaesthetic gases were stopped and she was given 100% O₂ until she became conscious again, which occurred rapidly. The subject was later driven home.

DISCUSSION

These results clearly demonstrated the effects of N_2O on performance. All of the tests except critical flicker frequency showed significant effects after N_2O , and subjective changes in dizziness and muzziness were also detected on the visual analogue scales. The results agreed well with those of Korttila et al., (1981) and Greenberg et al., (1985) who found statistically significant effects with 30% and 20% N_2O respectively using similar batteries of tests to the one used in the present study.

The sway dose-response curve showed exponential characteristics. Most of the other measures appeared to have linear dose-response curves initially, but the responses became variable at 40% N_2O . The tests which were most affected at the highest concentration of the drug were not necessarily the tests which were sensitive enough to detect low concentrations of N_2O , the exception being continuous attention errors. Some of the dose response curves also appeared to cross over confirming our hypothesis.

Reported lightheadedness/dizziness and ^aparesthesia were greater in the first five minutes after the highest doses indicating that there may have been some rapid tolerance to these effects. This is in agreement with the reports of apparently acute adaptation to the sensation of lightheadedness experience by deep sea divers at depth and in hyperbaric chambers (Fowler et al., 1985).

There also appeared to be acute tolerance to ^aparesthesia, but this was less certain since some subjects found difficulty in distinguishing between a reduced and an increased effect. Thus numbness, which is a stage beyond ^aparesthesia, was sometimes reported as reduced tingling, which may have appeared like acute tolerance. There have been several reports of tolerance

and acute tolerance to the analgesic effects of N₂O (Kripke and Hechtman, 1972; Whitwam et al., 1976; Stuart, 1989). Whitwam et al., (1976) demonstrated that rate was involved since a slowly increasing concentration did not produce acute tolerance in a tooth pulp shock paradigm. However, large interindividual differences in acute tolerance were noted over a 45 minute period using the same pain model (Stuart, 1989). Like Dworkin (1984), Stuart concluded that cognitive modification was responsible for the change in pain threshold in some individuals. Acute tolerance to the psychomotor effects, however, has not been demonstrated in humans.

Korttila et al., (1981) were unable to demonstrate acute tolerance to 30% N₂O using a battery of tests including tapping, critical flicker frequency, choice reaction time and free recall. Testing in this experiment started at 2 minutes after inhalation, and continued for 32 minutes. These authors reported that the maximum impairment occurred at two minutes and remained the same thereafter. The magnitude of the effect was the same on re-challenge. The apparent acute tolerance demonstrated in the present experiment may therefore be a purely subjective effect.

The visual analogue scales appeared to be less sensitive to N₂O than some of the psychomotor tests below 20% N₂O. This contrasts with the results we have previously obtained for visual analogue scales. Temazepam, for instance, was associated with visual analogue scale reports of drowsiness and dizziness for some hours after psychomotor test scores had returned to baseline values (Fagan, et al., 1984). Similarly, visual analogue scales were more useful than objective measures in detecting stimulant effects of mianserin, caffeine, theophylline and enprofylline (Swift et al., 1988; Fagan et al., 1988; Tiplady et al., 1990).

Bruce et al., (1974) and Bruce and Bach (1975, 1976)

reported in a series of open and single blind studies that trace concentrations of N_2O impaired memory for digit span. The results of these studies have never been replicated in any double-blind study despite a number of attempts using similar methodology (for reviews see Smith and Shirley, 1978; Venables et al., 1983). Indeed it is somewhat surprising that these authors consistently found impairments with digit span considering its well known insensitivity to drug effects. Cook et al., (1976) showed an 8% impairment of performance on digit span after 20% N_2O in volunteers. This is of the same order of magnitude as the effects found in the present study with a variety of other tests. There have been few reports of statistically significant effects on any performance measure below 20% N_2O , and the threshold for behavioural effects is considered to be around 10% N_2O (for review see Eger, 1985). Our results, and those of Cook et al., (1976), indicate that the threshold concentration is between 5 and 10% N_2O .

There appears to be no clear reason for the digit span test being approximately 1000 times more sensitive in the hands of Bruce and co-workers than other researchers and it seems likely that there is no specific effect of trace N_2O on memory. The open nature of the studies of Bruce et al., may have contributed to these discrepancies. The memory effects of N_2O will be discussed in Chapter 8 (General Discussion).

Critical flicker frequency was remarkably unchanged throughout. This lack of effect has been reported previously for N_2O (Wernberg et al., 1980; Korttila et al., 1981). Indeed both of these groups found that critical flicker frequency actually increased with N_2O , though we could not repeat this finding. Of the drugs reviewed by Smith and Misiak, (1976) and by Hindmarch (1980) only stimulants showed this pattern of effect. The reasons for the lack of effect of N_2O on critical flicker frequency are not clear. There was little

evidence of drowsiness, and as critical flicker frequency is said to be an index of arousal (Eysenck and Eysenck, 1985) it is possible that N₂O simply did not affect arousal. On the other hand, N₂O is known to increase pupil size, and this is also believed to increase critical flicker frequency scores (Smith and Misiak, 1976), though not via an increase in arousal, but via an increase in retinal illumination. Several authors (including Smith and Misiak, 1976) have recommended standardising pupil size using artificial pupils to avoid this problem. Artificial pupils were not used in our study, and are not thought to be necessary when critical flicker frequency measurements are foveal and recorded under constant lighting conditions as in our experiment (Bobon et al., 1982) However, further experimentation was clearly required to establish the reasons for this lack of effect, and this is addressed in the next section.

EFFECTS OF STANDARDISING PUPIL SIZE ON CRITICAL FLICKER FREQUENCY AFTER 40% N₂O

In the last experiment critical flicker frequency was virtually unaffected by N₂O even in concentrations that affected all of the other tests in the test battery. Critical flicker frequency is sometimes recommended as the test of choice in psychological testing for the effects of drugs (Smith and Misiak, 1976; Hindmarch, 1980; Bobon et al., 1982; Curran, 1990). It therefore seemed odd that the test was the least sensitive to change in an experiment where all of the other tests demonstrated impairments.

Critical flicker frequency is said to be a measure of arousal (Gortelmeyer and Wieman, 1982; Eysenck and Eysenck, 1985). One school of thought says that some or all of the changes in test performance after centrally depressant drugs may be a consequence of a reduction in arousal (Curran et al., 1986, 1988; Fowler et al., 1985, 1989). However, if N₂O produces performance impairment without affecting arousal, then it follows that performance impairment is not necessarily a by-product of sedation. Before making this conclusion, it therefore seemed relevant to investigate whether the lack of effect of N₂O on critical flicker frequency could be explained by other factors such as the methodology.

As discussed in the last section, one possible reason for the lack of effect on critical flicker frequency was that the subjects' pupils had dilated allowing in more light and therefore offsetting the decrease in critical flicker frequency that might otherwise have occurred. If this was the case, then standardising pupil size artificially should allow a decrease in critical flicker frequency to be seen with N₂O. It was the purpose of the following experiment to test this hypothesis.

METHODS

Nine healthy volunteers (6 males and 3 females) participated in this open study, 7 of whom had participated in the N_2O dose response study. The mean age of the subjects was 27 years (range 20-33 years) and the mean weight was 66 kg (range 47-80 kg). Individual demographic data is shown in Table 2.1. The experimental conditions were as outlined in Chapter 2. The experiment was performed in a single session for each individual so that arousal, the lighting and other conditions were the same.

The subjects received 0% and 40% N_2O in oxygen for approximately 30 minutes each under stable external lights-on conditions. Drug delivery and the apparatus used was identical to that described in the N_2O dose response study. In each case the subjects were tested with and without artificial pupils. Testing took place in a set order as follows:

normal vision - 0% N_2O ,
artificial pupils - 0% N_2O ,
artificial pupils - 40% N_2O ,
normal vision - 40% N_2O .

The artificial pupils were constructed from black card cut in the shape of a visor using the visor part of a pair of laboratory safety goggles as a template. The card was replaced in the goggles which could be worn in the usual way. Holes of approximately 2.5 cm diameter were cut in the middle front of each "lens" of the goggles, and these were covered with a black paper disc of about 4.5 cm diameter with 2 mm holes punched in the middle using a leather punch. After the first critical flicker frequencies were obtained with 100% O_2 , the goggles were put on, and the subjects positioned the black paper discs themselves so as to obtain the best binocular view. The discs were then taped to the card. The subjects were tested again on critical flicker

frequency. Once the values were obtained the artificial pupils were left in place, the 40% N₂O was delivered, and testing was carried out again after 10 minutes of steady-state. The goggles were then removed and the subjects were given a little time to adjust to the light again. Testing was carried out again on N₂O without the artificial pupils.

Besides the Leeds apparatus, another critical flicker frequency device (Lafayette) was used for comparison in order to determine whether the sensitivity of the apparatus contributed to the results.

Statistical comparisons

The results were compared using Wilcoxon Signed Ranks. The critical level of significance was taken to be 0.05%.

RESULTS

One "new" subject vomited after a few minutes on 40% N₂O, and the Leeds normal vision results were not obtained after N₂O with this subject. The other new subject became unconscious on 40% N₂O, and so had to be withdrawn from the study.

The results of the analysis for the Leeds critical flicker frequency are shown in Table 7.8.

Normal vision

There was no significant change in the mean critical flicker frequency scores after N₂O, although the mean (\pm s.d.) decreased slightly from 33.5 \pm 3.6 after O₂ to 32.0 \pm 4.1 after N₂O.

Subject	Increasing Critical flicker frequency				Decreasing Critical flicker frequency				Mean Critical flicker frequency			
	Normal Vision		Artificial Pupils		Normal Vision		Artificial Pupils		Normal Vision		Artificial Pupils	
	O ₂	N ₂ O	O ₂	N ₂ O	O ₂	N ₂ O	O ₂	N ₂ O	O ₂	N ₂ O	O ₂	N ₂ O
SW	36.4	33.0	27.2	27.6	36.8	30.8	30.1	24.0	36.6	31.9	28.7	25.8
CM	29.2	32.0	25.0	24.6	31.0	29.4	24.7	20.7	30.1	30.7	24.9	22.7
SD	38.7	39.0	30.4	34.0	39.1	36.9	30.0	23.5	38.9	37.9	30.2	28.7
DP	35.1	-	23.8	29.2	34.8	-	23.8	23.7	35.0	-	23.8	26.5
ML	29.8	23.7	25.0	21.1	28.9	30.3	26.0	20.5	29.3	27.0	25.5	20.8
AH	32.2	30.3	24.3	25.3	33.6	33.3	33.0	30.9	32.9	31.8	28.7	28.1
AL	29.4	30.5	24.5	26.4	29.9	25.6	23.2	19.2	29.6	28.0	23.8	22.8
EC	35.9	37.8	24.5	33.4	35.1	35.8	30.6	25.0	35.5	36.8	27.6	29.2
Mean	33.3	32.3	25.6	27.7	33.7	31.7	27.7	23.4	33.5	32.0	26.7	25.6
s.d.	3.7	5.1	2.2	4.4	3.5	3.9	3.7	3.6	3.6	4.1	2.5	3.1

Table 7.8. Effects of "artificial pupils" on critical flicker frequency in the presence of 100% oxygen (O₂) and 40% nitrous oxide (N₂O).

Artificial pupils

There was a significant reduction in the decreasing flicker rate after N_2O , but not in the increasing flicker rate or the mean. The decreasing critical flicker frequency was reduced in all 8 subjects after N_2O ($P < 0.02$). The mean percentage change was about 15%.

Normal vision vs. artificial pupils

Critical flicker frequency was reduced by the artificial pupil per se ($P < 0.02$). This change was the biggest effect in the study.

Lafayette

As with the Leeds tester, the only significant effect was on flicker rate decreasing which reduced from 34.2 ± 4.8 to 30.7 ± 3.0 Hz representing a 10% reduction in critical flicker frequency.

Discussion

The hypothesis was that the lack of change in critical flicker frequency was due to an increase in pupil size, and that if the pupil size was constant there would be a decrease in critical flicker frequency after N_2O . The results only partially support this argument since flicker rate decreasing, but not increasing, behaved in the expected manner. Indeed flicker rate increasing showed a tendency to increase with the artificial pupils after N_2O , in common with the findings of Korttila et al., (1981) and Wernberg et al., (1980).

The most likely explanation for these results was that the drug affected the response time, and that this offset the reduction in flicker rate increasing. However, if the response was delayed after N_2O this would have caused an overshoot in both increasing and decreasing critical flicker frequency. Thus any real

reduction in flicker rate increasing would be masked by an overshoot in the direction of increasing flicker. For flicker rate decreasing, overshoot would have produced a further reduction thereby exaggerating the effect.

Neither Korttila et al., 1981 nor Wernberg et al., 1980 mentioned using artificial pupils in their studies with critical flicker frequency, and this may be the reason for the results with N_2O (i.e. improvement of critical flicker frequency) that these authors have reported.

The effect of N_2O on arousal will be discussed further in Chapter 8.

SUMMARY

A dose response experiment was carried out with nitrous oxide as a model compound in order to test the hypothesis that dose response curves for different psychological tests, cross over. The effect of nitrous oxide on critical flicker frequency was assessed in a separate experiment.

Results from a five period crossover study using four doses of nitrous oxide and oxygen appeared to confirm this hypothesis.

The tests which were most sensitive at the lower concentrations did not necessarily show the biggest changes at the higher concentrations.

The most sensitive tests were choice reaction latency, digit symbol substitution, continuous attention and tapping showed significant changes at 10% N_2O . The least sensitive measure was critical flicker frequency.

In another experiment the effect of standardising pupil size on the critical flicker frequency response to N_2O was evaluated. The hypothesis that the lack of effect on critical flicker frequency was due to pupil dilation rather than lack of effect on arousal was only partially supported.

CHAPTER8 - GENERAL DISCUSSION

DISCUSSION

The purpose of this thesis was to establish the usefulness or otherwise of steady-state with constant plasma concentrations for studying the central effects of central nervous system depressants. The technique was applied using different routes of administration for three different drugs; chlormethiazole (intravenous), ethanol (oral) and nitrous oxide (inhalation). The venous plasma and/or breath concentration-effect relationships were studied over a relatively short period of time using both subjective and objective measures.

The battery of tests used to assess the behavioural effects of the drugs included a selection of some of the main human performance factors identified by various researchers (e.g. Kennedy et al., 1985; Bittner et al., 1986; Englund et al., 1986). The psychological functions covered included attention (sustained - continuous attention correct, continuous attention errors, visual vigilance; focussed and execution - digit symbol substitution), memory (paired word association), psychomotor speed (total choice reaction time, choice reaction latency, motor reaction time, decision making time, Gibson spiral maze time, tapping rate) and psychomotor accuracy (Gibson spiral maze errors). Other factors such as arousal (critical flicker frequency), mood (visual analogue scales) and standing steadiness (sway) were also assessed as recommended by Hindmarch (1980); Bond and Lader (1974) and Linnoila (1983). Fine hand-eye coordination and reasoning ability were the two main psychological functions not specifically assessed in these studies.

All of the tests except paired word association were shown to be reliable, consistent and stable between and within test sessions (Chapter 3). Of the 23 variables derived from the 10 performance tests, significant drug effects were revealed in 21. In addition significant effects occurred in 10 of the 11 visual analogue scales,

and in the symptom rating scale. No significant effects were revealed for digit symbol substitution errors, visual vigilance errors or for the pleasant/unpleasant visual analogue scale used in the N₂O study (Chapter 7).

The results showed clearly that steady-state with constant plasma levels was an extremely useful condition for observing the presence or absence of early acute tolerance and the pattern of impairments produced by central nervous system depressants with widely differing physical properties. The dose-response curve produced using this technique (Chapter 7) was also easier to interpret than the dose-response curves usually produced in psychomotor studies.

Since there were no rapid changes in plasma/brain concentrations of drug after the initial rise, the drugs could be evaluated and the tests compared under relatively stable, "clean" conditions and the distribution artifact (Goldstein; 1983) avoided. This was particularly important for the study of acute tolerance since in some pharmacokinetic studies the venous blood levels of drugs have been shown to continue rising some time after the arterial (and presumably brain) concentrations have started to fall (e.g. Tucker and Boas, 1971; Dundee et al., 1971). Using the steady-state methodology it could be seen that, in terms of cognitive and psychomotor performance, there was no acute tolerance to chlormethiazole or ethanol (Chapters 5 and 6) although there was evidence of acute tolerance to some subjective effects of chlormethiazole and N₂O (see Chapters 5 and 7). It thus seems unlikely that the duration of action of the drugs is limited by tachyphylaxis, or that bigger doses would be required to produce the same effect in the very short term.

Use of the steady state methodology also had the advantage of allowing the same battery of tests to be used to study the pattern of impairments produced by drugs with widely differing properties. For example,

the number and choice of tests in a battery is often limited by the length of time it takes to perform the tests (e.g. Sahakian, 1990). For the study of drugs with short and ultra-short half-lives, such as chlormethiazole and N_2O , the battery and the individual tests may, of necessity, have to be brief in order to usefully detect any effects of the drug. Because the plasma concentrations were maintained at steady levels in each experiment the half-lives became an irrelevant feature in the choice of tests used to assess the impairment pattern of the drugs. Three apparently markedly different central depressants, chlormethiazole, ethanol and N_2O , with very different plasma/time profiles could thus be studied using the same tests. The results showed that with all three drugs, the greatest effects were on continuous attention and body sway. From the N_2O dose-response study (Chapter 7) it was clear, however, that dose was an important consideration in the apparent profile of impairment. The tests which showed statistical significance at the lower doses were not necessarily those which demonstrated the biggest impairments at the highest dose. Conversely, the fact that a test was more affected at the highest dose did not mean that it was more affected at the lower doses.

The main disadvantage of using steady state with constant plasma concentrations was that greater effort had to be put into the initial planning stage in order to calculate suitable dosing regimes. However, it may not be necessary to individualise the regimes in all studies if the pharmacokinetics are known and the inter-subject variation is reasonably small. We successfully used group data to calculate loading and maintenance regimes for constant plasma infusions of enprophylline and theophylline in another recent experiment (Tiplady et al., 1990).

A further proposed use of the constant plasma regimes was for comparing the sensitivities of the tests in the battery. Analysis of test sensitivity is not easy

(Hindmarch and Bhatti, 1987). Comparing the sensitivities of different tests is even less so. However, although it may seem like comparing apples with pears, there is a good reason for making these comparisons. If, for example, attention tests are more sensitive than reaction time tests then it follows that significant effects on attention but not reaction time after drugs may reflect the test sensitivities rather than any specific effects of the drugs. Thus apparent profiles of impairment of drugs might simply be artifacts of using tests with different sensitivities.

In order to investigate this the test results were scaled to units which could be compared between different measures, viz. Z scores and % changes, thus allowing us to compare the magnitudes of the effects in each experiment. There were difficulties in interpreting the results analysed in this simplistic way. Not all of the tests have so far been validated in terms of Construct Validity (Isaac and Michael, 1981), and we had no accurate idea of how well the tests measured the functions they were meant to represent. In any one experiment it was therefore extremely difficult to distinguish between the resultant effects of test sensitivities, the sensitivities of the underlying central nervous system processes being assessed, and the apparent profiles of impairment caused by the drugs.

Wittenborn (1979, 1987), and Hindmarch and Bhatti (1987) have used an alternative approach to assessing test sensitivities. These authors calculated the proportion of times psychomotor tests gave statistically significant discriminations in drug studies relative to the number of times they were used. This value was then used as an index of test "efficiency". The authors assumed that lack of statistical significance was due to lack of test sensitivity rather than lack of difference between the drugs, and inferred that efficiency was closely related to sensitivity. Although these assumptions may be challenged, it does seem reasonable

to expect that the most sensitive tests would be statistically significant more often in a large number of studies.

For Wittenborn (1987) the rank order of efficiency for the tests relevant to this thesis was as follows (see explanation of abbreviations at foot of page):

DSST>sway>tapping>CFF>CRT>"attention" (unspecified).

For Hindmarch and Bhatti (1987) the order derived using a smaller selection of tests was:

"Vigilance" (unspecified) >CFF>CRT>"memory tests".

From the three main studies described in this volume the order calculated using the same method was:

CAT>DSST>tapping>sway>CFF, CRT>GSM errors>PWAT.

With minor differences for tapping and sway, the orders of test efficiency were the same. Wittenborn's attention task(s) appeared to be less likely to show significances, but were only used 4 times. Hindmarch's vigilance test was only used twice, but was significant both times.

The reliability coefficients recommended by Shrout and Fleiss (1979) and Kraemer and Korner (1976) had little predictive value for test efficiencies. The tests which most often showed statistically significant drug effects did not have the highest coefficients of reliability, stability or consistency (Chapter 3). Since

Abbreviations: DSST = digit symbol substitution test, CFF = critical flicker frequency, CRT = choice reaction time, CAT = continuous attention test, GSM = Gibson spiral maze, PWAT = paired word association test, CRT-T = total choice reaction time, CRT-L = choice reaction latency, CRT-M = choice reaction motor time.

reliability coefficients are based on correlations, the highest reliability scores were obtained from data where the intra-subject test score variation was small and the inter-subject variation was large. However, it was clear from the data that the most efficient tests (i.e. those which gave statistical significances most often) tended to be those with the smallest inter-subject variation.

The coefficients of variation (coefficient of variation = $100 \times (\text{standard deviation}/\text{mean})$, calculated from the baseline data (scores between and within sessions, Chapter 3) were better predictors than reliability scores of the statistically significant results after chlormethiazole, ethanol, and after the lower doses of N_2O . This gave rise to the following predicted order of test efficiencies (the smaller the coefficient of variation the higher the efficiency):

CAT>PWAT>tapping>DSST>CFF>CRT-T=CRT-L>CRT-M>sway

Although not an exact predictor, this order was in generally good agreement with the rank orders of efficiency obtained from the three main experiments in this thesis. The order also agreed well with those obtained by Wittenborn (1987), and Hindmarch and Bhatti (1987) from their meta-analyses of a large variety of studies assessing different types of drugs.

Only paired word association and sway had efficiencies which could not be predicted from the baseline coefficient of variation scores. Sway gave more significances and a higher efficiency than predicted (because it was exponentially affected by the drugs at higher doses/concentrations, and substantial doses may have been given in many studies to get a "good effect"), and paired word association because it was not impaired by any but the highest dose of N_2O (probably because the test was too easy).

From the above data it seems likely that the minimum size of detectable statistically significant effect (the sensitivity) differed from test to test, and in the approximate order of test efficiency. Thus even if a drug produced identical dose response curves on the tests, different tests would have begun to show the effects as statistically significant at different concentrations/dose levels. Theoretically, a non-specific drug having an identical size of effect on all of the behaviours assessed would produce a quite specific-looking pattern of behavioural effects. This pattern would change predictably as the dose increased and more tests demonstrated the effects significantly.

This has enormous implications for the way in which drug studies should be interpreted. Even using steady-state constant plasma concentration methodology and a standard battery of tests, non-equivalent doses of drugs with identical behavioural effects would produce apparently different impairment patterns. Some differences in impairment patterns between research centres would also be expected since researchers often use different tests with different sensitivities to assess the same functions (Hindmarch, 1980). The sensitivities of individual tests are often magnified (Poulton, 1965), or inadvertently reduced because of poor methodology (see Smith and Misiak, 1976). Indeed Gullion and Eckerman (1986) listed six different ways, proposed by various researchers, of increasing test sensitivity and thus improving the chance of obtaining statistical significance. The fact that different analyses of test efficiencies gave similar rank orders (Wittenborn, 1987; Hindmarch and Bhatti, 1987; the present work) suggests, however, that overall the tests in the battery used in these experiments behaved similarly in the hands of other researchers.

For a depressant drug the impairment pattern expected from the coefficient of variation data using our battery

of tests would be as follows: significant sustained attentional impairments with the smallest doses; memory (paired word), focussed attentional and tapping impairments at a slightly higher dose; reduced critical flicker frequency, prolonged choice reaction time and increased sway at yet higher doses. For reasons which will be discussed later, paired word association would not be expected to be consistently sensitive.

Nitrous oxide appeared to be a good candidate for a drug with non-specific acute actions in the central nervous system. N_2O is virtually unmetabolised by the body, although very small quantities are believed to be metabolised by intestinal bacteria (Trudell, 1985). The drug is known to inactivate vitamin B12 via inactivation of methionine synthetase (Nunn and Chanarin, 1985), but it is highly improbable that this has any immediate consequences on behaviour. The limited available evidence suggests that the effects of N_2O on behaviour is qualitatively indistinguishable from other inert gases such as hyperbaric nitrogen and the noble gases (Fowler et al., 1985, 1989).

The data from the N_2O study fitted in well with the non-specific pattern predicted using the coefficients of variation calculated from the pooled data used in the reliability studies (Chapter 3). Although the data from the reliability studies and the N_2O study are not entirely independent, the overlap of volunteers was small (<20%), and this should not have biased the results. Paired word association and critical flicker frequency were the exceptions to this non-specific pattern.

The apparent resistance to impairment of paired word association with N_2O has been noted previously (Biersner 1972; Mewaldt et al., 1988). The test appeared to show a substantial impairment the first time it was used with N_2O , but this was followed by an improvement of test performance with successive trials (Biersner, 1977;

Mewaldt et al., 1988). This may have been due to a change in rehearsal strategy (Fowler et al., 1987) and/or may simply be a practise effect. There tended to be a ceiling effect with paired word association, and practice effects may not have been seen after placebo because there was little room for improvement. Breakthrough practice effects are not uncommon after drugs (e.g. Scott et al., 1983), and Biersner et al., (1977) noted that this "improvement" with paired word association took place irrespective of treatment or test set. It is therefore possible that there were carryover effects from the ethanol study to the N₂O study with this test, since one preceded the other and there was a considerable overlap of volunteers (see Table 2.1).

The insensitivity of critical flicker frequency to N₂O has also been reported previously (Kortilla et al., 1981). The relative lack of effect of N₂O on this test was somewhat surprising in view of its reputed sensitivity (Smith and Misiak, 1976; Hindmarch, 1980). It may be explained, in part, by an effect of N₂O on pupil size (see discussion in Chapter 7, Experiment 2). Critical flicker frequency is considered a measure of arousal/sedation (Hindmarch 1980, Smith and Misiak, 1976; Eysenck and Eysenck, 1985), and there is little evidence of sedation among subjects even in relatively high subanaesthetic doses (Parbrook, 1967; Porter, 1972). Critical flicker frequency may therefore not have been sensitive to changes that were not linked to sedation. Arousal, sedation and critical flicker frequency will be discussed again later in this Chapter.

It was our hypothesis that chlormethiazole and ethanol would have different patterns of effect from N₂O because of differences in the sites and mechanisms of action of the drugs.

Chlormethiazole is believed to enhance gamma-aminobutyric acid transmission and glycine mediated inhibition in the

brain, possibly at the level of the gamma-aminobutyric acid receptor coupled ionophore (Ogren, 1986). Additionally, chlormethiazole appears to exert effects on some other neurotransmitter systems such as the dopaminergic, serotonergic and noradrenergic systems (Ogren, 1986). Assuming that these neurotransmitter systems are involved in aspects of behaviour, chlormethiazole appears to have the potential for a more specific action than N₂O.

Ethanol, on the other hand, might well have had the same pattern of effect as N₂O if the effects were nonspecific as would be expected from the general depressant action classically attributed to the drug (Meyer-Overton, 1901). However, it has recently been proposed instead that ethanol exerts a variety of very specific effects at quite different sites in the central nervous system.

Bloom (1987), reviewed a number of electropharmacological and whole animal behavioural studies with ethanol. The electropharmacological evidence showed some neuronal systems (e.g. the inferior olive and the locus ceruleus) to be much more sensitive to the drug than others. Furthermore, the effects of ethanol on these and other brain systems (e.g. cerebellum and hippocampus) were considerably greater when ethanol was delivered via intraperitoneal injection than applied directly by iontophoresis to the brain. On the basis of these results it was suggested that some areas of the brain might be specifically affected by a direct or indirect action of ethanol. Two of these areas, the hippocampus and the locus ceruleus, have been shown to be important for the processes of attention and memory (e.g. Devenport and Hale, 1989; Bloom, 1987). It might therefore have been predicted that ethanol would have a pattern of impairment demonstrating specific effects on these performance factors in particular. The behavioural effects of ethanol would therefore be expected to differ from those of both N₂O and chlormethiazole.

All of the tests were significantly affected by chlormethiazole as we have noted previously (Fagan et al., 1990), and the size of the effect was different for each test (Chapter 5). In contrast, the ethanol results clustered around 7-8% in at least one measure for several tests, but not all of these effects were statistically significant. The sway and continuous attention error scores showed the greatest impairments for both drugs.

Although the chlormethiazole and ethanol patterns looked different there were similarities, particularly with regard to the effects on sway and continuous attention errors. It was clear that the subjects were more affected in the chlormethiazole study than in the ethanol because the subjects were quite drowsy and the doses were not equipotent. From the discussion of test sensitivities, the patterns of statistically significant effects would be expected to differ for non-equipotent doses even if the effects were qualitatively the same. It therefore seemed possible that the results were similar, but were taken from different points along a similar dose response curve of central nervous system impairment.

Superimposing the results of the chlormethiazole and ethanol studies on the N₂O dose-response curves indicated that both drugs had similar patterns of impairments to N₂O (Figure 8.1), but at different parts of the dose-response curve. 1.3 µg/ml chlormethiazole appeared to have slightly larger behavioural effects than 40% N₂O, whereas 100mg% ethanol appeared to be approximately equivalent to 20% N₂O. The main exceptions were critical flicker frequency and paired word association which behaved differently with N₂O as discussed previously. The N₂O test results were also less consistent than those of ethanol, probably because vision and movement were occasionally restricted by the apparatus in the N₂O study.

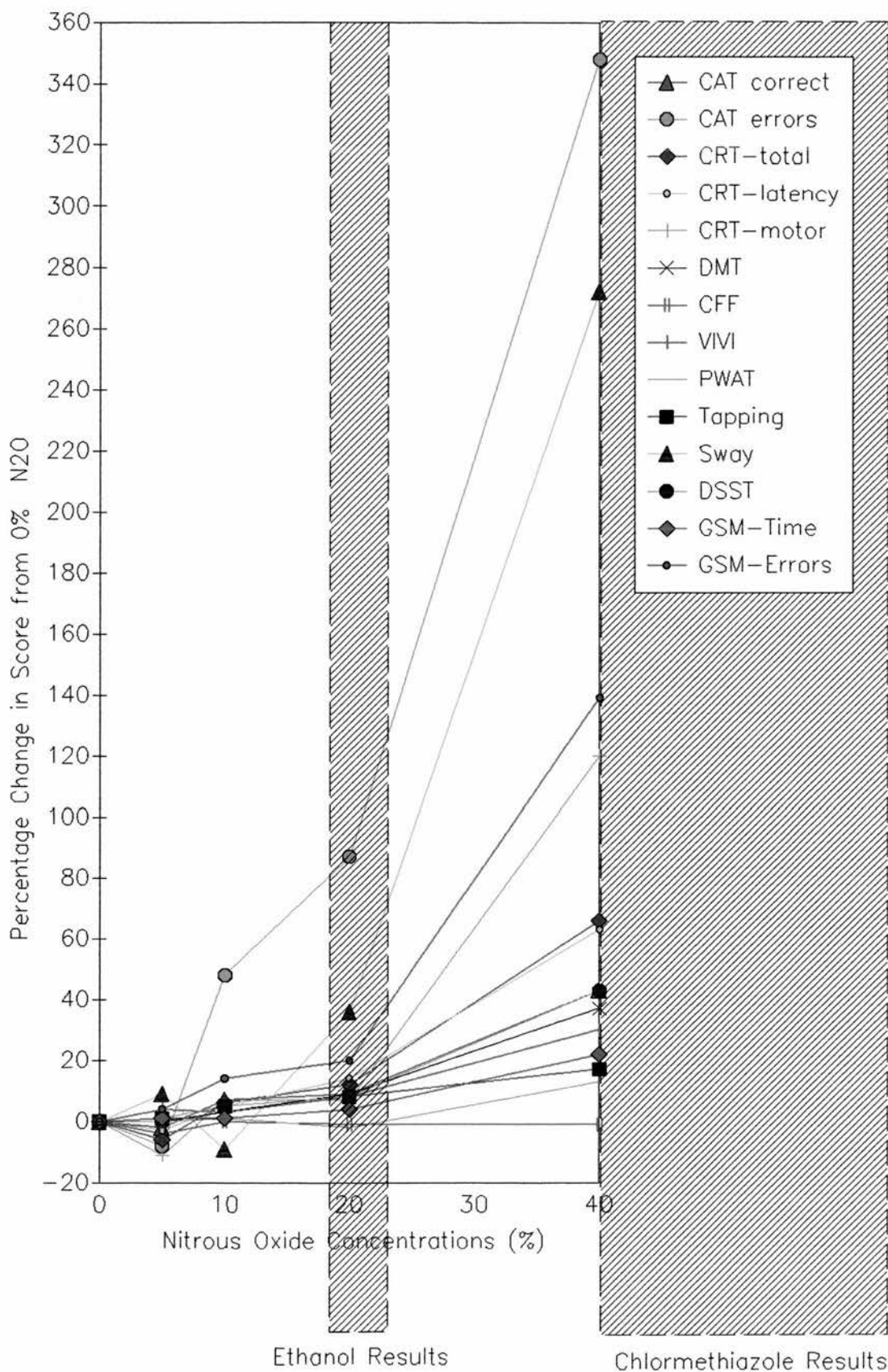


Figure 8.1. Ethanol and chlormethiazole results superimposed on the N₂O dose-response curves for the tests. The narrow shaded area represents the bandwidth in which the ethanol (100 mg.100 ml⁻¹) results appear. The exact location of the chlormethiazole (1.3 ug.ml⁻¹) beyond the N₂O results is unknown (hence wide shaded area). CAT = continuous attention test, CRT = choice reaction time, DMT = decision making time, CFF = critical flicker frequency, VIVI = visual vigilance, PWAT = paired word association test, DSST = digit symbol substitution test, GSM = Gibson's spiral maze. For the ethanol data see Table 6.4. column 8. The chlormethiazole data is from Table 5.3.

All three drugs showed bigger performance effects on sway than on the other tests. This test appeared to be exponentially affected by N_2O (Figure 7.6), and may also have been exponentially affected by ethanol and chlormethiazole. It was interesting, therefore, that the sway test was less sensitive than the other tests to N_2O in the lower doses. This suggested that a differential sensitivity of the balance process was responsible for the bigger sway effects rather than a more sensitive test apparatus or a specific effect of the drugs. Subjects therefore appeared to be able to compensate for effects on balance in the lower doses, but the balance system essentially collapsed when the dose increased. This finding clearly requires further investigation and clarification as to its generality. However, an exponential dose response curve for balance may explain why sway appears to behave synergistically with a number of combinations (e.g. N_2O and ethanol; CO_2 and hyperbaric air; benzodiazepines and age; benzodiazepines and ethanol) which otherwise appeared to be additive (Fowler et al., 1985; Swift, 1985). A combination of the distribution artifact (with peripheral venous concentrations lagging behind brain concentrations making the central effects look "early"), a large peak effect (because of an exponential dose-response curve) and the appearance of faster recovery (because of insensitivity at lower concentrations) may well give the impression of acute tolerance with functions such as sway.

Sustained attention, as assessed by the continuous attention test was reduced by all three drugs. In the N_2O experiment the size of the impairment of continuous attention correct was about the same as the decrements on most of the other tests, but it was less impaired than the others in the chlormethiazole study. However, the results were generally more variable at this much greater impairment level in the chlormethiazole study. The size of the impairment on continuous attention correct was

double that of the other measures in the ethanol study (14% as opposed to 7-8%). All three drugs showed large increases in errors on the continuous attention test.

This bigger effect of ethanol on continuous attention correct was the best evidence of a specific effect on attention as proposed by Bloom (1987). However, although double the size, the effect on continuous attention correct was neither convincingly large nor statistically significant, and random effects due to the small numbers could not be ruled out. Furthermore, digit symbol substitution and visual vigilance which assessed aspects of focussed attention (Mirsky, 1988) were no more affected than the other tests after ethanol, although the effect on digit symbol substitution correct was statistically significant in all three main drug experiments. There was no significant increase in digit symbol substitution or visual vigilance errors.

Several authors noted that ethanol impaired the ability to monitor more than one task simultaneously, i.e. the ability to "divide attention" (e.g. Moskowitz, 1973; Linnoila, 1978; Mills and Bisgrove, 1983). This was postulated as one of the early or "threshold" deficits of the drug (Mitchell, 1985), and is considered to be one of the specific effects of ethanol (Bloom, 1987). However, attention has been shown to be affected by low concentrations of many drugs including e.g. N₂O (Garfield et al., 1975), toluene (Stewart et al., 1975; Echeverria et al., 1989), benzodiazepines (Gruneberger and Saletu, 1980), neuroleptics (King, 1990), vasopressin and adrenocorticotrophic hormone (Wolkowitz et al., 1985) and it seems likely that these divided attention tests may simply be particularly good at detecting nonspecific changes.

The size of the impairments increased with task difficulty (Moskowitz, 1973; Moskowitz and Sharma, 1974), suggesting that increasing task complexity increased test sensitivity. Results of these studies

were also recorded in terms of error scores, and it is perhaps not surprising that the more complex attentional tasks produced fewer correct scores after ethanol. Both of these strategies (i.e. increasing task complexity and using error scores as the main measure) have been recommended by Poulton (1965) and Gullion and Eckerman (1986) for magnifying the sensitivities of tests, and may therefore have no further significance. However, the idea of this "specific" effect of ethanol on attention is an appealing and plausible theory, and it has consequently been intensively studied (with positive results). This may have had the effect of falsely increasing its importance.

The subjects were quite drowsy in the chlormethiazole study, and they had some difficulty in performing the tests. The results may therefore have reflected the nonspecific effects of sedation as postulated by Curran et al., (1986, 1988). However, the subjects' performance was also quite disrupted with the highest dose of N₂O, despite the degree of reported sedation being small (<10mm mean change from the baseline visual analogue scale alert-drowsy score). This suggests that the relationship between impaired performance and sedation is not necessarily a close one, or that very small changes in the state of alertness may lead to very large effects (Lezack, 1983). It was interesting therefore that the objective measure of arousal, critical flicker frequency, was hardly affected by N₂O. Wernberg et al., (1980) noted an increase in critical flicker frequency with N₂O which is generally considered to indicate a stimulant effect. Although these results could be explained partly as an effect on pupil size, it was interesting that Lader and Norris (1969) and Jarvis and Lader (1971) also found that, after N₂O, subjects reported a small degree of subjective sedation which was not paralleled by changes in the N wave of the electro-encephalogram (also proposed as an index of arousal; Wilkinson, 1967). However, as pointed out by Bond and Lader (1974), single visual analogue scale

subjective ratings are influenced by other subjective feelings, and it is possible that the subjects were not drowsy, but were registering the fact that they did not feel normal. It would be interesting to find out if this apparent dissociation between impairment and sedation would be seen with low steady-state constant plasma concentrations of chlormethiazole and benzodiazepines.

Relatively few studies involving drugs and psychomotor impairment have used steady-state methodology which allows drug effects to be assessed in a clear way. However, three areas where these effects have been studied together in some detail are in the fields of anaesthesia, undersea biomedical research and neurobehavioural toxicology.

In the neurobehavioural toxicology area, guidelines for the standardisation of test batteries to evaluate health hazards were set down some years ago by the World Health Organisation and the National Institute of Occupational Safety and Health (Seppalainen et al., 1983). These were aimed to assess a range of behavioural functions. Thus many researchers in the neurotoxicology field have approached the assessment of behavioural impairments in a standardised manner which allows reviews of the data to yield quite useful information. An example of a single study and a review is illustrated below.

Echeverria et al., (1989) looked at the neurobehavioural effects of "threshold" doses of the solvent toluene (0, 75 and 150 ppm) on healthy volunteers over seven hour periods in an acute inhalation chamber. A large battery of psychological tests were used to assess impairment, and the results were analysed in a similar manner to the results in this thesis, although only the statistically significant results were presented as percentage changes. These authors found that most statistically significant decrements clustered around 5-7% (visual and verbal short term memory - pattern memory, digit span; manual dexterity - number and time of movements), but that a

perceptual (attentional?) task (pattern recognition) was more affected (12%). The false positive responses on another test (symbol-digit) were also more affected (26%).

The general findings of these authors was in close agreement with the effects we found for ethanol (and for N₂O since neither critical flicker frequency nor paired word association were assessed), however there were two major discrepancies between their data and ours in the observed sensitivity of similar tests. If toluene was behaving in the same way as ethanol or N₂O we would have expected tapping and continuous performance to be significantly affected which was not the case. However, on close inspection, the discrepancies appeared to be caused by differences in methodology which made these two tests less "sensitive" in the hands of these workers. Tapping in Echeverria's study was only performed for 10 seconds, and in the continuous performance test the reaction time was used as the main measure rather than the correct and/or error scores. The test was also performed very near "ceiling" as evidenced by the very small numbers of omission and commission errors (1 each), and the test may therefore have been too easy.

Despite these differences, the methodology used was similar enough to that used in the present work to note the strong similarities in the patterns of impairment between these drugs. From their steady-state toluene data Echeverria et al., (1989) also noted that positive results were found on the most stable measures, and that performance decrements could be more easily detected on tests with little interday variation for the group.

Ten doses of toluene were assessed in 9 acute steady state inhalation studies (for review see Echeverria et al., 1989) for periods of 3.5 - 8h using batteries of tests assessing a wide range of functions.

The results at each dose level are summarised below:

- 50 ppm - no effect
- 75 ppm - symbol digit improved
attention, perception, vigilance impaired
- 100 ppm - fatigued and sleepy, components of manual
dexterity impaired
- 150 ppm - digit symbol substitution and memory impaired
- 200 ppm - tapping and simple reaction time impaired
- 300 ppm - choice reaction time impaired
- 600 ppm - sway impaired (ataxia and incapacitation)
- 700 ppm - "reasoning" impaired.

Based on the evidence from the same (or equivalent) tests, the effects of toluene appeared to be similar to those of ethanol and N_2O . In higher doses the effects were similar to those of chlormethiazole.

Using steady-state methodology, toluene thus appeared to demonstrate a non-specific global pattern of acute impairment similar to that which we found with ethanol and N_2O , apart from the drowsiness. However, the ethanol and toluene experiments ran for a much longer period of time than the N_2O experiment, and this time factor may have contributed to the increased drowsiness.

The doses at which the drugs demonstrated statistically significant effects on different functions are shown in Table 8.1. The results from a number of other studies are also shown. N_2O appeared to be approximately 2000 times less potent than the solvent toluene for equivalent behavioural effects. 100 mg% ethanol (i.e. 25% over the U.K. legal driving limit for ethanol) also appeared to be equivalent in behavioural terms to between 300 and 600 ppm toluene.

It was clear that the limits of sensitivity of the tests determined the size of the effects that could be detected

Measures affected (in order of increasing impairment)	Chlormethiazole (µg/ml)	Ethanol (mg%)	N ₂ O (%)	Toluene (ppm)
Improved performance	?	↑	<5?	75
Impaired attention, perception, vigilance				75
Impaired manual dexterity (repetitive movement)				100
Impaired digit symbol substitution, "memory"				150
Slowed tapping rate, simple reaction time			10	200
Slowed choice reaction time		100	20	300
Marked, noticeable ataxia			40	600
Impaired "reasoning"	1.3			700

Table 8.1. Concentrations of chlormethiazole, ethanol and nitrous oxide at which various performance measures (and all those above) were affected. Toluene is also shown for comparison. Toluene data were taken from a review by Echeverria et al., 1989.

as statistically significant. It was striking therefore that with steady state constant concentrations of ethanol, N₂O and toluene several tests demonstrated the same percentage size of impairment irrespective of statistical significance. As there was no reason a priori to expect that this would be the case, it made this result all the more interesting. It seems likely that this percentage change represents the actual size of a global effect on the cortex. The "threshold" significant changes occurred when the size of the effect was about 5%.

Since it appears to be possible to predict in healthy young volunteers what types of functions are likely to be statistically significantly affected for different degrees of global impairment, this may be useful in providing an index of general impairment for the drugs. A graphical representation of the percentage global impairment for different concentrations of drug is shown in Figure 8.2.

Fowler et al., (1985, 1989) have suggested that the fundamental change in inert gas narcosis is a slowing of speed of processing linked to a decrease in arousal. The present results suggest that there may indeed be a common mechanism underlying these changes, although the nature of the involvement of arousal is not clear and requires further investigation.

One of the reasons postulated for the similarity of action of N₂O and the noble gases is that they are small, inert molecules which probably behave in the same non-specific way (Porter, 1972). Toluene, although organic, is also a comparatively small molecule and this may explain its similarity of action. It is interesting, however, that these are all drugs which are capable of being evaluated in inhalation chambers or using anaesthetic techniques.

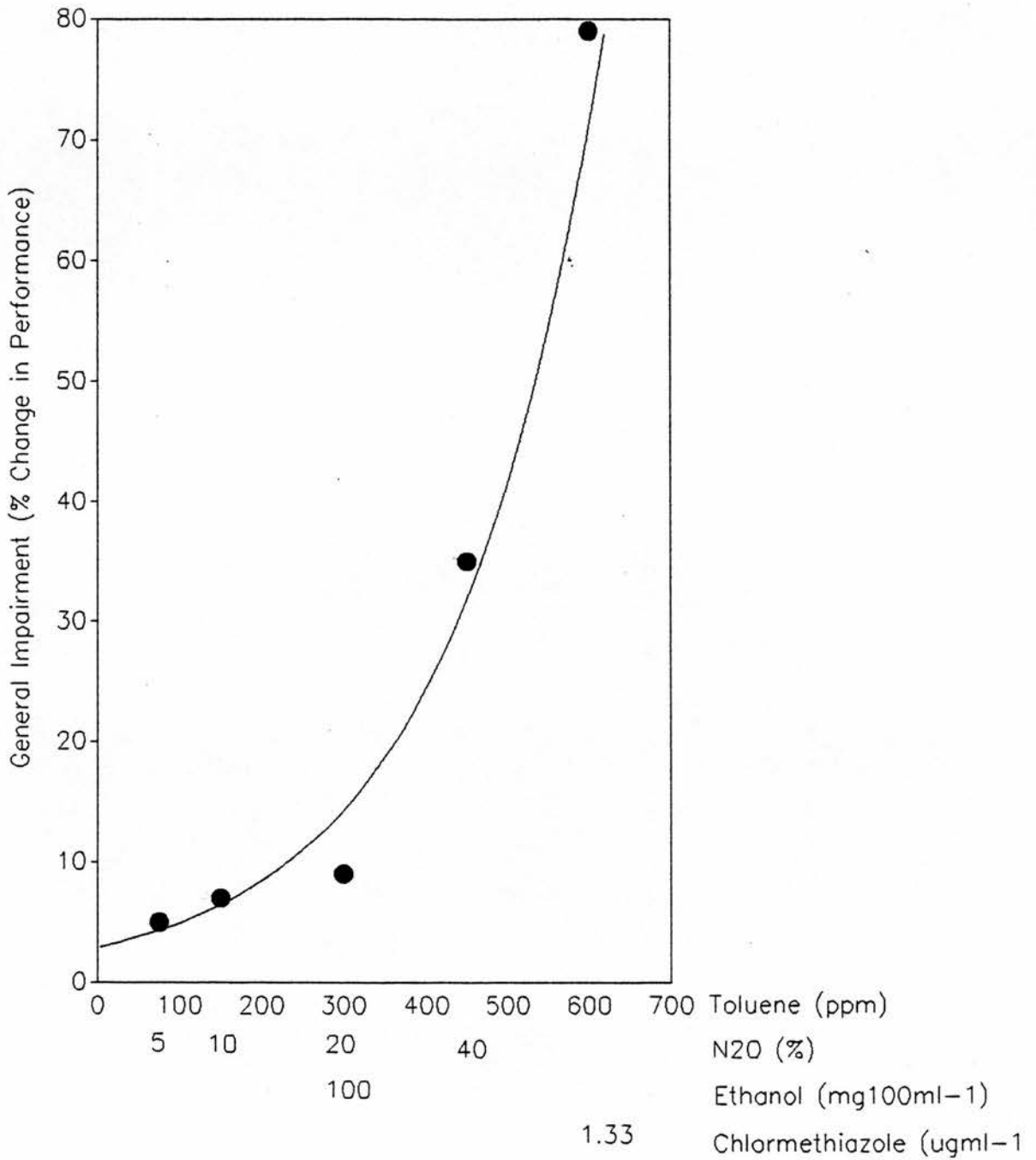


Figure 8.2. General impairment (average percentage change in performance on choice reaction time, digit symbol substitution, tapping rate, memory and attention tests) at various concentrations for the drugs used in this thesis, and toluene for comparison. Toluene data were taken from a review of Echeverria et al., (1989). N.B. The impairment for sway would be much steeper.

This has allowed testing to be carried out under relatively "clean" steady-state constant plasma conditions which has enabled the similarities to be seen more clearly.

Although some researchers such as Moskowitz (1985) and Hindmarch et al., (1990) assert that all psychoactive drugs have "unique profiles" of impairment, it is likely that many apparently specific effects and differences between drugs are artifacts of the experimental methodology. Given that the pattern of behavioural effects is dose-related, and that drugs with different pharmacokinetic profiles have often been compared in non-equipotent single doses, there would appear to be a vast scope for overinterpretation.

Some specific differences between drugs will therefore be critically discussed from the viewpoint of a null hypothesis which states that all central nervous system depressants have the same behavioural effects on the brain.

Over the past twenty years or so there has been an intensive effort to elucidate the effects of drugs on memory. One of the initial spurs for this was the observation made by Dundee and Pandit (1972) that the benzodiazepine diazepam had a seemingly unique effect on memory. Patients injected intravenously with the drug experienced, subsequently, a period of amnesia for conversation and events that occurred while they were still conscious and able to communicate. Since then, almost every conceivable aspect of memory has been studied, in relation to benzodiazepines and to a host of other drugs (for reviews see Judd et al., 1987; Warburton and Rusted, 1989).

Memory does not appear to be a unitary process, and several theoretical models have been developed to describe it. Three types of information processing models are popular in the evaluation of memory and drugs.

These consist of multi-stage models such as that proposed by Atkinson and Shiffrin (1968) which divide memory into short and long-term; single trace theories which emphasise the role of meaningfulness in learning and memory via encoding processes (Craik and Lockhart, 1972); and models which assume that the memory trace is plastic and can be transformed biologically (consolidated) into permanent memories (Weingartner and Parker, 1984). There are therefore a large number of research paradigms used to evaluate these processes.

Subhan and Hindmarch (1984) used the Sternberg test to evaluate the effects of single doses of 3 benzodiazepines, lormetazepam, triazolam, flunitrazepam and the cyclopyrrolone zopiclone on memory.

The Sternberg test (Sternberg 1975) allows the speed of processing of several components of memory to be assessed. In this test subjects are shown a set of digits (usually 1, 3 or 5) on a monitor screen. A sequence of probe digits is then presented, and the subject responds by pressing a YES or a NO button depending on whether each probe was in the initial set or not. It is thus possible to observe the changes in reaction time for length of string (scan in memory), digit discriminability, and response selection (i.e. time to YES or NO).

Using analysis of variance (to avoid type I errors - i.e. false positive conclusions) these authors asserted that simple main effects for flunitrazepam and triazolam suggested selective drug impairment of encoding. The authors also pointed out, however, that the patterns of reaction times suggested that these two drugs further impaired serial comparison and response time. Zopiclone, on the other hand, exerted its action on serial comparison and response selection with no selective action on encoding. Lormetazepam apparently had no effect.

Several things were striking about this study. The null hypothesis that the authors appeared to start with was that there were no specific differences between the drugs. Thus any statistically significant differences between the drugs were regarded as indicating "selective" differences. Additionally, the entire interpretation of the results was from a psychological viewpoint, whereas it was quite clear that there were some obvious pharmacological explanations.

The drugs were given in single doses with no mention of whether they attempted to compare equipotent doses or how the two testing times might have related to the peak concentrations. It was clear that they were not comparing like with like since the magnitude of the reaction times scores varied considerably between drugs. The drugs all slowed reaction time acutely on all on components of the test, and with one main exception (the effect of zopiclone on low discriminability at one testing time), virtually always in the same order. However, the lack of significance on the discriminability part of the test using analysis of variance was used as evidence that the drug had a selective effect elsewhere.

A plausible alternative explanation is that the drugs affected all aspects of memory tested as indicated by the reaction time data. The failure to observe a statistically significant effect of zopiclone on the encoding part of the test was because of a rogue value at one testing time (type II error), whereas the dose of lormetazepam was insufficient (or the timing was inappropriate) for allowing effects to be demonstrated. There was therefore little to suggest that the drugs behaved differently on different stages of memory.

Several years later, it has been accepted that different benzodiazepines have qualitatively the same effect on memory. However, in the intervening period it has been suggested that benzodiazepines have a different effect on

memory compared to anticholinergic drugs (e.g. Sunderland et al., 1986, 1989). Other evidence (e.g. Broks et al., 1988) using dose response curves, a battery of tests, counterbalanced order of testing and assessment at the time of peak plasma concentrations has indicated that the effects are qualitatively the same. Although there was a distinctive pattern of impairment, it was the same for both drugs. Thus the same tests appeared to be consistently sensitive or insensitive. One of the insensitive tests, digit span, has been extremely popular in memory studies. Lack of impairment on this test has often been interpreted to mean lack of effect of the drug on short term memory. Obviously, since the test lacks sensitivity this is not necessarily so, and false conclusions have probably been made many times in studies where this test has been used. It would therefore be of interest to look at the effects of these drugs (i.e. benzodiazepines and anticholinergics) on memory and on other aspects of performance at steady state to determine whether the pattern of effects is different from N₂O and ethanol, or whether tests of memory are actually more sensitive than other tests, or whether the idea of differential effects on memory was simply based on false conclusions.

Recent advances in the study of neurotransmitters and receptors (see for example Farde et al., 1988) have increased the belief in a one-to-one relationship between chemical structure and individual behavioural effects. This notion of different drugs having different intrinsic effects on behaviour is appealing. However, while this type of relationship may hold true for several pharmacological effects, it has not been shown to be consistently true for behaviour (Hughes et al., 1988). Furthermore many of the methods used have led to false conclusions. Methodological difficulties may persist for decades (Editors, Psychopharmacology and Reaction Time, 1988). However, as many of these difficulties are overcome over the next few years, future prospects may be more hopeful.

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ADDENDUM - UNANSWERED QUESTIONS AND FUTURE STUDIES

FUTURE PROSPECTS - FUTURE STUDIES - UNANSWERED QUESTIONS

A large number of questions arising from the work in this thesis remain unanswered, and require further investigation.

Acute tolerance

1. Assuming that apparent acute tolerance to the psychomotor effects of drugs can be explained mainly by pharmacokinetic differences between concentrations in brain vs venous blood, can test sensitivities explain the pattern of acute tolerance since some tests appear to show greater tolerance than others?

Hypothesis 1: Least sensitive test = last impaired = first recovered.

Hypothesis 2: Functions with steep dose response curves are more likely to show apparent tolerance than those with gradual dose response curves.

2. Does acute tolerance to the subjective effects (lightheadedness) of drugs appear when high concentrations of drugs are reached more slowly? (Cf. Sensation of speed while accelerating to sensation while "cruising" at high speed.)
3. Alternatively, can the subjective "rush" be explained in terms of increased cerebral blood flow initially? (Test hypothesis by using a small quantity of labelled Xenon as tracer, and measure the cerebral blood flow when test drugs such as ethanol and nitrous oxide are given).

Sedation

1. Does N₂O produce drowsiness at all before unconsciousness? What about the other inert gases and the general anaesthetics?
2. How does the shape of the dose-response curve for sedation compare with the dose-response curve for other functions (for benzodiazepines, N₂O, Xenon, chlormethiazole, anticholinergics, antihistamines etc)?
3. If large doses of drugs produce nonspecific effects because of sedation, can specific effects be detected with smaller doses (with constant plasma concentrations) where sedation is less of a problem? For the drugs used in this thesis, this is of particular interest with chlormethiazole since there have been reports of restless legs and anxiety with very slow subclinical infusions. This is not seen with fast infusions or with clinical (higher) doses. There have also been occasional reports of disinhibition with benzodiazepines in low doses.
4. Do subjects eventually become fatigued/ drowsy after several hours of low concentrations of N₂O in the same way as seems to happen with ethanol (and possibly toluene)?
5. How does critical flicker frequency relate to the dose-response curves for arousal/sedation for drugs using steady-state with constant plasma concentrations?

Sensitivity

1. Do coordination tests have exponential dose-response curves at steady-state (since increased task complexity increases sensitivity)?

2. Are memory tests (particularly acquisition and encoding tests) any more sensitive than other tests? How do they fit in with the general pattern of impairment?

Hypothesis: Some memory tests are sensitive, but no more so than other sensitive tests such as attention tests and tapping. The size of change is the same as for other tests when drug concentrations are constant.

3. Since statistical significance was more likely to be achieved where test scores were homogeneous in the test group, it is of interest that the subjects in these, as in most, studies were usually College or University graduates. Therefore it is to be expected that they should be homogeneous for some characteristics, possibly attentional and memory capacities, since these correlate with intelligence. Would the pattern of significant "effects" be the same if another selection criterion such as sporting ability was chosen to be the homogeneous factor?
4. Although it was not assessed in this thesis it is interesting that tests of saccadic eye movements, which are becoming more popular because of their "sensitivity" also have very small coefficients of variation (5-14%, Richens et al., 1984). As this test is supposed to be simple and quick to use and does not require practise, how would this test compare with others in the battery?

Other miscellaneous studies

1. How do the dose response curves of other inhalation anaesthetics compare with that of N₂O and the other drugs used in this thesis?
2. What do the dose response curves for other drugs such as antihistamines, opioids, local anaesthetics look like? Are they any different?

3. As it would not expect to react with anything at all, Xenon would be ideal as the model compound for the Index of General Impairment, preferably assessed in an inhalation chamber.
4. Do the elderly have different dose-response curves to sway after drugs compared with young people, or is there a shift to the left indicating increased sensitivity at all drug concentrations, or do the elderly simply have a different starting point (threshold) on the same basic curve?
5. Can different dose-response curves on different tasks explain the (apparent?) synergism that is sometimes seen in interaction studies? Can this be demonstrated at steady state? (Test hypothesis by infusing subjects with ethanol to steady state, and then performing a dose response to e.g. N_2O on top. Compare the shape of the curves with those from N_2O alone.)
6. Can the apparent improvement on some tests in small doses be explained in terms of speed accuracy tradeoff?
7. Which kinetic/dynamic model best fits the data from studies using Wagner's/Sheiner's Models?

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