Behavioural and Pharmacological History in Drug Discrimination Studies

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Zoë Sarah Burgess

March 2001

Burgess, Z.S. Behavioural and Pharmacological History in Drug Discrimination Studies

The aim of the first study reported was to investigate the effect of drug history on caffeine discrimination in rats in a two lever operant task. Three groups, the 'discriminators', were trained to discriminate amphetamine, chlordiazepoxide (CDP) or nicotine (0.5, 10 or 0.3 mg/kg respectively). Three groups, the 'non-discriminators' received non-contingent injections of the respective drugs. 'Controls', received saline. The animals learnt to discriminate all three drugs to approximately 90% accuracy in circa 100 sessions. All animals were then trained to discriminate caffeine (10 mg/kg escalated to 15 then 20 mg/kg) versus saline. The caffeine dose was increased progressively as the rats were not learning the discrimination. The 'Controls' learned the caffeine cue to a level about 85% correct, only after very extensive training (circa 150 trials). Amphetamine trained rats initially generalised partially to caffeine at 10 mg/kg. However, they did *not* show the expected *facilitation* of acquisition of the caffeine cue relative to controls. CDP and nicotine trained rats initially showed no generalisation to caffeine at 10 mg/kg. In addition, they did *not* show the expected *retardation* of acquisition of the caffeine cue relative to controls. The patterns of data obtained with the 'non-discriminators' did not differ from those obtained with the 'discriminators'. It is suggested that the data deviate from theoretical expectations due to the very low and unexpected discriminability of caffeine at 10 mg/kg. This led to the overall conclusions of no effect of drug history. CDP and nicotine discriminations groups possibly had their drug history extinguished and the amphetamine groups showed no effect, possibly due to development of cross-tolerance during discrimination training.

Due to the complex results obtained with caffeine, it was decided to study receptor pharmacological history rather than drug history. The effects of pharmacological history were studied by investigating clozapine, and not caffeine, because it had proved difficult to train animals on the caffeine cue. Clozapine was studied because it is used chronically as an antipsychotic. Animals were trained to discriminate clozapine (5 mg/kg) versus vehicle. Once accuracy was 100%, time- effect curves were determined for all drugs used to induce tolerance (clozapine) and cross-tolerance (olanzapine, JL13, cyproheptadine and CDP), with the aim of administering drug doses which would facilitate tolerance development by acting for prolonged periods of time. Once such doses had been established, a series of studies were run in which the clozapine dose-effect curve (DEC) was computed three times: - i) Prior to drug treatment (DEC 1); ii) After 10 days chronic drug treatment with discrimination training suspended (DEC 2); and iii) 16 days after cessation of chronic drug treatment (DEC 3). The results showed that: - a) Clozapine (10 mg/kg b.i.d.), olanzapine (5 mg/kg b.i.d.), JL13 (10 mg/kg b.i.d.) and cyproheptadine (40 mg/kg daily) all induced tolerance or cross-tolerance to clozapine and this was spontaneously reversible; b) CDP (40 mg/kg b.i.d.) did *not* induce cross-tolerance to clozapine despite being administered at a very high dose. These results show that tolerance and cross-tolerance to the clozapine discriminative stimulus (which is presumed to be pharmacodynamic), was *only* induced by clozapine and clozapine-like compounds.

Acknowledgements

Reaching the end of my Ph.D. and being able to compile this document has not been achieved without the quite considerable help of various people. It would therefore have been very remiss of me to forego the opportunity of recording here their much appreciated assistance.

Firstly I must say an enormous thank you to my supervisor Andrew Goudie. I have considered the opportunity to work under his guidance at Liverpool University a great privilege and honor. Without his constant support and the benefit of his vast experience I should not have gained so much academically and I am sure would not have found the whole process so fulfilling.

My thanks must also go to Mike Taylor, Jane Burwood and Sue Courtney who devotedly fed and cared for the animals. These Laboratory Technicians were shoulders to cry on, ears to bend when the going got tough and friends to rejoice with when success needed to be shared, not to mention great suppliers of regular coffee.

Acknowledgment must be made to Barbara Sykes who with great patience took on the onerous task of proof reading my thesis. Despite residing in Switzerland and communicating via the vagaries of E-mail she resolutely soldiered on chapter by chapter.

Penultimately I must thank my family network of supporters. To my parents Arthur and Susan Burgess thanks for unstinting support both of the emotional and financial kind. A special thanks to my dad for his example in the belief of further education. To my kind aunt Doreen Mackarel I give thanks for the generosity of letting me move temporarily into her home in Liverpool and then stay for three years.

Lastly, but by no mean least, I thank my husband Richard Hickman for the unending patience he has shown over the past four years. First in travelling to Liverpool to support me and latterly, during the first few months of our married life, in giving me total encouragement to devote all my time to completing this mammoth task.

Chapter 1.0

Drug Discrimination

1.1 Drug stimuli

A discriminative stimulus is a term derived from the pioneering work carried out by B. F. Skinner on operant or instrumental conditioning (Goudie & Leathley, 1993). Skinner believed the main factor determining animals' behaviour is the reinforcing stimulus, e.g. water or food for deprived animals. The reinforcement is delivered when the animal produces the correct response e.g. lever pressing for rats, key pecking for pigeons. However, Skinner also believed that other stimuli could be used to alter the animals' behaviour. Such information could be processed and used by the animal to determine which response to make, e.g. a rat can be trained to press a lever for a food reward only when a light is switched on. If the light is switched off the response will not produce a reward. The stimuli that can be used to determine the animals' behaviour in this manner are called "discriminative stimuli". Either external (exteroceptive) stimuli can be used to guide behaviour e.g. the light being on or off, or internal (interoceptive) stimuli i.e. a drug cue, where the animals' assess their internal state and then make the correct response in order to obtain a reward (Goudie & Leathley, 1993).

It is usually believed that the drug stimulus that animals respond to for a drug is similar to the subjective effect in humans, e.g. amphetamine is believed to be a stimulant in animals and the subjective effect in humans is a stimulant.

The specificity of a drug cue can be demonstrated during testing with drugs other than the training compound. Studies have shown that animals do not respond simply

for drug or no drug, but will respond on the drug lever only if the two drugs have comparable stimuli. Animals learn to attend to the presence or absence of the specific training drug, rather than any drug treatment (Jarbe & Swedberg, 1982).

1.2 Accuracy of lever selection

Accuracy of lever selection during discrimination training can be measured by recording the FRF, that is the total number of responses on both levers before the first reinforcement for each animal on the FR schedule.

This as a very important measurement because once a reward has been presented the animal could use the reward itself to decide which lever to select (Goudie, 1977) e.g. in a two-lever FR30 schedule of reinforcement, the animal could learn a win/stay – lose/shift strategy, where the animal quickly makes 30 responses on one lever and if it gains a reward, it wins and stays on that lever. If the animal fails to receive a reward, it switches to the other lever. In this case, the animal is using the presentation or lack of presentation of a reward, instead of the discriminative properties of the drug to guide its lever selection. It is therefore very important in assessing the accuracy of discrimination learning to study each animal individually and to only consider their behaviour before the presentation of the reward (Colpaert & Janssen, 1984). When animals are trained under an FR-30 schedule of reinforcement, then an FRF (First ReinForcement) is important. This is because the animals must make 30 responses on the correct lever before making 30 response on the incorrect lever. An FRF value of 59 or less indicates the animal made a correct lever selection whilst an

FRF value of more than 59 indicates it chose the incorrect lever. As discrimination training continues, the FRF values typically decrease, so in well trained animals they will be at or very close to 30 (Goudie & Leathley, 1993).

The animals are subjected to an FRF schedule within sessions and each animal must make a correct FRF to indicate that they are learning to discriminate between the drug and saline. The animals were also trained to a specific criterion and after many trials they are defined as having learnt or not learnt the discrimination. The second criterion was compared for each animal across sessions. It involved comparing the animals FRF values over 10 days and checking their reliability. The criterion required the animals to not only make a correct FRF within a session but to also maintain that behaviour across sessions. So to determine the criterion, the number of correct FRF sessions was examined in a ten day period. The criterion can be weak e.g. 8 correct lever selections out of 10 consecutive sessions or more stringent e.g. 20 correct lever selections in 20 consecutive sessions. The actual training criterion used is determined by the experimenter and works on a cost/benefit basis. It is possible for a weak criterion to be reached by chance, especially if there has been a large number of training sessions. However, if the drug studied has a weak discriminative stimulus it may be necessary to use a weak criterion along with extensive training (Goudie, 1982). The results need to be studied carefully to ensure that post-criterion the animals maintain a high level of accuracy. Using a more stringent criterion could mean extensive training even with a highly discriminable drug. The benefits gained,

i.e. more accurate discrimination, will be offset by the increased costs incurred in terms of the increased training required (Overton & Hayes 1984).

1.3 Importance of training dose

One essential factor in drug discrimination is the training dose that is used. The discrimination learning rate depends upon the dose of the drug used and the "discriminability" of the drug (Holtzman, 1990). Therefore, in most discrimination studies, a reasonably large dose of the drug should be chosen to ensure that the stimulus is learnt at a reasonably fast rate. There may be other factors determining the training dose that necessitate it being smaller than the optimal discriminable dose. Another factor to be taken into consideration when choosing the training dose is the direct effect that the drug will have on the animal's response rate. Most drugs when initially administered to animals suppress responding, but if the dose is too large the animals stop responding completely and never receive rewards. An optimum dose is therefore one that has a reasonably potent discriminative stimulus but does not totally suppress the response rate (Overton & Hayes, 1984).

It is generally accepted that the lower the training dose of a drug the less specific the stimulus will be compared to higher doses of the same drug (Colpaert, Niemegeers & Janssen, 1980). So, with any novel compound it is important to establish the degree of specificity of the training dose or doses being studied. This could be done by training different groups of animals on different doses of the novel compound and

then by carrying out generalisation tests with drugs from different pharmacological classes.

1.4 Drug discrimination procedures

A drug discrimination operant assay requires the training of animals to choose one of two levers to press. The simplest type of drug discrimination is the presence or absence of a drug. It is vital that for the un-drugged condition that the animals are injected with vehicle. The injection with vehicle prevents the animals from using the injection procedure as a discriminative stimulus with which to decide which response to make. It has been noted that injection procedures in animals are very salient. It has been previously shown that animals can learn to guide their own behaviour depending on the route of administration or route of injection e.g. intra-peritoneal versus subcutaneous (Goudie & Leathley, 1993). Therefore, the presence or absence of an injection or even a change in the route of administration must be considered as a potential discriminative stimulus. It is extremely important to ensure that throughout the training and testing procedure the only stimulus that animals can use is the presence or absence of a drug cue.

1.5 Generalisation testing

Once animals have been trained to discriminate between drug or saline to a specific criterion, and are responding stably, then generalisation or substitution tests are carried out.

Generalisation tests are usually conducted with at least two training days separating each test day. The training days that separate the test sessions are important because they allow the accuracy of the animals' responses under the training drug to be assessed. It has been shown that sometimes generalisation tests with novel compounds disrupt baseline lever selection (Colpaert *et al*, 1975). If the animals' criterion as a group falls too low it will be necessary to carry out more training days before the next stage of generalisation testing.

The most common procedure for generalisation testing is where the animals are rewarded on one lever, chosen by the individual animal, throughout the whole of the test session. The animal makes a lever selection and after 30 responses (the usual level in training sessions) it receives its initial reward, and more rewards are obtained for lever pressing on the chosen lever (Goudie & Leathley, 1993). Therefore, the animal selects either the drug or saline lever in the generalisation test and this selection is reinforced by the feedback that the animal receives. Hence, it is possible to define at any specific dose of drug, the percentage of animals that selected the drug lever in a generalisation test (Colpaert *et al*, 1975).

Generalisation tests are carried out to determine one of two factors. The first factor is a dose/response curve for the training drug. This involves varying the dose of training drug administered to the animals and calculating the percentage drug lever selection at each dose. The second factor is the degree to which other drugs generalise to the training drug. Generalisation between the training drug and novel

compounds will occur if the two drugs have similar pharmacological properties. If the drugs have different pharmacological properties then the animals will respond on the saline lever. However, these drugs may produce discriminable properties of their own if the animals were trained on that drug alone rather than being used in generalisation tests (Goudie, 1982).

In dose/response curves the doses used should be counterbalanced over a time period and across the groups. This allows a double checking process to ensure that the responses seen with the animals at any one particular dose are a true effect of the drug and not an anomaly of that day. In a generalisation dose/response curve, as the dose of drug decreases the number of drug lever selections typically decreases.

The time/effect curve is another important tool. The animals are tested for percentage drug lever responding at various time intervals after administration of the drug. Studies show that initially most of the animals respond on the drug lever and as the time interval from injection to testing increases the percentage of drug lever selection decreases. The number of drug lever selections decrease until all the animals are responding on the saline lever. Most drugs have a duration of action of several hours; however, after some hours they may still produce a degree of drug lever selection. However, some drugs are extremely short acting e.g. beta-phenylethylamine is undetectable after 60 minutes (Goudie, 1982). Some very long acting compounds may interfere with the training day after the test day because they leave active residues behind.

Results show that test compounds generalise to the training drug if they are in the same pharmacological class. The classification of drug stimuli occurs according to their stimulus attributes e.g. sedative/hypnotic, opiates, CNS stimulants etc. However, the classification of the drugs into broad pharmacological classes does not necessarily explain why the drugs can be differentiated with respect to their discriminative stimulus even when they are in the same class. An example of this is barbituates and benzodiazepines, both types of drugs are sedatives and anxiolytic, but they can be discriminated to some extent from each other in the drug discrimination assay in a drug versus drug discrimination (Jarbe, 1989).

1.6 Drug discrimination and tolerance

Tolerance can occur in two forms, the first is genetic. Some animals are more tolerant to the effects of drugs as can be seen when assessing their response rates after the first administration of the drug (Jarbe, 1989).

The second type of tolerance is learnt or acquired tolerance. For many years it was generally believed that no tolerance occurred to the discriminative stimulus of a drug because it was believed tolerance would hinder detection of the drug stimulus. This in turn would reduce the efficiency of the drug in producing its discriminative control of the animals' behaviour (Goudie & Leathley, 1993). Studies have shown that tolerance occurs to the rate suppressant effects of drugs. A more complicated form of tolerance is developed to the discriminative actions of the drugs on suspension of discrimination training. During the suspension of the discrimination training

supplemental doses of the drug are administered. The animals are tested again and a shift to the right of the dose/effect curve indicates that tolerance has occurred (Young & Sannerud, 1989).

The development of tolerance can be measured easily. When a drug is administered over a long period of time increasing amounts of the drug are necessary to maintain the same effect. This loss of potency is often called tolerance (Jarbe,1989).

1.7 Conclusions

There is no one "correct" way to run drug discrimination studies. Also there is no "pure" drug stimulus that can be assayed in one particular manner (Goudie & Leathley, 1993). Instead, there are several different types of schedules of reinforcement that can be used. The most commonly used schedule of reinforcement is the fixed ratio, as described previously and is used in the following studies. Discriminative drug studies are frequently used to determine what drugs generalise to each other and to try and find other compounds that are similar to novel compounds.

Chapter 2.0

Drug History

2.1 Introduction

That the effects of drugs depend upon behavioural history, drug history or both is recognised as determinants of behaviour in drug discrimination experiments (McMillan *et al*, 1996). It has been suggested that drug discrimination is really a study of drug history in the animal (McMillan *et al*,1996). This is because the training drug is administered chronically and the dose of the training drug and chronic administration of other drugs are important determinants in stimulus control (Young *et al*, 1992).

2.2 Drug history

Squirrel monkeys were trained under a fixed-interval stimulus shock termination schedule after the administration of d-amphetamine, pentobarbitone or morphine. It was shown that low rates of punished responding were increased by d-amphetamine (Bacotti & McKearney 1979) and decreased by morphine (Glowa & Barett, 1983). However, the effects of pentobarbitone were dependent upon the drug studied immediately beforehand. Thus, if amphetamine was administered before, then pentobarbitone increased responding (Glowa & Barrett, 1979). Whilst if morphine was administered previously then pentobarbitone decreased responding. It was shown that once responding was significantly decreased by morphine, it could be increased by administering pentobarbitone interspersed with brief exposures to amphetamine to speed the process up. Thus, the effects of pentobarbitone were dependent upon the drug, which had been administered previously providing conditions under which pentobarbitone can have opposite effects. Therefore,

pharmacological history may either diminish or enhance a particular effect of a drug (Barrett *et al*, 1989).

Drug history can have different effects on animals behaviour. It has been shown that animals attach significance to innocuous events i.e. route of injection, person injecting the animals. So to ensure that the effects observed in a study are due to drug history, then the experimenter needs to standardise certain effects:

1) animals in different groups should have the same number of drug and saline sessions,

2) all the groups should have the same length of time for training under the initial drug and saline before moving onto the second drug,

3) all animals should be trained under the same schedule of reinforcement if the aim of the study is to investigate the effects of drug history,

4) saline should ideally be administered during the tests sessions to ensure that the drug is being discriminated and results observed are not simply due to random responding (Barrett *et al*, 1989).

2.3 Behaviour under drug

An example of how behavioural consequences, which occur under the influence of a drug, can alter the subsequent activity of the drug is where key pecking in pigeons was maintained (Smith & McKearney, 1977). In this study the pigeons were trained under a procedure in which key pecking produced food only if at least 30 seconds elapsed between successive pecks (DRL30). An increase in responding after initial d-

amphetamine administration was observed which led to a decrease in the frequency of food presentation. This is because the time between responses was often less than the 30 seconds the schedule required. However, after a second injection of amphetamine, increases in responding were less than observed after the initial injection. Subsequent injections of amphetamine led to further decreases in the rate-increasing effects of amphetamine, and after the fourth injection, no increase in response-rate was observed at all. The same effect was seen with pentobarbitone, suggesting that experience with drugs (e.g. amphetamine and pentobarbitone) which induce increases in the rate of responding, which in turn led to animals receiving fewer food rewards, decreased with subsequent injections (Smith & McKearney, 1977). The continuous administration of amphetamine and pentobarbitone eventually decreased the general effect of increasing responding allowing the animals to gain more food rewards. Therefore, tolerance occurred and prior history altered drug effects that would ordinarily be detrimental to the animal (Smith *et al*, 1978).

Therefore, the aforementioned studies indicate that behavioural and pharmacological histories, as well as drug-induced changes in behaviour, can exert effects on several drugs. There are several mechanisms by which behavioural factors e.g. previous history can influence both acute and chronic effects of drugs and produce modifications of drug activity that resemble tolerance or sensitisation i.e. modification of the basic dose-response function, e.g. the effects of d-amphetamine on punished responding are changed from rate decreasing to rate increasing after a shock avoidance history (Barrett *et al*, 1989). The drug induced changes in behaviour

are not limited solely to one class of drugs, but instead are observed across many different drug classes e.g. amphetamine, pentobarbitone, nicotine. However, all the drugs which show the changes appear to be drugs of abuse (Barrett *et al*, 1989). It is possible that all drugs of abuse have the potential for producing a variety of effects but under different conditions i.e. d-amphetamine may show effects which chlorpromazine does not show under the same conditions (Bacotti & McKearney, 1979).

2.4 Drug mixtures

As most drug stimuli are believed to be compound (mixture) stimuli, then mixtures have been used in the drug discrimination paradigm to attempt to understand how drug stimuli are perceived and processed by animals. It was initially thought that when a mixture of drugs (e.g. amphetamine and pentobarbitone, nicotine and midazolam) were administered, rats might perceive the mixture cue as a new entity rather than a mixture of two compounds (Stolerman, Rauch & Norris, 1987). Little evidence has supported this idea however.

There are two types of training paradigms used in mixture training (Stolerman & Mariathasan, 1990). One type is called 'AND-discrimination' – in this animals learn to press one lever after administration of a mixture and the other lever after the administration of vehicle. The second type of discrimination learning is called 'AND-OR discrimination' – in this, animals learn to press one lever after the

administration of a mixture and the second lever after the administration of either drug. Thus, the group may have :-

e.g. lever 1 pentobarbitone and amphetamine mixture

lever 2 pentobarbitone or amphetamine

It has been shown that the two types of discrimination training can alter animals' behaviour and their pattern of generalisation. In 'AND' discriminations there is almost complete generalisation seen between the training mixture e.g. pentobarbitone and amphetamine mixture and the individual drugs e.g. amphetamine and pentobarbitone separately (Stolerman & Mariathasan, 1990). However, under 'AND-OR discriminations', then no generalisation was seen between the training mixture and the individual components even after 'AND discrimination' training (Stolerman & Mariathasan, 1990). Thus, 'AND-OR discrimination' training history modifies the characteristics of a later 'AND discrimination'. This could suggest that the sequence in which drugs are used in generalisation tests could influence the outcome. Hence, a history of experimenting with drugs may change an individual's perception of the mixture effect which could alter the pattern or extent of drug abuse (Stolerman & Mariathasan, 1990).

2.5 McMillan's and colleagues work

McMillan *et al* (1996) taught pigeons to discriminate between pentobarbital and vehicle. Once the initial discrimination of pentobarbital was learnt, the animals were split into two sub-groups, A and B. The two groups were trained to discriminate

drugs in different orders and generalisation tests were carried out between each subsequent discrimination.

Table 2.1: Order of discriminations in the two groups and the compounds that generalised after each one.

Drug number	Group A	Compounds which generalise (i.e. cause responding) to the drug key	Group B	Compounds which generalise (i.e. cause responding) to the drug key
1	Pentobarbital versus saline			
2	Morphine	Full – morphine, pentobarbital, diazepam. Partial – PCP None – amphetamine, Haloperidol, vehicle	Amphetamine	Full – amphetamine, pentobarbital, diazepam, Partial – PCP, None – Haloperidol, vehicle, morphine
3	Amphetamine	Full – morphine, amphetamine, pentobarbital, diazepam, Partial – PCP, None – haloperidol, vehicle	Morphine	Full – morphine, amphetamine, pentobarbital, diazepam, Partial – PCP, None – haloperidol, vehicle

Group A was trained to discriminate morphine from saline after pentobarbital and the following compounds generalised fully:- morphine, pentobarbital and diazepam; PCP showed partial generalisation and amphetamine, haloperidol and vehicle showed no generalisation. Group B were trained to discriminate d-amphetamine from saline after pentobarbital and amphetamine, pentobarbital and diazepam showed full generalisation; PCP partial generalisation and haloperidol, morphine and vehicle showed no generalisation. In the second part of the experiment, morphine and d-amphetamine training were reversed in group A and B, (see table 2.1) and this time the same compounds generalised in both groups. The results showed that morphine, d-amphetamine, pentobarbitone and diazepam all showed full generalisation in both groups. PCP however only showed partial generalisation whilst haloperidol and vehicle did not generalise at all. These data appear to show that the pigeons "remembered" all of the prior trained discriminations, because the animals responded on the drug lever after administration of the drugs that they had been trained under.

Thus, the results showed that pigeons can learn a series of drug discriminations even when the training drugs come from different pharmacological classes. The data showed that, as each new discrimination was learnt and added to the sequence then previous discriminations were retained. Not only were the old discriminations retained, but they also continued to exert a discriminative effect, because when compounds were tested in generalisation tests the previous training drugs still engendered responding on the appropriate key. Therefore, training with the new training compounds did not affect the retention of the previous drug discrimination.

Although the data showed that once a discrimination was learnt it was not lost, the amount of stimulus control maintained by a training drug was weakened if it was not continuously administered in discrimination training (McMillan *et al*, 1996).

In another study Li & McMillan (1998) showed that pigeons could be trained to discriminate between pentobarbital and saline and then they could be trained to acquire the methamphetamine cue. The data showed that after both discriminations had been learnt, the pigeons responded on the drug key after either pentobarbital or methamphetamine. The same authors also showed that pigeons could be trained to discriminate morphine from saline after a prior history of learning the buspirone cue from saline. Again, the data showed that both buspirone and morphine engendered drug key pressing in these subjects (Li & McMillan, 1998). These results show that the pharmacological class of the first compound does not matter in relation to the second drug, as long as the two drugs produce discriminably different effects (McMillan *et al*, 1996). The data showed that not only was the training drug discriminated but all the other drugs that normally substitute for the training drug were also discriminated (McMillan *et al*, 1996; Li & McMillan, 1998).

The results of the McMillan *et al* (1996) study are very important because there is a suggestion that the discriminative stimulus of an abused drug can produce drug-seeking behaviour for the drug (Griffiths *et al*, 1980). The results suggest that drug stimuli and possibly the "memories" of previous drug stimuli can combine to produce discriminative stimulus effects. They also show that once a discrimination is

learnt then it is retained over very long periods of time (McMillan *et al*, 1996). However, there was no effect at all of drug discrimination history because all the animals learnt the discriminations regardless of the order of presentation of the drugs

2.6 Summary

There are many effects seen with drug and behavioural history. The effects are observed both when drugs are administered acutely and chronically. The mechanisms of action are unknown and more work needs to be carried out to determine these. However, the results show that drug history causes an effect as previously discussed. Some of the differences could depend upon the drugs studied, the different species of animals used, and the different schedule of reinforcement used in the studies. All these factors may play an important role in the studies.

The aim of this experiment was to determine if drug history had an effect. The animals were going to be trained to discriminate initial drugs with each group being administered a different drug and then trained to discriminate another drug to investigate whether the initial drugs could alter how the animals perceived the second drug. This is different from previous studies in that in this study the effects of subsequent drug discriminations on future discriminations was studied, rather than whether animals could retain subsequent drug discriminations. Three drugs i.e. amphetamine, nicotine and cyproheptadine were chosen for the initial drugs and

caffeine was chosen as the second drug. The effects of all four drugs are discussed in the next chapter.

Chapter 3.0

Effects of the drugs used in Study I

3.1 CAFFEINE

3.1.1 Introduction

Caffeine is probably the most widely used psychoactive drug in the world (Evans & Griffiths, 1992). It is estimated that world wide per capita caffeine consumption is 70 mg per day, which is about a large cup of instant coffee for every man, woman and child (Evans & Griffiths, 1992). The consumption of caffeine far exceeds the usage of alcohol and nicotine (Griffiths & Woodson, 1988).

3.1.2 Discrimination of caffeine

Several studies of caffeine have used the drug discrimination paradigm. The discriminative effects have been studied mainly in non-human species, and the subjective effects mainly in humans.

Caffeine has been shown to have discriminative properties in both humans (Holtzman, 1996) and animals (Silverman *et al*, 1994). Studies have been carried out with several animal species, although rats are the most commonly used.

Discrimination of caffeine has been shown in previous studies (Holtzman, 1996). Caffeine doses of 10-125 mg/kg have been used as the training stimulus (Griffiths & Mumford, 1996). It has been shown that the rate of acquisition of the caffeine cue is dose-dependent. Therefore the higher the dose the quicker caffeine discrimination is learnt (Griffiths & Mumford, 1996).

3.1.3 Discrimination of caffeine at low doses

There is evidence to suggest that the discriminative stimulus effects of low caffeine training doses (10-30 mg/kg, i.p. in the rat) may be related to its behavioural stimulant effects (Holtzman, 1996). It has been suggested that because other behavioural stimulants e.g. d-amphetamine, cocaine, mazindol, theophylline occasion caffeine-appropriate responding at low caffeine training doses, so the discriminative cue may lack pharmacological specificity. As the dose of caffeine increases then the cue becomes very specific and only methylxanthines will generalise e.g. theobromine and theophylline (Gilbert, 1976).

However, several compounds from various pharmacological classes e.g. yohimbine, IBMX, fenfluramine do not occasion caffeine-appropriate responding even at low caffeine training doses. This suggests that there is some specificity in the low-dose caffeine interoceptive stimulus (Griffiths & Mumford, 1996). Caffeine does not always occasion drug-appropriate responding in animals trained with other behavioural stimulants alone (Griffiths & Mumford, 1996).

3.1.4 Discrimination of caffeine at high doses

The discriminative stimulus of high caffeine doses appears to be qualitatively different from that of low-caffeine doses (Mumford & Holtzman, 1991). High doses of caffeine (56 mg/kg) in humans produce several unpleasant effects that include anxiety, dysphoria and depression (Mumford & Holtzman, 1991). Whereas lower doses of caffeine (10-25 mg/kg) produce more pleasant effects including wakefulness

and a feeling of more energy (Griffiths & Woodson, 1988). In rats, caffeine training doses of 10 and 56 mg/kg produce different patterns of generalisation to novel drugs (Mumford & Holtzman, 1991). This was shown as animals trained on low doses of caffeine, generalised to behavioural stimulants, whereas animals trained on high doses of caffeine showed no generalisation with behavioural stimulants. In addition, the length of time that animals took to learn the caffeine discrimination was significantly higher in the low caffeine dose compared to the high caffeine dose.

The lower the dose of caffeine the slower the rate of acquisition. So rats learning to discriminate 10 mg/kg of caffeine needed about 93 training sessions, whilst rats learning to discriminate 56 mg/kg caffeine only needed about 43 training sessions. This suggests that the limits of caffeine discriminability had almost been reached at 10 mg/kg (Mumford & Holtzman, 1991).

3.1.5 Mechanisms of action

The following are possible ways in which caffeine produces discriminative effects on the CNS (Modrow *et al*, 1981).

Caffeine affects the CNS by inhibition of phosphodiesterases (Nehlig & Debry, 1994) which in turn inhibit cyclic 3'5'-adenosine monophosphate (cAMP) which is a secondary messenger (Rang & Dale, 1994). cAMP is continually produced and deactivated by phosphodiesterases.

Griffiths & Mumford (1996) suggested that the inhibition of cyclic nucleotide phosphodiesterases by caffeine might contribute to its discriminative stimulus effects at low doses. However most selective phosphodiesterase inhibitors are depressants (Griffiths & Mumford, 1996), so it seems unlikely that phosphodiesterase inhibition would be involved in low-dose caffeine discriminative effects since caffeine at these doses is known to produce psychomotor stimulation (Mumford & Holtzman, 1991).

In addition, if inhibition of phosphodiesterases were a significant mechanism for the caffeine cue, all methylxanthines would be expected to generalise to caffeine (Mumford & Holtzman, 1991) as all methylxanthines inhibit phosphodiesterase, with varying degrees of potency. IBMX is a potent phosphodiesterase inhibitor which did not occasion caffeine-appropriate responding (Mumford & Holtzman, 1991). This suggests that phosphodiesterase inhibition does not contribute to the discriminative stimulus effects of a low dose of 10 mg/kg of caffeine in rats (Mumford & Holtzman, 1991).

If inhibition of phosphodiesterases was the mechanism for the caffeine cue, it could be expected that compounds producing stronger phosphodiesterase inhibition would produce a stronger discriminative cue. However, theophylline which is a more potent phosphodiesterase inhibitor than caffeine is less potent in producing a discriminative cue (Modrow, Holloway & Carney, 1981).

Another hypothesised mechanism for the discriminative stimulus effects of caffeine

is the antagonism of adenosine receptors (Finn & Holtzman, 1986).

Actions of adenosine are mediated by two receptors, A_1 and A_2, linked respectively to inhibition and stimulation of adenylate cyclase, so reducing or increasing intracellular cAMP formation. Adenosine's actions include constriction of the bronchi, inhibition of platelet aggregation and dilation of blood vessels, nucleic acid formation and ATP biosynthesis (Snyder, 1981).

The suggestion that caffeine may act as a discriminative cue by blocking adenosine receptors results from the observation that it blocks adenosine's actions on cAMP (Griffiths & Mumford, 1996). Caffeine blocks both A_1-mediated lowering and A_2-mediated enhancement of adenylate cyclase. Adenosine has nanomolar affinity for A_1 receptors but micromolar affinity for A_2 receptors and the affinity of caffeine at A_1 and A_2 receptors appears to be quite similar (Griffiths & Mumford, 1996).

IBMX (a potent adenosine antagonist and a behavioural stimulant) did not engender caffeine appropriate responding, however, CGS 15943 (a non-xanthine adenosine antagonist and a behavioural stimulant) generalised completely to the caffeine cue at low caffeine doses (10 mg/kg) (Mumford & Holtzman, 1991). Previous studies have shown that adenosine agonists do not (Holloway et al, 1985), or only partially, (Holtzman, 1986) reverse the discriminative effects of caffeine. This suggests that adenosine antagonism may contribute to the discriminative stimulus effects of 10 mg/kg caffeine, but it is not the only way in which the cue can be induced (Mumford

& Holtzman, 1991).

Dopamine can act as a neurotransmitter in certain parts of the brain (Rang & Dale, 1994). The dopamine D_2 receptor may be involved in the discriminative stimulus effects of caffeine. D_2 receptors are closely associated with adenosine A_{2a} receptors being co-localised in the striatum and they possibly interact with each other (Garrett & Holtzman, 1995). It has been suggested that the interaction between adenosine and dopamine receptors may mediate the behavioural effects of caffeine (Garrett & Holtzman, 1995).

Another possible mechanism for the action of caffeine is the intracellular mobilisation of calcium in the skeletal muscle (Nehlig & Debry, 1994). Intracellular calcium acts as a second messenger that regulates a variety of enzymes (Rang & Dale, 1994). The mobilisation of intracellular calcium by methylxanthines leads to the initiation and potentiation of muscle contraction. This effect is achieved by lowering the threshold of muscle excitability and by prolonging the duration of the active period of muscle contraction. The muscle contraction is lengthened by increasing the release of and inhibiting the uptake of calcium. This allows more ions to be available for muscle contraction. The overall effect is that the striated muscles are strengthened and are less susceptible to fatigue (Nehlig & Debry, 1994), when caffeine has been administered.

3.1.6 Conclusions

Caffeine is a compound that is found naturally and that has been around for many years. Caffeine use has increased over the years, and it is a compound that is accepted in most cultures and societies. The discriminability of caffeine is full of mixed reports and appears to be dose dependent. Caffeine is accepted as a stimulant compound which generalises to other compounds including cocaine, amphetamine, nicotine – all of which have known discriminative stimulus stimulant cues. Also, the higher the dose of caffeine used e.g. 32 mg/kg, then not only does it produce a more discriminable cue but the cue is also a specific methylxanthine cue. Whilst lower doses of caffeine e.g. 10 mg/kg are less discriminable and produce a more general stimulant cue. The precise mechanism of the caffeine cue has not yet been determined and it may involve several receptor systems, although it appears likely that the adenosine receptors will be involved either directly or indirectly. The likelihood that the caffeine discriminative stimulus cue requires several systems made it an ideal candidate for this experiment. This experiment needed the second compound to have a complex, general discriminative stimulus cue – which caffeine appears to have at low doses. However, the discriminability of caffeine at very low doses has mixed data, about which is the lowest dose of caffeine that animals can discriminate.

3.2 AMPHETAMINE

3.2.1 Introduction

Amphetamine is a psychomotor stimulant widely abused by people in many countries (Jaszyna *et al*, 1998). It is in the class of indirectly acting sympathomimetics, structurally related to noradrenaline (Leonard, 1992).

3.2.2 Drug discrimination and amphetamine

Tolerance develops quickly in animals to amphetamine's rate suppressant effects, so rats can be trained to lever press under amphetamine (Young, Walton & Carter, 1992). It has also been shown that humans can reliably discriminate between amphetamine and saline (Chait, Uhlenhuth & Johanson, 1986). A study carried out in human volunteers has shown that there is a disassociation between mood and the discriminative stimulus effects of a drug (Chait *et al*, 1986). This study showed that the discriminative stimulus effects of d-amphetamine generalised to mazindol, but the subjective effects profile of mazindol is different to that of amphetamine. Hence, this indicates that there is a difference between mood and discriminative stimulus effects.

A study (Cole, 1970) has shown that low doses of amphetamine (0.5 and 1 mg/kg) actually facilitate the learning of food-motivated discrimination, however higher doses of amphetamine (2 mg/kg) do not have the same effect. Another study showed that the behavioural effects of amphetamine are tolerated out to when the action of

the drug causes the animals to receive a decrease in the number of food rewards (Schuster *et al*, 1966).

A study investigating the effects of amphetamine in human subjects has shown that the drug discrimination stimulus and self-reported effects whilst are not identical may actually overlap at a range of doses tested (Kollins & Rush, 1999).

It has been shown that dopamine agonists produce not full generalisation but instead produce at most 70% drug-appropriate responding in animals that have been trained to discriminate amphetamine versus saline (van Groll & Appel, 1992). Also not all psychomotor stimulant have the same cueing properties (Druham, Fibiger & Phillips, 1991).

Amphetamine has been shown to partially generalise to nicotine in the drug discrimination paradigm. It was also shown that the partial generalisation between amphetamine and nicotine was not blocked by haloperidol, which suggests a minimal role for the D_2 receptors in nicotine-like discriminative stimulus effects of nicotine as shown by amphetamine in the drug discrimination paradigm (Mansbach *et al*, 1998). So this data indicates that the activation of D_2 receptor subtypes play a role in the partial generalisation between amphetamine and nicotine, but the precise receptor subtype involved is not yet known.

The dose of amphetamine used in discrimination training is critical in determining the nature of the discriminative stimulus. It has been suggested that low doses of amphetamine (less than 2 mg/kg) act peripherally and high doses of amphetamine (more than 2.5 mg/kg) act centrally. It has been suggested that in rats, central dopaminergic containing neurones play a role in the discriminative stimulus properties of psychomotor stimulants such as amphetamine (Woolverton & Cervo, 1986). However, many studies including this one use 1 mg/kg or less because that is the highest dose at which the animals will respond under before becoming suppressed.

Studies have shown that dopaminergic systems play an important role in mediating the effects of amphetamine (Brauer *et al*, 1997). It has been suggested that stimulation of D_1 receptors is not sufficient alone to induce generalisation to the amphetamine cue, however they may play an enabling role and allow the specific effects of D_2 receptors to be expressed (Clark & White, 1987).

In a study where subjects were trained to discriminate between cocaine and placebo, the results showed that cocaine, amphetamine and caffeine all produced dose-related increases in cocaine-appropriate responding (Oliveto *et al*, 1998). The study also showed that in human subjects cocaine and amphetamine were similar in discriminative performance and stimulant-like effects but the subjects did not rate caffeine like cocaine or amphetamine, which is different to the animal data.

Apart from cocaine, it has been shown that other stimulants will generalise to the amphetamine cue in the drug discrimination paradigm. These drugs include pseudoephedrine which at high doses i.e. 40 mg/kg will fully generalise but at lower doses i.e. 20 mg/kg, then only partial generalisation is observed (Tongjaroenbuangam *et al*, 1998)

3.2.3 Amphetamine and caffeine

The following study was to investigate the effects of amphetamine experience on acquisition of future caffeine discrimination. So the interactions between amphetamine and caffeine are of interest. Caffeine and amphetamine show partial generalisation to each other (Chait & Johanson, 1988). It has been shown that caffeine produces a profile of subjective effects in humans that are very similar and partially overlap those produced by amphetamine (Chait & Johanson, 1988). Caffeine does share some stimulus functions with amphetamine, but only under specific dose ranges and training conditions. Caffeine also has a relatively lower dependence potential than amphetamine (Heischman & Henningfield, 1992).

Caffeine and amphetamine produce similar effects on behaviour despite the fact that they modulate different neurotransmitter systems. However, it has been suggested that caffeine as well as acting directly on adenosine receptors may also remove the negative modulating effects of adenosine from dopamine receptors, which in turn would lead to stimulating dopaminergic activity. This mechanism of action may explain why caffeine and amphetamine have similar behavioural effects e.g. both

increase locomotor activity, both increase turning in 6-hydroxydopamine-lesioned rats, both have stimulant-like discriminative stimulus effects and self-administered effects. Also, some of caffeine's and all of amphetamine's effects on the aforementioned behaviours can be blocked by dopamine receptor antagonists (Garrett & Griffiths, 1997). It has also been shown that both amphetamine and caffeine produce bi-phasic response curves over a range of doses, whilst cocaine produces a dose-dependent response curve (Antoniou *et al*, 1998).

One study showed that neither amphetamine at a low dose (0.05 mg/kg) nor caffeine (15 mg/kg) alone produced amphetamine-like responding in rats trained on amphetamine (0.8 mg/kg) from saline. But it was shown that the co-administration of amphetamine and caffeine induced amphetamine-like responding; the animals responded as if they had been injected with the original training dose of amphetamine (Schechter, 1977).

Another study carried out to investigate the effects of caffeine on amphetamine involved placing caffeine in the animals drinking water and comparing the effects of caffeine and amphetamine in the caffeine-drinking rats and the water-drinking rats (Jaszyna *et al*, 1998). The study showed that the animals tolerated out to the effects of caffeine after exposure for 5 days in the drinking water. Once the animals had tolerated out, then the animals were administered amphetamine and nicotine. The results showed that caffeine potentiated the behavioural response to amphetamine but not nicotine – indicated by the fact that nicotine affected equally both the water- and

caffeine-drinking rats whilst amphetamine affected the caffeine-drinking rats more than the water-drinking rats (Jaszyna *et al*, 1998).

3.2.4 Summary of amphetamine

Amphetamine is a commonly used drug of abuse that has stimulant properties. Many of the stimulant properties are due to the dopaminergic effects of amphetamine. Studies have shown that amphetamine can easily be discriminated from other drugs and from saline in both humans and non-humans. The studies also show that amphetamine and caffeine have similar but not identical properties to each other and are both stimulants. It was because of the similarities between amphetamine and caffeine in other paradigms and the similarity between the two drug discrimination cues, that it was predicted that previous exposure to amphetamine would facilitate acquisition of the caffeine cue.

3.3.NICOTINE

3.3.1 Introduction

Nicotine is a naturally occurring compound that is widely used by people in all countries of the world. Smoking spread to Europe in the 16th century (Rang & Dale, 1994).

3.3.2 Nicotine and nicotinic-cholinergic receptors

Nicotine is classed as a CNS stimulant (Gauvin & Holloway, 1993). It acts by binding to the nicotinic cholinergic receptor (Collins, 1990) which appears to initiate many actions in different neuronal areas including the release of dopamine (Hisoaka & Levy, 1985).

3.3.3 Drug discrimination of nicotine

Animals can be trained to discriminate nicotine from saline at many different doses of nicotine (Perkins *et al*, 1994). In the drug discrimination paradigm, then nicotine can act as both a stimulant and a sedative depending upon the dose, time interval since administration and the environment of the administration (Perkins *et al*, 1994). Nicotine shows partial generalisation to the amphetamine and caffeine cue (Gauvin & Holloway, 1993). Tolerance quickly develops in the drug discrimination paradigm to the depressant effects of nicotine (Stolerman & Jarvis, 1995).

The higher the training dose of nicotine used, then the quicker that the drug discrimination is learnt in (Garda *et al*, 1993). Some rats show more innate tolerance than other, this phenomenon is also found amongst humans (James *et al*, 1994).

In fact, animals can discriminate nicotine from vehicle at doses associated with plasma concentrations of nicotine similar to those of cigarette smokers who inhale the smoke (Stolerman & Jarvis, 1995). Other studies with rats in the drug discrimination paradigm use nicotine and another drug to try and investigate the effects of a mixture (White & Stolerman, 1996). Rats do not learn to self-administer nicotine spontaneously in situations where they have to press a lever to obtain an injection of nicotine. However, rats will learn much quicker if they have previously been injected with nicotine (Stolerman & Jarvis, 1995). This can be explained by nicotine being classed as a weak reinforcer unlike cocaine which is a strong reinforcer. However studies have shown that nicotine can act as a strong reinforcer, but under limited conditions (Stolerman & Jarvis, 1995).

Monkeys have been trained to smoke by the use of successive approximation. This involves the monkeys being gradually rewarded with food reinforcement for smoking behaviour until they smoked cigarettes and received the reward from the nicotine itself and not another reinforcement. One study showed that the monkeys continue smoking if the cigarettes contain nicotine, but not if nicotine-free tobacco is used (Griffiths & Henningfield, 1982).

Drug discrimination of nicotine in humans is difficult to carry out because nicotine dosing is imprecise in smoking (Pomerleau *et al*, 1989). This is because in drug discrimination, the dose must be standardised in order to be able to compare the effects of nicotine on different subjects. The other problem with drug discrimination of nicotine in humans is that the other methods of administering nicotine lack the rapid uptake that is common to smoking and they may have a different pharmacokinetic profile compared to smoking. The different pharmacokinetic effects may produce different subjective effects (Henningfield & Keenan, 1993).

3.3.4 Nicotine and caffeine interactions

The data generally show drug discriminative cues of nicotine are more like amphetamine and cocaine than caffeine (Stolerman & Jarvis, 1995).

There are suggestions that caffeine and nicotine have pharmacological interactions and that the effects of the two drugs together are different from those alone (Kerr, Sherwood & Hindmarch, 1991). It has been shown that consumption of caffeine and nicotine correlates positively (Matarazzo & Saslow, 1960) and that there is a higher intake of caffeine in smokers when compared with non-smokers. Furthermore, caffeine is eliminated from the body of smokers quicker than from the body of non-smokers (Parsons & Neims, 1978).

Coffee consumption and cigarette smoking have been strongly correlated in several studies (Dawber *et al*, 1974; Lang *et al*, 1983; Klatsby *et al*, 1973). However some

studies have shown that there are no caffeine induced dose related increases in cigarette smoking (Chait & Griffiths, 1988; Kozlowski, 1976).

Although long term studies have shown a correlation between caffeine and nicotine, short term studies have yet to show a direct pharmacological effect (Marshall *et al*, 1987). One study showed an increase in smoking when drinking either caffeinated or decaffeinated coffee, e.g. whether caffeine or no caffeine was present. Smokers tend to have higher heart rates and catecholamine excretion when they are smoking compared to when they are abstaining from nicotine and this effect is independent of caffeine intake (Benowitz, 1986).

It has been shown that cigarette smokers consume more caffeine than non-smokers and so that cigarettes are more likely to be smoked during caffeine consumption (Hrubec, 1978). It has been suggested that more caffeine is consumed during cigarette smoking because the half-life of caffeine is shorter for smokers compared with non-smokers, i.e. smokers require a greater amount of caffeine to produce a given effect (Emurian *et al*, 1982). However one study has shown that coffee with or without caffeine, actually increases the number of cigarettes smoked (Marshall, Epstein & Green, 1980).

Under a limited range of conditions, caffeine and nicotine interact and alter subjective arousal. Nicotine has a stimulant effect when the subject is in a low arousal state, but a depressant effect if the subject is in a high arousal state. In a study

nicotine and caffeine were administered together no effects on arousal were seen (Rose & Behm, 1991).

The data generally show drug discriminative cues of nicotine are more like amphetamine than caffeine in a drug discrimination paradigm (Stolerman & Jarvis, 1995).

There are suggestions that caffeine and nicotine interact pharmacologically and the effects of the two drugs together are different from each other separately (Kerr, Sherwood & Hindmarch, 1991).

3.3.5 Summary of nicotine

Nicotine is a naturally occurring compound that has been around and indeed used for many decades. Studies have been carried out in both humans and animals on the effects and discriminability of nicotine. Studies have shown that nicotine and caffeine together can have different effects compared to the drugs alone. The studies showed that caffeine and nicotine sometimes have differences and sometimes have similar effects, which is why it was unsure what effect previous exposure to nicotine would have on the acquisition of the caffeine cue.

3.4 CDP

3.4.1 Introduction

Chlordiazepoxide (CDP) is a member of the benzodiazepine (BDZ) family (Rang & Dale, 1994). Benzodiazepine's including CDP are used to decrease anxiety and to act as a sedative (Rang & Dale, 1994).

3.4.2 Mechanism of action

CDP was believed initially to act as a non-specific depressant, like anaesthetics. It has been shown that there are specific benzodiazepine binding sites on the $GABA_A$ receptor-ion channel complex. There is also mutual augmentation of binding between GABA and benzodiazepines on the $GABA_A$ receptor-ion channel complex. GABA and benzodiazepines both increase the affinity of the sites of the other without affecting the total number of sites (Rang & Dale, 1994).

Benzodiazepines bind to the $GABA_A$ receptor/chloride ionophore complex and with a lower affinity to the "peripheral" benzodiazepine sites. The peripheral sites are believed to be associated with calcium channels (Maragos *et al*, 1982).

3.4.3 Drug discrimination of CDP

Studies have shown that it is possible to train animals to discriminate CDP from saline. It has been shown that it takes 90 days to train rats to discriminate CDP (7 mg/kg) from saline (Bronson & Chen, 1996). It has also been shown that once animals have been trained to discriminate between CDP and saline, then the

discrimination is very robust and remains even after 30 extinction sessions (Rjinders, Jarbe & Slanger, 1993).

The nature of the CDP discriminative stimulus is complex (Andrews, 1992). It has been shown that animals can discriminate between both high and low doses of CDP versus saline, however once animals have been trained to discriminate between a high dose of saline, then they can no longer discriminate between a low dose of CDP and saline (Rjinders, Jarbe & Slanger, 1993). The cueing properties of benzodiazepines are well correlated with sedation (Barry & Krimmer, 1977).

Studies have shown that many benzodiazepines generalise to the CDP cue, as do many barbiturates. Thus, the discriminative cue of CDP is benzodiazepine and barbiturate specific (Colpaert, 1977). Also, cross-tolerance occurs between the discriminative stimulus of CDP and bentanzil (Bronson & Chen, 1996), however, the exact mechanism by which either compound produces its discriminative stimulus cue is unknown (Andrews, 1992).

3.4.4 Interactions with caffeine

The following study was to investigate the effects of previous CDP experience on future caffeine discriminations. So it is relevant to investigate the effects and interactions, if any, between CDP and caffeine. It has been shown that caffeine (56

mg/kg) blocked the discriminative stimulus properties of CDP (5 mg/kg) and shifted the CDP discrimination dose-response curve to the right (Gauvin, Pierce & Holloway, 1994). This indicates that a complex interaction can occur between caffeine and CDP in some situations. Also, it was shown that if animals were trained to discriminate between CDP and saline, then a mixture of caffeine and CDP did not alter CDP appropriate responding (Holloway, Modrow & Michaelis, 1985).

One study (Holloway, Modrow & Michaelis, 1985) showed that CDP did not generalise to the methylxanthine cue in caffeine trained rats, but instead produced dose-related decreases in response rates. CDP and caffeine combinations produced dose-related decreases in drug-lever responses.

Another study by Quenzer, Feldman & Moore (1974) has shown that CDP suppresses spontaneous activity whilst caffeine increases it, so when the two compounds were administered together then antagonistic effects were observed compared to the individual compounds alone. However, it has also been shown that CDP suppresses shock-induced aggression and caffeine suppresses aggression, so when administered together the mixture has additive effects when compared to the individual drugs alone (Quenzer, Feldman & Moore, 1974).

Another study has shown that both CDP and caffeine increased response rates whilst decreasing reinforcement frequencies in animals trained under a DRL schedule.

Therefore, in this paradigm, then caffeine and CDP administered together have again more additive effects compared to either drug alone (Sanger, 1980).

3.4.5 Summary of CDP

CDP is a member of the benzodiazepine family. It has many properties that include a decrease in anxiety and sedation as well as anticonvulsant properties. It is possible to train animals to discriminate between CDP and saline. CDP and caffeine have different discriminative stimulus effects, caffeine is a stimulant cue and CDP is a sedative cue, which is why it was expected that previous experience of CDP would retard the acquisition of the caffeine cue.

3.5 Rationale for the drugs used in this study

The training drugs of amphetamine, CDP and nicotine were chosen because they all have different discriminable effects. The training drugs were also chosen because of their well-documented discriminative effects in rats (Jaszyna *et al*, 1998; de Vry & Slangen, 1986; Stolerman & Garcha, 1989). It was assumed that the animals learning the caffeine discrimination might "attend" to different components of the caffeine cue due to their different prior training on different drugs. Amphetamine was chosen because in the discrimination paradigm, rats attend to a "stimulant" cue (Woolverton & Cervo, 1986), whilst animals trained on CDP learn to attend to a "sedative" cue (Holloway, Modrow & Michaelis, 1985). The final drug nicotine was

chosen because nicotine was another different cue that can resemble a stimulant cue weakly (Stolerman, Garcha, Raff & Kumar, 1984).

Caffeine at 10 mg/kg was chosen because it is a low dose and studies have shown that this dose is not a specific methylxanthine cue but is a broad "stimulant" cue (Mumford & Holtzman, 1991). It was vital that the second compound had a general discriminative stimulus cue, to allow any differences between the ways the animals responded after the three initial different drugs to be observed. If the second drug had a specific cue, then the animals would have been trained to discrimination one drug followed by another drug, but no differences of drug history could have been observed. Thus, the caffeine cue needed to be a general, stimulant cue and not a specific methylxanthine cue.

It was expected and predicted that any drug which generalised to the caffeine cue would induce very rapid, if not immediate, acquisition of the caffeine cue. This was because under the training drug the animals had learnt to respond on the left lever under saline and the right lever under drug. So if the second drug generalised fully then the animal would be expected to choose the same levers under the new drug/saline conditions. Therefore, by definition the animals should have acquired the cue faster than drug naïve controls. In contrast, if the second drug did not generalise at all then the animals should have responded on the left or saline lever under both drug and saline conditions. So, by definition these animals should have much slower acquisition of the new drug cue compared to drug-naïve controls.

Chapter 4.0

Methods for Study I

4.1 Animals

Stock Sprague-Dawley Wistar rats were obtained from Interforna (Cambridgeshire, UK) and bred at Liverpool University Psychology Department to produce the experimental animals. Eighty-five experimentally naive adult female Sprague-Dawley rats were group housed (n=6) from weaning (21 days of age) until the beginning of the experiment. A week before the beginning of the experiment, the animals were singly housed in cages measuring 38 cm x 22 cm x 22 cm. The animals weighed between 190 and 300 grams with a mean of 262.9 $\pm$ 2.09 (S.E.) grams when the experiment began.

The animals were able to see, hear and smell other rats at all times during the experiment. They were handled regularly for 12 days before the start of the experiment and once a day for weighing and injection purposes during the course of the experiment. All animals were housed in the same room, under conditions of: controlled temperature (21^0C), humidity (53%), lighting (dark period 1800-0600 h) and given free access to standard laboratory chow (Bantin & Kingman, U.K.) and water, except when this was modified for an experimental procedure.

Each rat was assigned an experimental number from 1 to 85, which was also their cage number.

The animals were divided into 7 groups of 12 or 13 each by the use of their cage numbers, as follows:

Table 4.1: The experimental numbers of the animals and their initial treatment

Experimental Number	Initial Treatment	Number in the group
1-12	Amphetamine discriminators	n = 12
13-24	Amphetamine	n = 12
25-36	CDP discriminators	n = 12
37-48	CDP	n = 12
49-60	Nicotine discriminators	n =12
61-72	Nicotine	n =12
73-85	Saline (controls)	n =13

CDP – Chlordiazepoxide

One group acted as the control group and the rats received daily injections of saline only and, by definition, received no discrimination training prior to caffeine discrimination training. The remaining 72 animals were split into 3 groups (n=24). Each group received a different training drug (see Table 4.1). Each group consisted of two subsets (n=12). One subset for the respective training drug was called the "discriminating set" and the other subset was termed the " non-discriminating set ".

The "discriminating sets" received discrimination training in operant boxes. They were trained to discriminate between saline and their respective training drugs. The "non-discriminating set" received daily yoked injections of either saline or drug. The

"discriminating set" was used to assess whether previous drug discrimination *training* altered the caffeine cue. The non-discriminating set was used to assess whether discrimination training per se altered the drug cue rather than simply drug *experience* (i.e. cross-tolerance or cross-sensitisation).

The 7 groups and their initial and final treatments were as follows: -

Table 4.2: The groups of animals and their treatments

Initial Treatment	Planned Treatment
Saline (controls)	Caffeine discrimination
Amphetamine Discrimination	Caffeine discrimination
Amphetamine	Caffeine discrimination
CDP Discrimination	Caffeine discrimination
CDP	Caffeine discrimination
Nicotine Discrimination	Caffeine discrimination
Nicotine	Caffeine discrimination

The aim of the experiment was to see if previous drug history (i.e. discrimination training or non-contingent treatment) affected the discriminative cue of caffeine. The doses of the drugs, amphetamine (2 mg/kg), CDP (10 mg/kg) and nicotine (0.3 mg/kg) were chosen because it was known that the drugs at those doses were discriminable (Jaszyna *et al*, 1998; Barry & Krimmer, 1977; Corrigall & Coen,

1994). Ideally, the animals in each of the three training sets would learn the discriminations of their individual drugs in a similar number of training sessions. However, I believed that it was more important that all the animals learnt their initial drug discrimination to a high level (90% or higher) than that the animals all learnt at the same rate. The rats injected with saline only would allow the assessment of whether any differences between the groups were due simply to the daily injections.

4.2 Apparatus

The operant chambers were supplied by Med.-Associates, USA. Each chamber consisted of a white outer compartment with a door that had a spy hole in the centre. The inner box was made of clear perspex, had a wire floor and measured 29 cm x 28 cm x 25 cm. One of the side walls of the inner chamber contained two non-retractable levers positioned 3 cm from the ground and 12 cm apart. Situated between the two levers was a pellet dispenser which delivered one 45 mg banana pellet (P.J. Noyes Company Inc.) every time it was activated. The operant chambers were controlled and data recorded by OPN software for an IBM compatible personal computer. The OPN software was a modified version of the package used by Spencer & Emmett-Oglesby, (1985) and was supplied by Emmett-Oglesby (Texas College of Osteopathic Medicine, USA).

Each animal was assigned an experimental chamber and throughout the whole of the experiment during training and test sessions the animal remained in the same box. The assignment of the rats to the experimental boxes meant that the animals always followed the same animal in each training and test session and that the olfactory cues

remained the same between rats during the sessions. As rats are sensitive to olfactory cues, a pseudorandom schedule was used to determine drug or saline injections for each group of animals, so that animals could not use such cues to guide lever selections (Extance & Goudie, 1981). The pseudorandom schedule meant that the animals had to learn to discriminate their internal cue and they could not press the same lever as the animal in the box before them.

A 2.8-watt light bulb was positioned 2.5 cm from the top of the wall on the side of the chambers on which the levers were situated, and a house light was illuminated continuously during the operant sessions. Masking white noise was present at all times throughout the sessions.

4.3 Drugs

Nicotine ([-] Nicotine hydrogen tartrate) and Chlordiazepoxide (Chlordiazepoxide hydrochloride - CDP) were both obtained from Sigma Chemicals UK. They were used as salts at training doses of 0.3 mg/kg and 10 mg/kg respectively. Amphetamine (dexamphetamine sulfate) was obtained from Smith, Kline Beecham. Initially amphetamine was used at a dose of 2 mg/kg as a salt, but this dose was found to be too high as it suppressed operant responding substantially, so the dose was reduced after 6 days of training to 1 mg/kg. The response rate data still showed that the dose was too high, so the dose was further reduced to 0.75 mg/kg after 34 days, and finally reduced to 0.5 mg/kg after 3 more days. Caffeine (anhydrous) was obtained from Sigma Chemicals UK and was used initially at a dose of 10 mg/kg as the base.

All the drugs were dissolved in 0.9% isotonic saline to the correct concentrations and were freshly prepared before each training and test session. The injection volume was 1 ml/kg of rat. The animals were injected once daily (5 out of 7 days) with either saline or the relevant training drug, and all injections were given i.p.

4.4 Training procedures

All groups of rats were food-deprived to about 80% of their free-feeding body weights. In order to increase the probability of the animals working for food reward they were only fed at the end of the day after the training session. Initially the animals were habituated to their operant chamber for one 30 minute session on a "shaping" programme to make a response on either lever in order to obtain a food reward. At the beginning of the training session either lever needed to be pressed once only for a reward to be given (FR 1). Once either lever had been pressed ten times and the rat received 10 rewards, the number of lever presses required for a reward was increased. From this point in time the levers needed initially to be pressed three times before each reward was given (i.e. a Fixed Ratio 3 schedule on each lever). The number of times that the levers needed to be pressed was gradually increased until a schedule of Fixed Ratio 8 was reached. This meant that the rats had to press the levers a total of 8 times before a reward was received. The training programme was run for 30 minutes/day for each animal.

Once all animals were reliably obtaining rewards under the FR 8 schedule, stability of responding was assessed by studying the raw data daily and comparing the total number of responses made throughout the training sessions. When the animals

responses were stable, the next stage of training was started. These programmes required the animals to respond for reward on one lever only (left or right). If the animals responded on the wrong lever, no reward was received. The programme initially required the animals to press the lever once for a reward, once 10 rewards had been given under the FR 1 schedule, the lever needed to be pressed 3 times (FR 3) before a reward was received. Once the animals had received 9 rewards then the fixed ratio was increased to 5. Then the fixed ratio was increased further within the session to 9, and after 8 rewards the number of times that the lever needed to be pressed was increased further to 16. Once the animal had received 30 rewards the fixed ratio was increased further to 30 and was kept at this level until the end of the session. Different programmes were used to train the animals on the left and right levers. At the end of each training session, the data for each animal were analysed and lever bias was assessed by determining the number of lever presses made on each lever. Any animals that showed lever bias were retrained on the appropriate programme (i.e. left or right lever).

The rest of the animals (i.e. those not showing signs of bias) were maintained at their food-deprived state of about 80% of their free-feeding body weight, but received no more training at this stage. The next stage of training was begun once none of the animals showed lever bias, and were responding reliably on both levers. Until this point of time in training, all sets, i.e. the "discriminating set" and the "non-discriminating set" and the "controls" were trained to respond for food reward in the operant chambers. From this point on the "discriminating set" received

discrimination training, the "non-discriminators" simply received yoked injections and the "controls" received injections of saline.

The next stage of training required the animals to be injected with either saline or a training drug. After drug treatment, the animals were required to respond 30 times on the right lever to receive a reward. Animals responding on the left lever received no reward. If treated with saline the animals had to respond 30 times on the left lever before a reward was given. The drug and saline programmes had 2 components to each session. Component One continued until the animal had made its first 30 responses on the single active lever for that session (i.e. left or right). The total number of responses on *both* levers in component one was called the FRF, i.e. response to First ReinForcement. The FRF is the total number of responses on both levers before the first reinforcement is given. Component Two ran for the remainder of the training session. Thus, the programmes recorded both the total number of responses that the animals made and the number of rewards received, as well as the accuracy of lever selection.

The animals were trained to discriminate the training drug from saline under a two-weekly alternating pseudo-random schedule as shown below:-

M	Tu.	W	Th.	F
S	S	D	S	D
S	D	D	S	D

[Derived from Colpaert *et al* (1975)]

In the pseudorandom schedule S means that the animals were injected with saline and D means that the animals were injected with the respective training drug. The "discriminating" animals were trained to discriminate the respective training drug from saline. Each session the animals had to have a correct lever selection, defined as the FRF value being less than or equal to 59. As discussed earlier a correct FRF also requires the animals to make 30 response on the correct lever before making 30 responses on the incorrect lever. As the animals learnt the discrimination of the drug versus saline, then the FRF value decreased to or close to 30. Also the animals had to 3reach a between session criterion of 9 correct lever selection days (FRF values less than 59) in 10 consecutive training sessions. The animals were constantly monitored to check that the FRF values were still below 59 daily and that they also maintained the between session criterion, until both criterion were reached and stable. The "non-discriminating" animals were injected under the same pseudorandom schedule as the "discriminating" animals. The same number of injections were administered to all groups. This meant that the "non-discriminating set" injection pattern was yoked to that of the "discriminating set" for each drug.

Once the "discriminating sets" had reached criterion on their respective training drugs, the "non-discriminating sets" and "controls" were retrained to respond for food rewards in the operant chambers. The rats were initially retrained on the shaping programme until all the animals had reached a fixed ratio of 8. Then the animals were trained for one day on each of the FR10 programmes (one for the left lever and one for the right lever). The programme required the rats to make 10 responses on the single active lever before a reward was received. This programme

was used so that the animals were accustomed to pressing one lever several times before receiving a reward. This training programme was used because the previous training programmes allowed the animals to obtain rewards easily by gradually increasing the Fixed Ratio from 1 to 30. The animals were then trained on the FR-30 left and right lever programmes so that the animals were accustomed to making 30 responses on both levers before receiving a reward.

It was decided to increase the training time from 15 minutes to 20 minutes on day 58 of initial drug training (i.e. CDP, amphetamine or nicotine) to allow the animals longer to respond in the operant boxes and therefore to obtain more rewards per test session. The total number of responses made by each animal during the training sessions was relatively low in these specific operant chambers, compared to other data obtained from other operant chambers in the same laboratory. The mean number of responses on session 53 under saline was on average 637.2 and for amphetamine was 557.8, for CDP was 1083.7 and for nicotine was 616.4. However once the increased session time was programmed the mean number of responses on session 61, for saline was on average 795.8 and for amphetamine was 622.6, for CDP was 1097.1 and for nicotine was 897.8. Therefore, the increase in session time allowed the total number of responses to increase. This increased the number of rewards earned, and thus may have facilitated acquisition of the various drug discriminations which were acquired relatively slowly (see results).

4.5 Caffeine discrimination training

Once all the animals in the "discriminating sets" had reached the criterion of learning for all the training drugs and the "non-discriminating set" and "controls" were responding for rewards, caffeine discrimination training commenced. The animals were initially trained to discriminate between caffeine (10 mg/kg) and saline. The drug and saline programmes were used as before. The daily training sessions were kept at 20 minutes and the data analysed daily for each animal to check their performance. However, the data showed that the animals were not learning the discrimination at 10 mg/kg, so the dose of caffeine was increased to 15 mg/kg on session number 60. After several sessions the data was reviewed and they showed once more that the animals were still not learning the caffeine cue, so a decision was made to increase the dose once more this time to 20 mg/kg on session number 67. The dose of 20 mg/kg of caffeine is still classed as a low general "stimulant" dose (Mariathasan & Stolerman, 1992). The dose was not further increased because studies have shown that a dose of 32 mg/kg is a specific methylxanthine cue and it was important that the caffeine cue was not a specific methylxanthine cue.

The aim at the start of the experiment was to investigate whether animals could be trained to discriminate initial drugs and then a second compound with a general discriminative stimulus cue. Going on from this, other substitution tests would have been carried out to investigate if the initial drugs had altered how the animals perceived the general cue from the second compound.

The data were analysed daily to check for the animals reaching the specified criterion of 9 correct lever selections out of 10 consecutive sessions (as previously defined).

The speed at which the animals learnt the discrimination between caffeine (20 mg/kg) and saline was monitored at all times and the FRF recorded daily. The accuracy of the animals learning the caffeine cue was recorded and compared within groups to check if there were subsets of animals *within* the groups that had learnt the caffeine cue (data not shown). It was possible that subsets within the groups had learnt the discrimination whilst other animals had not learnt the discrimination at all.

Chapter 5.0

Results for Study I

The animals were weighed every day and a record kept at all times. The mean body weights of all seven groups were compared to ensure that all groups followed the same trend and also to ensure that the body weights stayed constant at about 80% of the animals' free feeding body weights.

Fig 5.1 shows the mean bodyweights of the groups for all the "discriminating" animals for all the training drugs: - amphetamine (0.5 mg/kg), CDP (10 mg/kg) and nicotine (0.3 mg/kg).

The graph shows that the animals showed the same trends in increasing and decreasing body weights over the same time period. The graph shows that over the Christmas break (around sessions 10) all the animals put on weight which gradually decreased once the animals were placed back under the food-deprivation schedule to keep the animals at 80% of their free-feeding body weight.

Figure 5.1:

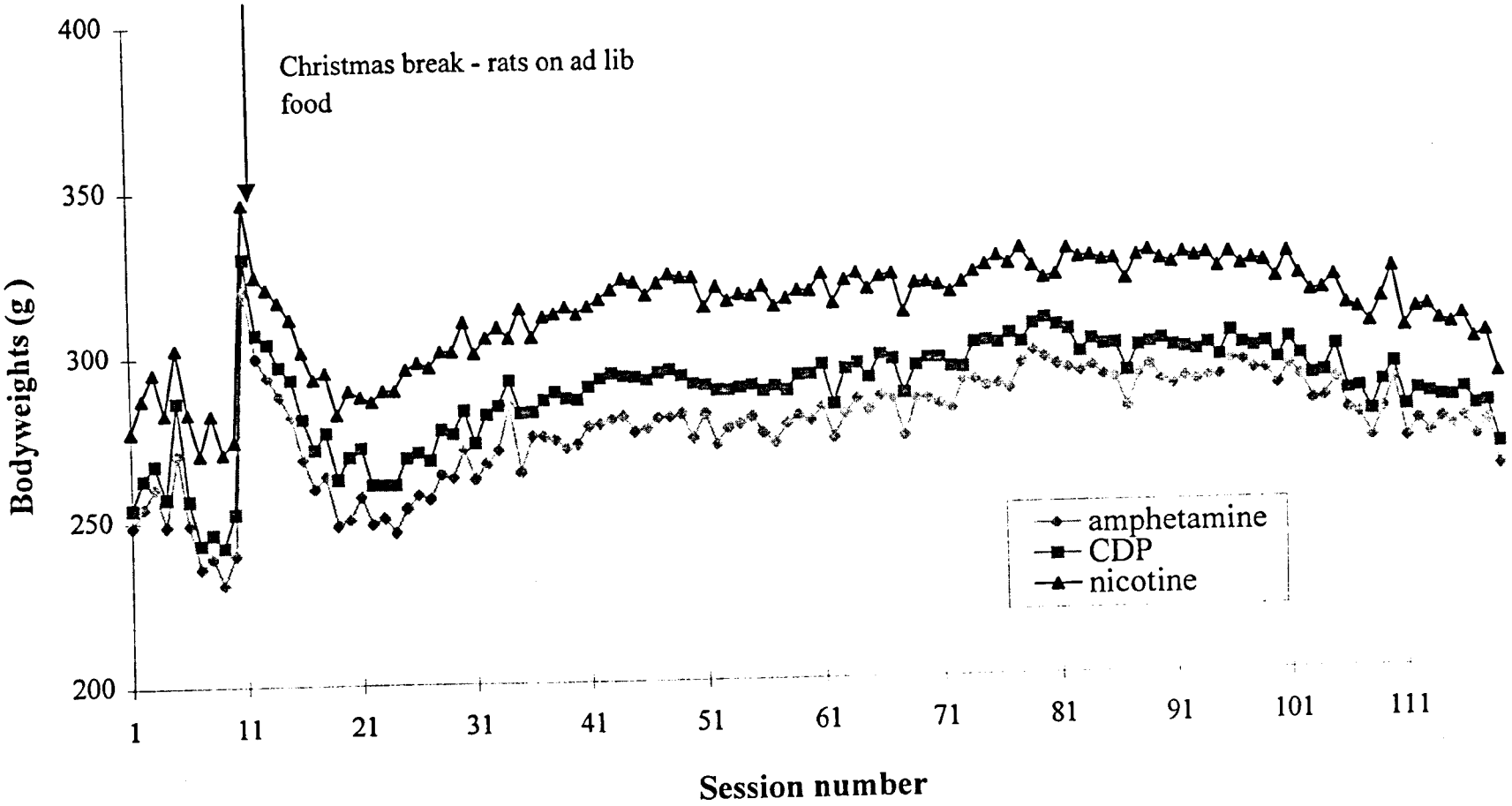
Christmas break - rats on ad lib food
Bodyweights (g)
400
350
300
250
200
1
11
21
31
41
51
61
71
81
91
101
111
Session number
amphetamine
CDP
nicotine

Fig 5.1: The graph represents the mean body weights of the animals in the "non-discriminating" groups. The drugs used were amphetamine (0.5 mg/kg, n=12), CDP (10 mg/kg, n=12) and nicotine (0.3 mg/kg, n=12).

The "non-discriminating" and "control" animals also followed the same trends, as can be seen in figure 5.2. The "non-discriminating" animals were injected with the same doses of "training" drugs as the "discriminating" groups, i.e. amphetamine (0.5 mg/kg), CDP (10 mg/kg) and nicotine (0.3 mg/kg) i.p. daily

Figure 5.2:

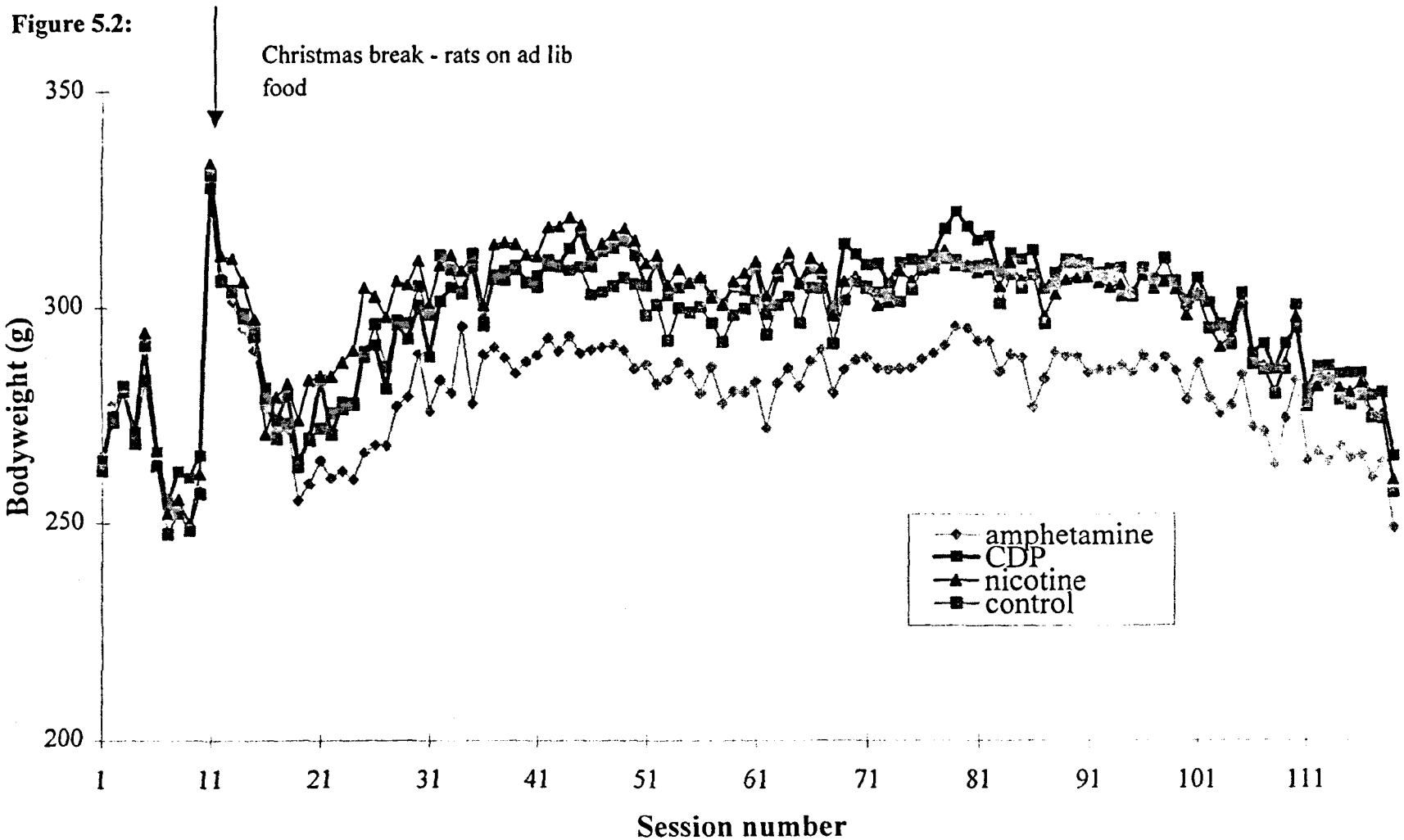
Christmas break - rats on ad lib food
350
300
250
200
Bodyweight (g)
1
11
21
31
41
51
61
71
81
91
101
111
Session number
amphetamine
CDP
nicotine
control

Figure 5.2: The graph shows the mean body weights of the "non-discriminating" animals treated with amphetamine (0.5 mg/kg, n=12), CDP (10 mg/kg, n=12) and nicotine (0.3 mg/kg, n=12). The animals were injected with saline or the respective "training" drug i.p. daily. The trends also show that the animals gained weight at session 11, due to the Christmas break.

The total responses of the "discriminating" animals were analysed daily to ensure that the animals made a response and that the response rates were increasing as the animals learnt to respond under drug. The data for the animals that were taught to discriminate between their training drug and saline were analysed and plotted as percentage drug lever selections. The data included all the animals in any one particular group that had received saline or the relevant drug. The percentage of animals that had made a correct lever selection was plotted as a proportion of the number of animals that had received either saline or drug. In addition, any animals that failed to make a lever selection were discounted from that day's data.

However, the results are presented with the data pooled over three successive training days i.e. days 1, 2 & 3; 4, 5 & 6 etc. The data were pooled over three days because if plotted daily the data showed no clear pattern. Therefore it was decided to display the data pooled over three days as this produced a clearer curve, which in turn allowed a pattern to be seen more easily. The graphs (figures 5.3, 5.4 and 5.5) showed that the animals initially responded on the drug lever at or below the chance level of 50%, this was because some of the animals in the amphetamine groups only (see figure 5.3) were initially biased towards the left or saline lever. The lever bias

was not seen until the animals were taught to discriminate between saline and the training drug, when the lever bias was very prominent. The lever bias was assessed in all three groups and calculated to assess whether all the animals were biased to the same degree. The animals that received amphetamine (results in Figure 5.3) responded 79.13% on the saline lever over session numbers 1-10. The animals that received CDP (results in Figure 5.4) responded 42.46% on the saline lever over session numbers 1-10 and the animals that received nicotine (results in Figure 5.5) responded 43.29% on the saline lever over session numbers 1-10. The responding was calculated by calculating the total percentage selection of responding on the saline lever, (disregarding whether the animal received saline or drug) for the first 10 days on training and then dividing by 10. The data showed that in actual fact *only* the amphetamine group of animals was lever biased at the beginning of the experiment, because they were the only group that showed significantly more or less than about 50% responding on the saline lever. The results shown in figure 5.3, indicate that the amphetamine group were clearly lever biased. The animals showed a preference towards the saline lever after injections of amphetamine and saline. One suggestion for the lever bias observed is that during discrimination training before the dose was lowered to 0.5 mg/kg, the dose of amphetamine administered was too high and suppressed the animals' responding. So for several sessions (not shown on the graph) the animals were injected only with saline to allow them a chance to recover from the affects of the amphetamine. More importantly this allowed response rates for some of the animals to increase and also allowed animals to press the lever at all. Before the period of chronic saline, several of the animals had stopped responding completely under either saline or amphetamine. Hence, this period of injection with

saline only may have induced saline lever bias because the animals were required to press the left lever for several sessions consecutively, instead of alternating between responding on the left and right levers.

The data are presented as percentage drug lever selection with three successive training sessions pooled together and group means taken. This form of graphical representation allows the data to show easily at which point the animals started to acquire the discriminations between saline and the drugs. As the animals learnt to recognise the drugs, then the number of saline lever selections decreased and the number of drug selections increased.

The results demonstrated that the dose of amphetamine, which was initially 2 mg/kg, was too high because the animals' response rates during the sessions were very low (data not shown). On average, the response rate on session 4 was only 21 responses per session. The dose was lowered to 1 mg/kg (see figure 5.3) and the average response rate on session 37 improved to 175 responses per session. The dose of amphetamine was further lowered to 0.75 mg/kg (see figure 5.3) and at this dose the average response rate on session 38 was 277 responses per session. A further lowering of the dose of amphetamine to 0.5 mg/kg (see figure 5.3) resulted in the average response rate on session 48 increasing to 394 responses per session. Once the dose of amphetamine was tolerated out, then all the response rates were stable.

The doses of CDP (10 mg/kg) and nicotine (0.3 mg/kg) were not altered, as once the animals had tolerated out the suppressant effects of the drugs, then response rates

were considered adequate for the purpose of the experiment (for CDP on session number 2 the mean number of responses was 476 and for nicotine on session number 4 the mean number of responses was 466).

Fig 5.3 shows the percentage drug lever selection for the amphetamine group with the data pooled over three training sessions. The graph shows that the animals learned to discriminate between the training drug and saline to a level of about 95% correct after about 90 sessions (30 blocks).

Figure 5.3

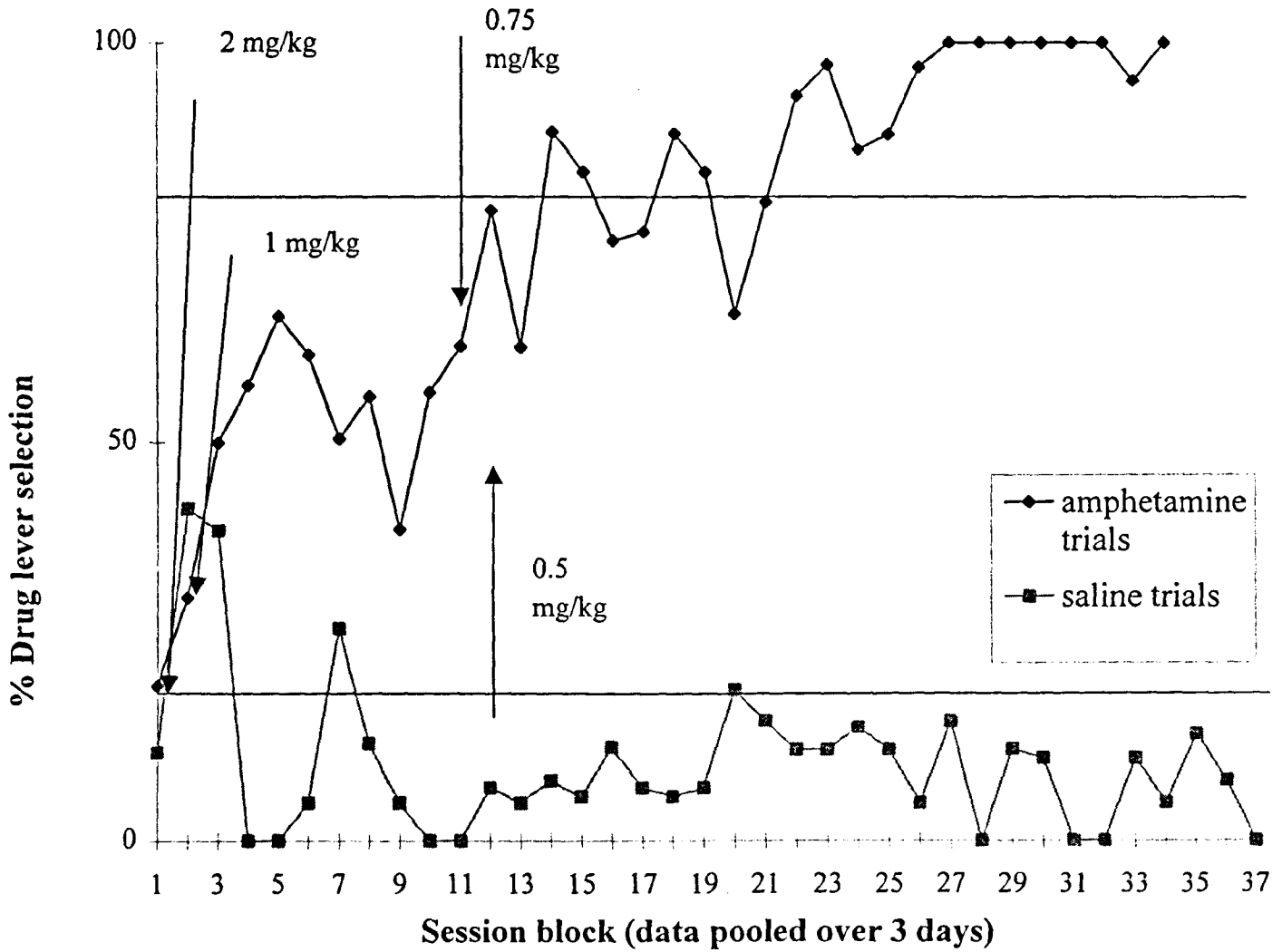
100
50
0
2 mg/kg
0.75
mg/kg
1 mg/kg
0.5
mg/kg
amphetamine trials
saline trials
% Drug lever selection
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37
Session block (data pooled over 3 days)

Figure 5.3: The graph shows the group of animals (n=12) trained to discriminate between saline and amphetamine. Amphetamine was administered i.p. daily on a pseudorandom schedule. The group size was reduced to 11 on session number 97, because one of the animals was ill and had to be removed from the study. The data show that until session block 3 (the equivalent to 9 training sessions) the animals showed marked saline lever bias, as most of the responses occurred on the saline lever even if the animals received amphetamine (as previously discussed). The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent.

Fig 5.4 shows drug lever selections for the CDP training group. The graph shows that the animals learnt to discriminate between CDP (10 mg/kg) and saline at a level of about 80% correct after circa 33 blocks. However, the graph in figure 5.4 also shows that the CDP discrimination was learnt at a slower rate and to a lower level of accuracy than the amphetamine discrimination.

Figure 5.4

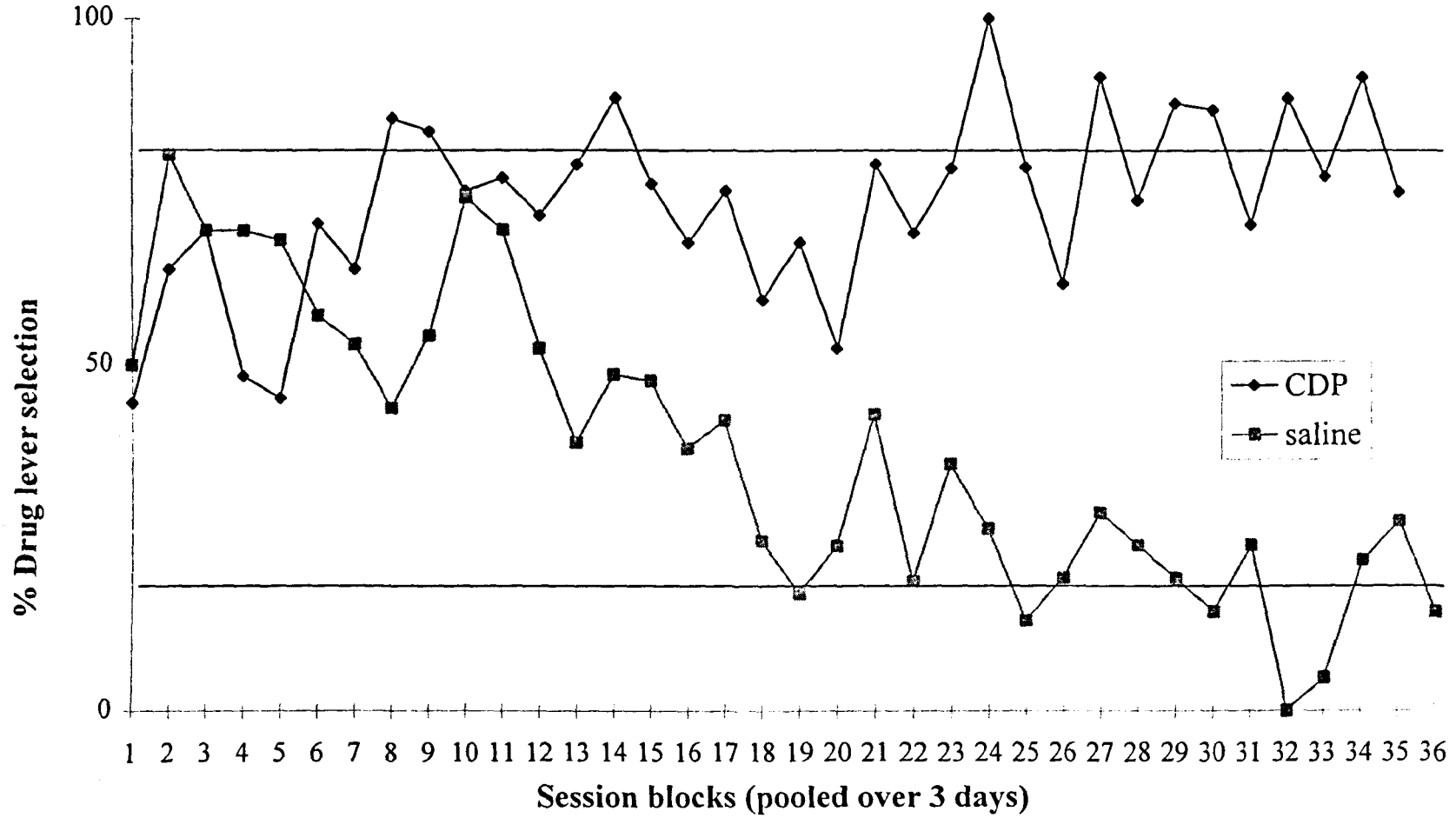
100
50
0
% Drug lever selection
CDP
saline
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Session blocks (pooled over 3 days)

Figure 5.4: The graph represents the training data for the animals (n=10) that learnt to discriminate CDP (10 mg/kg) from saline. The group size was decreased to 11 on session number 65 due to illness. CDP was administered i.p. daily under a pseudorandom schedule of CDP and saline. The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent.

Fig 5.5 shows drug lever selections for the nicotine group. The data show that the animals learned to discriminate between saline and nicotine (0.3 mg/kg), but again at a slower rate and lower level of accuracy than the animals learning to discriminate amphetamine (0.5 mg/kg) from saline.

Figure 5.5

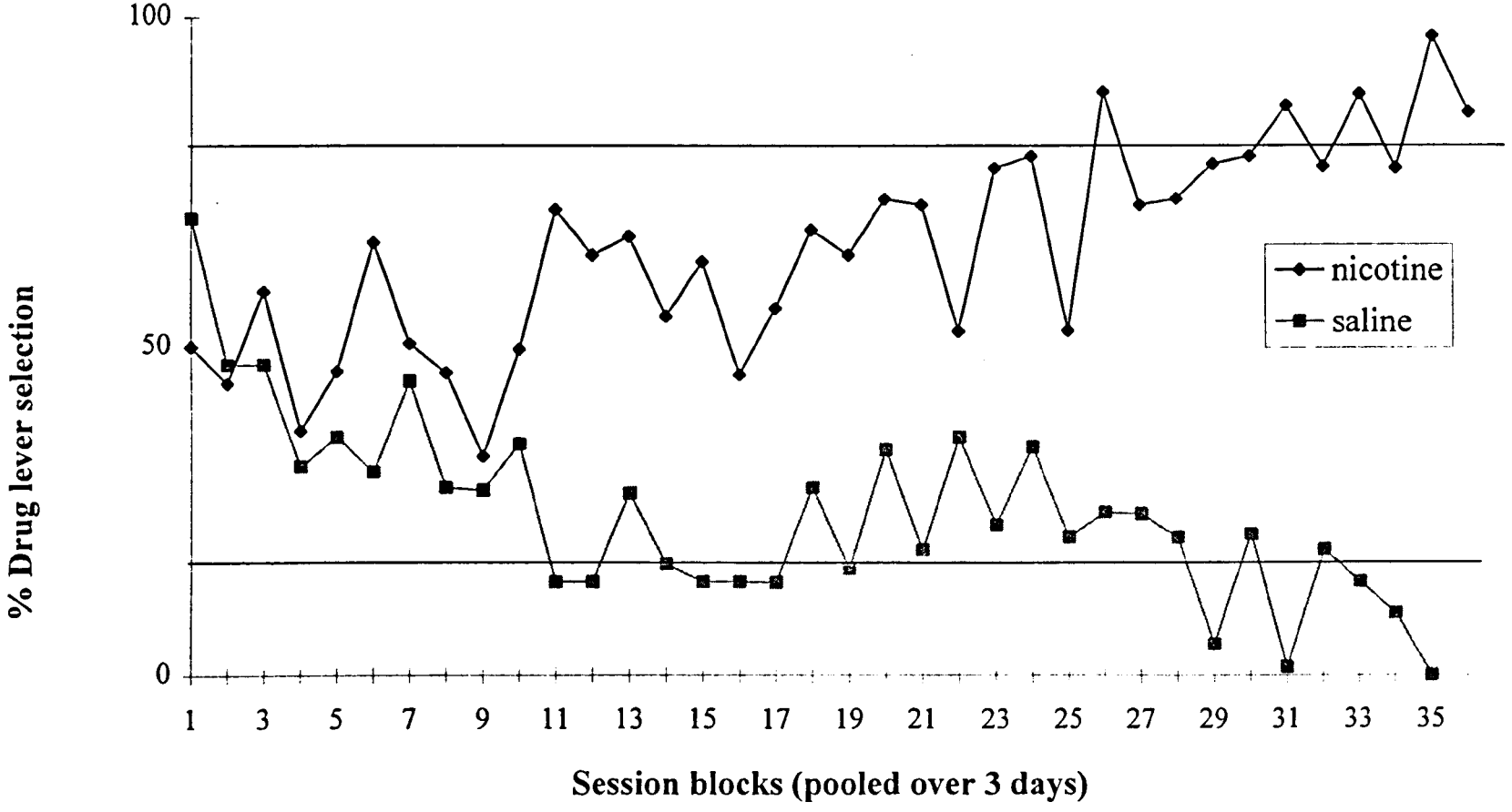
100
50
0
% Drug lever selection
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
Session blocks (pooled over 3 days)
nicotine
saline

Figure 5.5: The graph shows the group of animals (n=12) trained to discriminate nicotine (0.3 mg/kg) and saline. Nicotine was administered i.p. daily under a pseudorandom schedule with saline. The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent.

The data in each of the previous graphs show that the animals learned to discriminate amphetamine (0.5 mg/kg), CDP (10 mg/kg) and nicotine (0.3 mg/kg) from saline. The data also show that the animals trained with amphetamine learned the discrimination more quickly and to a higher level of accuracy than the animals trained on CDP or nicotine. This can be seen graphically by the steepness of the learning curves in figure 5.6.

Figure 5.6 is a comparison of all three initial discriminations. The graph represents the percent drug lever selection on *drug trials only*. The saline trials were omitted because they added too much data for the graph to be fully comprehensible.

Figure 5.6

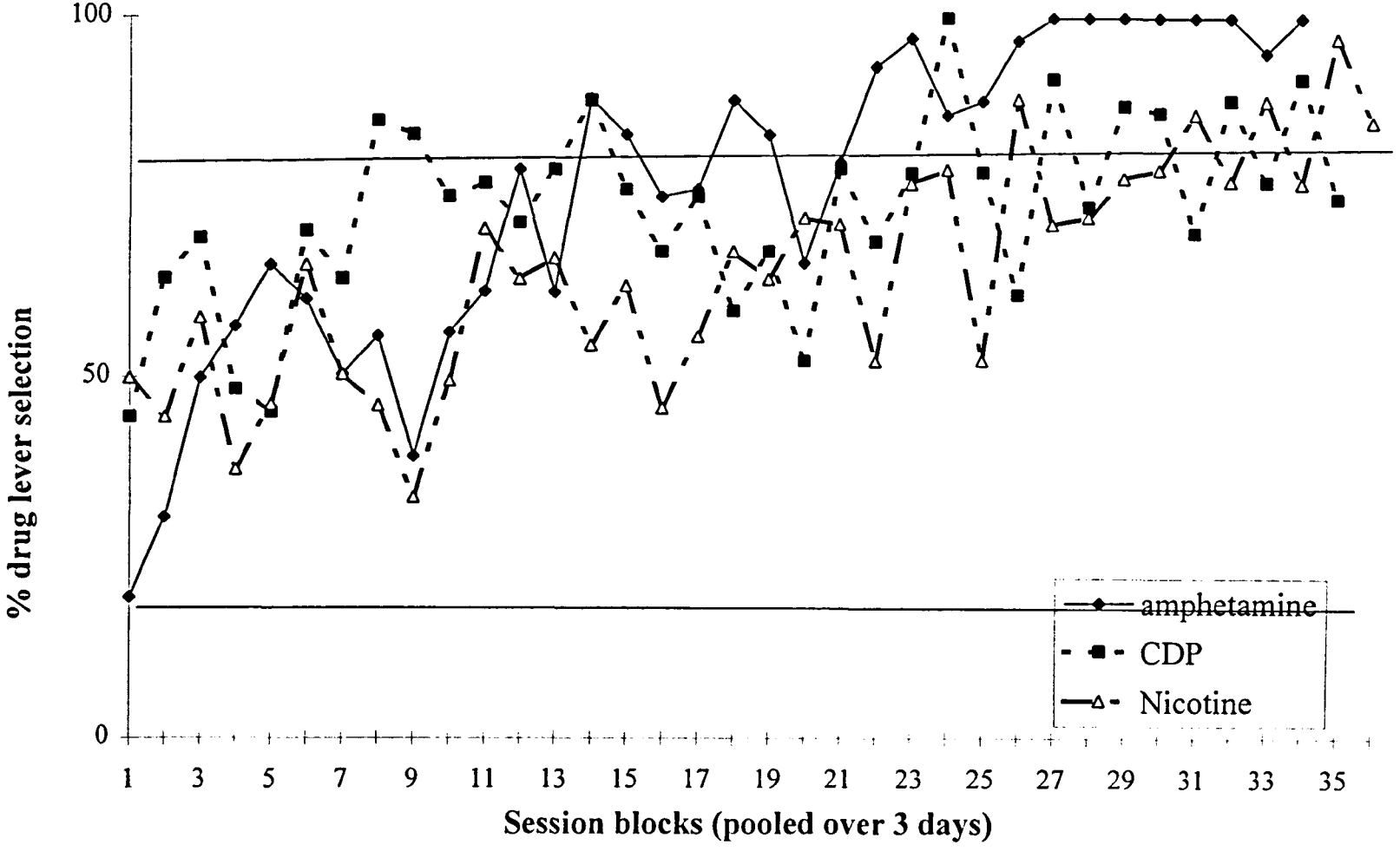
100
50
0
% drug lever selection
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
Session blocks (pooled over 3 days)
amphetamine
CDP
Nicotine

Figure 5.6: The graph shows a comparison of the three groups learning to discriminate amphetamine, CDP, and nicotine on drug trials only. At the beginning of the experiment, the amphetamine animals showed saline lever bias, but the other two groups did not, as they responded at the 50% level. The graph shows that for the amphetamine animals, the gradient of the curve is quite steep (possibly due to the lever bias, which meant that the accuracy level started out at below 50%) and that the animals learned relatively quickly. The animals that learnt to discriminate between saline and nicotine or CDP learnt *slightly* more slowly and to a lower level of accuracy than the amphetamine group which were trained to the highest level. The amphetamine animals were at the 100% drug lever selection level after 27 session blocks, whilst the CDP and nicotine groups were only at about 95% drug lever selection. The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent.

Hence, the graph in figure 5.6 shows that all the animals learnt to discriminate their respective training drugs from saline to a relatively high level of accuracy. Had more time been available then the CDP and nicotine animals could have been trained to a higher level of accuracy. However, due to time constraints it was necessary to start the second part of the study which involved the animals learning the caffeine discrimination.

One way of analysing rate of learning is to study sessions to criterion. For each animal, the number of training sessions (saline and training drug together) before the animal made 9 correct lever selections out of 10 consecutive sessions was measured.

The sessions to criterion data are below:

Table 5.1: The number of sessions to criterion for each animal.

Rat Number	Session No.	Rat Number	Session No.	Rat Number	Session No.
Amphetamine		CDP		Nicotine	
1	25	25	33	49	53
2	22	26	35	50	Never learnt
3	27	27	53	51	44
4	18	28	92	52	48
5	21	29	72	53	24
6	65	30	93	54	62
7	23	31	115	55	95
8	45	32	37	56	124
9	22	33	Died of unrelated illness to the drug	57	112
10	67	34	66	58	37
11	38	35	Died of unrelated illness to the drug	59	52
12	Never learnt	36	58	60	99
Amphetamine group mean ± S.E.	33.9 ± 5.1 n=11	CDP group mean ± S.E.	65.2 ± 8.3 n=10	Nico group mean ± S.E.	68.2 ± 9.6 n=11

Key to table 5.1:

The mean values do not include any rats that did not learn the discrimination and were removed from the study.

Amph = amphetamine

CDP = Chlordiazepoxide

Nico = Nicotine

Never learnt = never reached 9 out of 10 criterion

The number of sessions to criterion can be compared between the groups by studying group means. The mean value for animals learning to discriminate amphetamine (0.5 mg/kg) was 33.9 ± 5.1 (S.E.) sessions to reach the specified criterion. That of the animals learning to discriminate CDP (10 mg/kg) was 65.2 ± 8.3 (S.E.), whilst for the animals under nicotine (0.3 mg/kg) it was 68.2 ± 9.6 (S.E.) sessions to reach criterion. The session to criterion data were compared between groups by t-tests. The amphetamine and CDP groups were compared and were shown to be significantly different ($t=-3.04$, $p = 0.0083$ d.f. = 15) and the amphetamine animals learnt more quickly than the CDP groups. The amphetamine and the nicotine groups were compared ($t=-3.01$, $p = 0.0089$, d.f. = 15) and the amphetamine animals again learnt their discrimination more quickly compared to the nicotine group. The nicotine and the CDP groups were compared and were shown to be not significantly different ($t=0.22$, $p = 0.83$, d.f. = 18, NS), so the results showed no difference between the

nicotine and CDP animals. Hence, as shown in figure 5.6, the animals that were taught to discriminate amphetamine from saline learnt the discrimination more quickly than those taught to discriminate either of the other two training drugs, which learned at about the same rate.

Once the animals had learnt to discriminate their respective training drugs, then all the animals were initially taught to discriminate caffeine (10 mg/kg) from saline. The left lever remained the saline lever and the right lever remained the drug lever, but the training drug was now caffeine and not amphetamine, CDP or nicotine.

The training session time also remained at 20 minutes. The specific experimental box that the animals used during the training sessions remained constant. However, the olfactory cues of the animals in the operant chamber changed for some of the animals. This was because the order in which the animals were run had to be altered in order to allow the non-discriminating animals to be slotted into the experimental regime.

The data in all the graphs are represented as percentage drug lever selections regardless of whether the animals received saline or caffeine. In addition, as above, the data are presented as the percentage number of selections over three successive training sessions.

Comparison of saline and amphetamine with caffeine

Figure 5.7 shows the percent correct lever selections pooled over both drug and saline sessions for the "control" drug-naive animals, amphetamine "discriminators" and the amphetamine "non-discriminators" whilst being trained to discriminate caffeine (10-20 mg/kg) from saline. The "control" drug naïve animals had previously only received daily injections of saline. The amphetamine "discriminators" had previously received discrimination training between amphetamine (0.5 mg/kg) and saline, whilst the amphetamine "non-discriminators" had been previously injected with either saline or amphetamine (0.5 mg/kg).

Figure 5.7

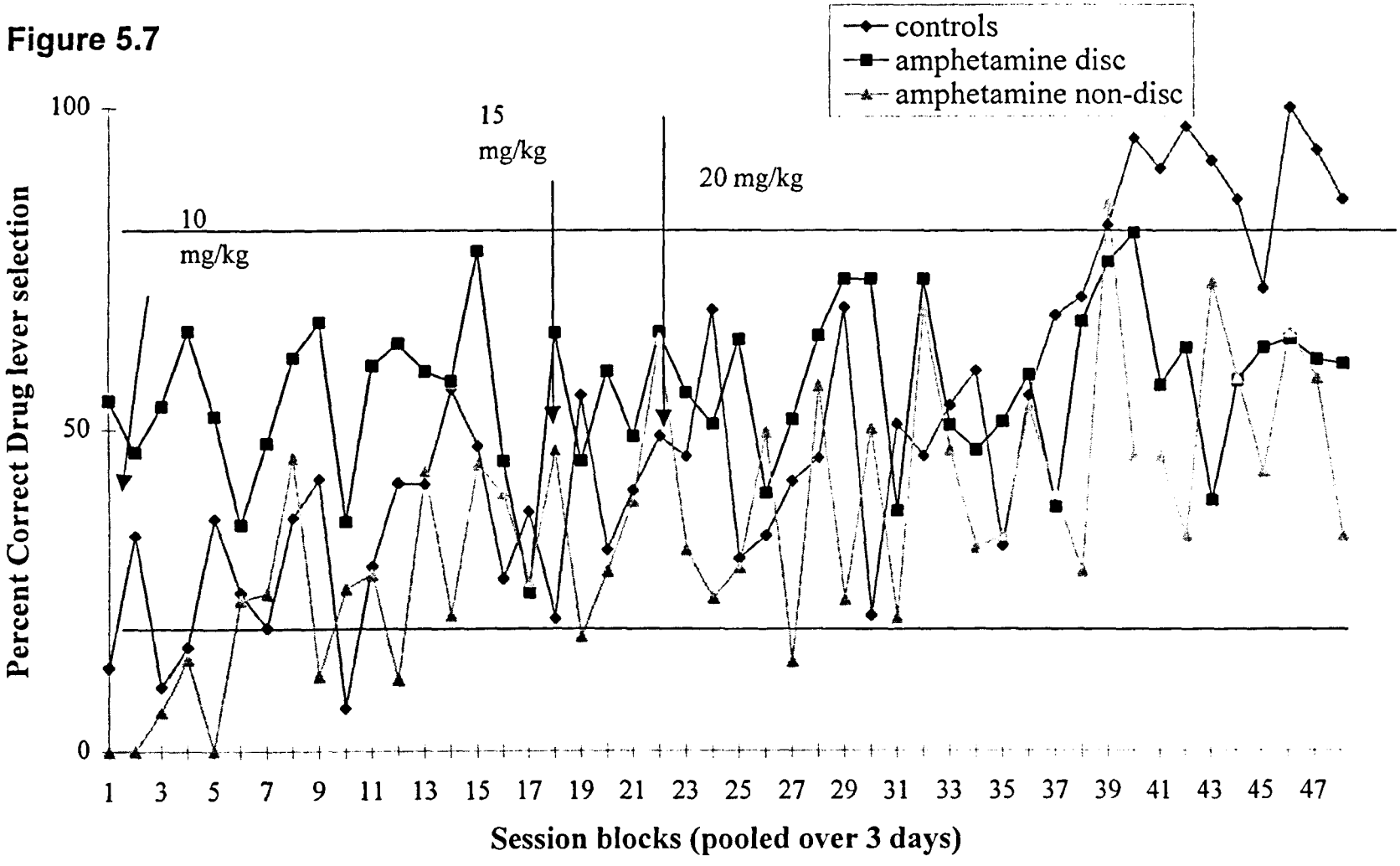
controls
amphetamine disc
amphetamine non-disc
100
50
0
10 mg/kg
15 mg/kg
20 mg/kg
Percent Correct Drug lever selection
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
Session blocks (pooled over 3 days)

Figure 5.7: The graph in this figure shows the data as percent correct lever selections for drug sessions. The data allow a comparison of both the amphetamine groups compared to the control group. The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent. The arrows on the graph indicate increases in the caffeine dose from 10 mg/kg through 15 mg/kg on session block 18 or training session 54 up to a final dose of 20 mg/kg on session block 22 or training session number 66 (see methods). Caffeine (10-20 mg/kg) and saline were administered i.p. daily (5 out of 7 days) under a pseudorandom schedule. The "control" and amphetamine "non-discriminating" animals appear initially to show saline lever bias on drug days only despite showing no lever bias during training.

Hence, Figure 5.7 shows that the "control" animals learnt to discriminate between caffeine (10 -20 mg/kg) and saline to a reasonably high level of accuracy (93%), but they failed to reach perfection on a stable basis. It took about 150 sessions for the animals to reach this stage and the whole study took about 20 months, so there was not enough time to try and see if the animals would learn the caffeine cue to a higher level of accuracy. It was believed that a dose of 10 mg/kg of caffeine would be discriminable because previous studies had shown it to be so (Mumford & Holtzman, 1991). This poor discriminability of caffeine (10 to 20 mg/kg) was not therefore predictable from previous studies. The data in figure 5.7 also shows that *initially* partial generalisation occurred between amphetamine and caffeine on drug days. This is shown by the fact that the animals responded on the drug lever on 50% of the drug trials from the *beginning* of the experiment. Therefore, the animals recognised the caffeine cue as being similar in some respect to the amphetamine cue, without any

explicit caffeine discrimination training. Despite the initial 50% partial generalisation, full (100%) drug lever selection was *never* seen in this group on *any* drug trials, despite very extended training over circa 50 session blocks which is about 150 training sessions. Figure 5.7 also shows that the amphetamine "non-discriminators" were lever biased towards the saline lever at the beginning of discrimination training, as shown by the animals responding on the saline lever irrespective of whether they had received saline or caffeine, despite showing no lever bias during training.

Hence, Figure 5.7 suggests initial partial generalisation occurred between caffeine (10 mg/kg) and amphetamine (0.5 mg/kg) and that the two amphetamine groups ultimately learnt the caffeine discrimination to a similar level of accuracy, which was about 60%. However, surprisingly the animals never learnt the caffeine cue fully on any of the drug trials. The animals had learnt the discrimination and were not responding randomly data (not shown) indicated that the animals had learnt the detect the absence of the caffeine cue, as shown by the fact that their response choice decreased to 0% drug lever selection on saline days. This indicates that the animals had learnt the caffeine cue partially but not fully.

Suggested explanations for these results are 1) the development of cross-tolerance between caffeine and amphetamine or 2) the lack of *expected* facilitation. The controls ultimately learnt the caffeine cue slightly better than the two amphetamine groups, and these two groups showed no difference with respect to caffeine relative to the controls despite extensive discrimination training. If the animals in the "non-

discriminating" group had learnt the caffeine cue to a high level of accuracy, this would have suggested that the animals in the amphetamine "discriminating" group were still under the influence of the amphetamine cue and this was hindering the acquisition of the caffeine cue. However, this was not the case.

Hence, all the groups ("controls", amphetamine "discriminators" and the "non-discriminators") learnt the caffeine discrimination to a reasonable level but never to perfection. Cross-tolerance was tentatively suggested as one explanation for the results observed after amphetamine treatment and then caffeine discrimination training. Cross-tolerance is defined as a reduction in the effect of a drug induced by chronic treatment with another drug (Le & Khanna, 1989). Both the amphetamine groups received chronic treatment with amphetamine prior to caffeine experience. The amphetamine "discriminators" received chronic amphetamine and discrimination training, whilst the amphetamine "non-discriminators" received chronic amphetamine experience only. The amphetamine "discriminators" only showed initial partial generalisation to caffeine (see Figure 5.7, trials 1-10), probably because both caffeine and amphetamine have stimulant properties, and this finding is in agreement with other studies showing partial generalisation between the drugs (Holloway, Michaelis & Huerta, 1985). However, the most likely explanation for the results the lack of expected facilitation between the caffeine and amphetamine cues. A *very* unusual finding was that despite initial partial generalisation to caffeine, the amphetamine "discriminators" never learnt the caffeine cue to as high a level as controls. The amphetamine "non-discriminators" at the start of the experiment were

lever biased, but after about 9 training session, the animals started to respond on both levers. The amphetamine "non-discriminators" slowly reached about 60% drug lever selection, but never increased their accuracy beyond that. However, both amphetamine groups, after extensive training were consistently above chance (50%), hence they had learned the caffeine cue partially.

Therefore, the amphetamine trained rats showed partial generalisation to the caffeine cue at 10 mg/kg however, the animals did not show the *expected* facilitation of acquisition of the caffeine cue.

Comparison of saline and CDP with caffeine

The next compound studied for the effects of discrimination training and drug experience was CDP. The animals in this group had received exactly the same treatment as the amphetamine animals except that the drug used was CDP and not amphetamine.

Figure 5.8 shows the percent correct lever selections pooled over both drug and saline sessions for the "control" drug-naive animals, CDP "discriminators" and the CDP "non-discriminators" whilst being trained to discriminate caffeine (10-20 mg/kg) from saline. The "control" drug naïve animals had previously only received daily injections of saline. The CDP "discriminators" had previously received discrimination training between CDP (10 mg/kg) and saline, whilst the CDP "non-discriminators" had been previously injected with either saline or CDP (10 mg/kg).

Figure 5.8

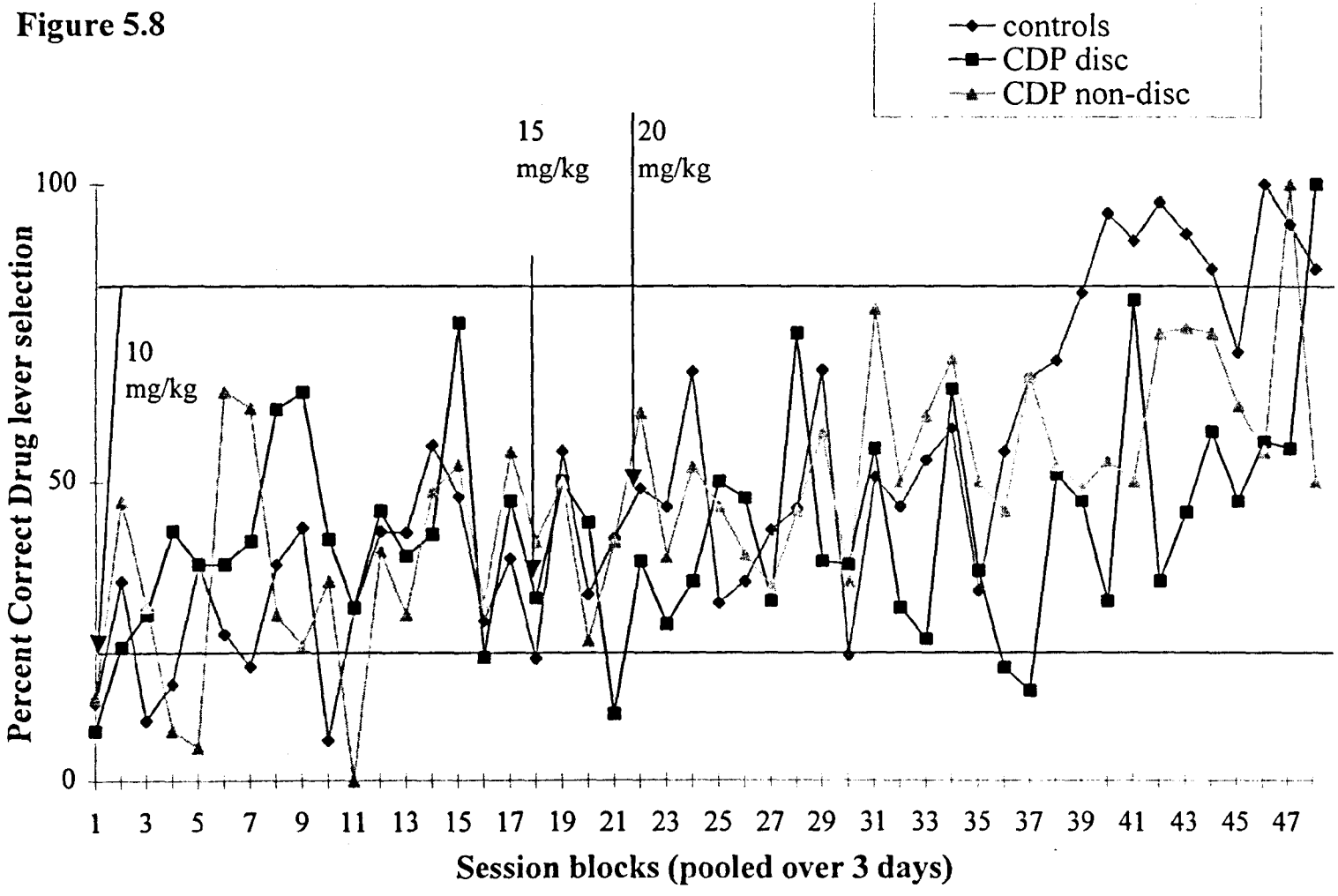
controls
CDP disc
CDP non-disc
15
mg/kg
20
mg/kg
10
mg/kg
100
50
0
Percent Correct Drug lever selection
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
Session blocks (pooled over 3 days)

Figure 5.8: The graph compares the two CDP groups and the "controls" under the caffeine cue. The arrows indicate where the dose of caffeine was increased from 10 mg/kg through 15 mg/kg up to 20 mg/kg. The animals were initially injected with caffeine (10-20 mg/kg) or saline i.p. daily (5 out of 7 days) under a pseudorandom schedule. The graph shows clearly that the animals in all groups learnt the caffeine and saline cue to the same extent, and although they all learnt the caffeine versus saline cue to a relatively high level of accuracy, none of them learnt to perfection, even after very extended training. The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent. The group size for the CDP "discriminators" decreased from 12 at the beginning of the experiment to 10, because two animals became ill. The CDP "non-discriminators" group size was decreased to 11 because one of the animals became ill and had to be dropped from the study.

The data show that there was no initial generalisation between caffeine and CDP at all, as shown by the fact that on caffeine days *all* the selections were on the saline lever. The animals did not recognise the caffeine cue as being CDP-like and therefore chose the saline lever on drug (caffeine) days, but instead they responded at chance levels (circa 50%).. The graph in Figure 5.8 shows the CDP "discriminators" learning the caffeine cue and that excluding the very last session block the animals failed to learn the cue to much above the 60% level. In the very last session block, the animals acquired the caffeine cue to 100%.

The results show that the CDP "non-discriminators" were initially lever biased for the left lever despite showing no lever bias during training. The results show that the CDP "non-discriminators" failed to learn the caffeine cue to perfection, except on one session block (number 46) and that overall they learnt the caffeine cue to about 70% accuracy.

All three groups of animals acquired the caffeine cue at the same rate and they reached the same level of accuracy. Thus, the results show that a prior history of CDP had no effect on the animals ability to acquire the caffeine cue compared to the "controls". The lack of generalisation between CDP and caffeine was expected and in agreement with previous studies (Holloway, Modrow & Michaelis, 1985).

By the end of the experiment all the groups had reached 100% on at least *one* session block. Nevertheless, the data also show that although the animals learnt the caffeine cue, it was not very stable or reliable, even after very extensive training.

The data show that CDP trained rats did not generalise to the caffeine cue at 10 mg/kg. Also the data show that the animals did not show the *expected* retardation of acquisition of the caffeine cue after CDP discrimination training. Retardation was expected because initially there was no generalisation between CDP and caffeine, so all the lever selections were on the saline lever.

The last compound studied for its effects on caffeine was nicotine and the following results were found.

Figure 5.9 shows the percent correct lever selections pooled over both drug and saline sessions for the "control" drug-naive animals, nicotine "discriminators" and the nicotine "non-discriminators" whilst being trained to discriminate caffeine (10-20 mg/kg) from saline. The "control" drug naïve animals had previously only received daily injections of saline. The nicotine "discriminators" had previously received discrimination training between nicotine (0.3 mg/kg) and saline, whilst the nicotine "non-discriminators" had been previously injected with either saline or nicotine (0.3 mg/kg).

Figure 5.9

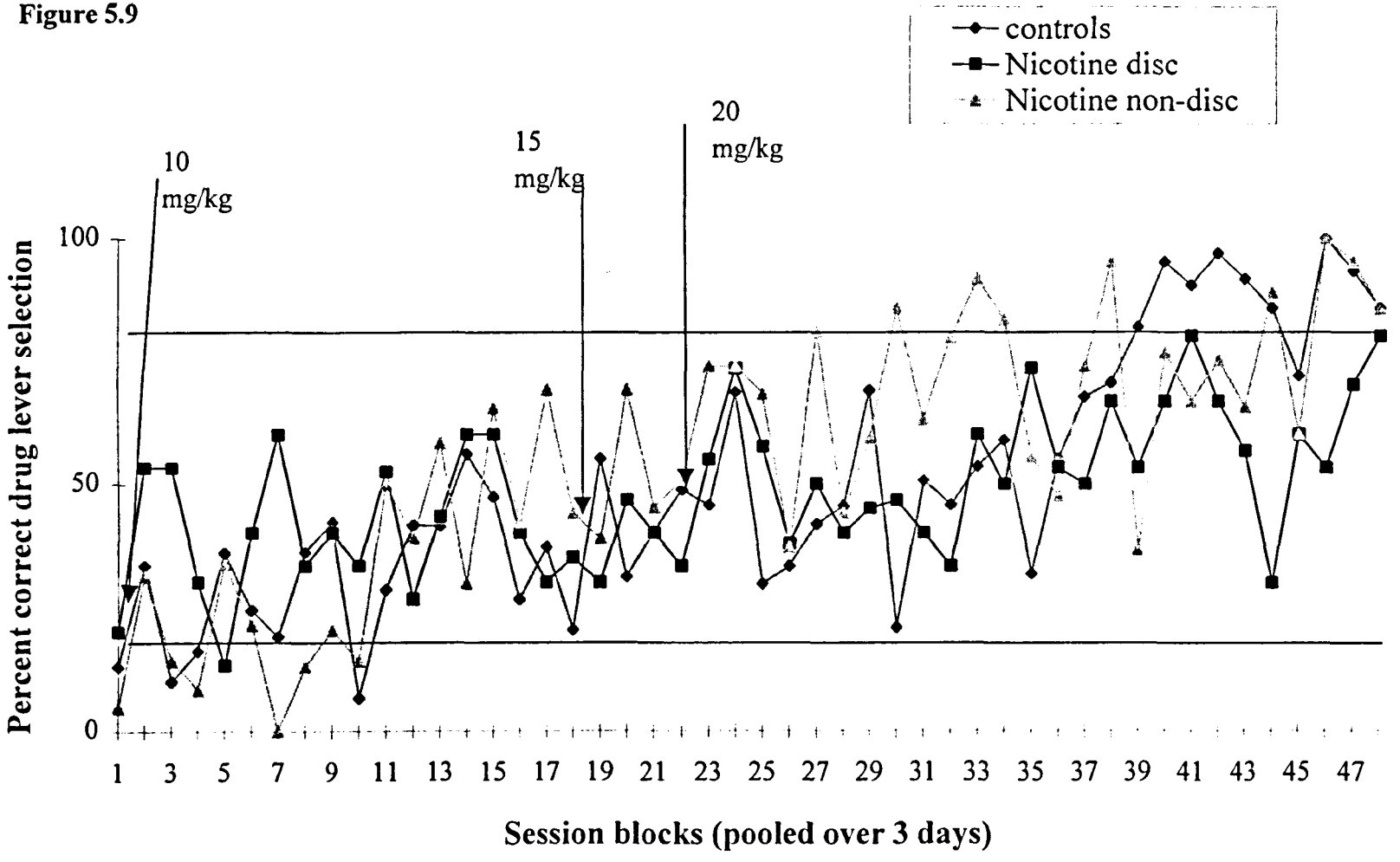
controls
Nicotine disc
Nicotine non-disc
10 mg/kg
15 mg/kg
20 mg/kg
Percent correct drug lever selection
100
50
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
Session blocks (pooled over 3 days)

Figure 5.9: The graph shows the data analysed by percent correct lever selections regardless of whether the animals received drug or saline. The arrows on the graph indicate at which session blocks the dose of caffeine was increased from 10 mg/kg through 15 mg/kg up to 20 mg/kg. Caffeine (10-20 mg/kg) or saline was administered i.p. daily (5 out of 7 days) under a pseudorandom schedule. The nicotine "discriminators" groups size was decreased because one of the animals failed to reach the specified criterion of the nicotine discrimination and consequently was dropped. The two horizontal lines across the graph represent percentage drug lever selection at 20 and 80 percent.

The data in figure 5.9 show that the "controls" and the nicotine "non-discriminators" were initially lever biased (for the left lever) but only on drug days, despite showing no lever bias during training.

At the start of the training caffeine and nicotine showed no partial generalisation on initial caffeine trials, as the nicotine "discriminators" chose the saline lever when under caffeine and there was a low level (circa 20% drug selection on drug trials). Also data (not shown) shows that on vehicle days there was ultimately 100% vehicle lever selection.

The graph in figure 5.9 shows that the nicotine "non-discriminators" acquired the caffeine cue to a high level of accuracy and even reached perfection on one session block. However, caffeine cue acquisition was not stable or reliable and the accuracy level varied over session blocks.

The graph shows that all three groups learnt the caffeine versus saline discrimination cue at a similar rate. The data also show that by the end of the experiment all three groups had learnt the caffeine cue to the same "reasonably" high level. The results also show that the overall trend was of no difference between the three groups, and that by the end of the study all three groups had acquired the caffeine cue to a similar level.

Therefore, as with CDP, nicotine trained rats did not generalise to the caffeine cue at the initial dose of caffeine used. Also, like the CDP animals, the nicotine animals did not show the *expected retardation* of acquisition of the caffeine cue compared to the controls. Retardation was expected because again nicotine did not initially generalise with caffeine, so all the initial lever selections were on the saline lever.

In conclusion, the data show that all the groups, except the controls, failed to learn the discrimination of the caffeine cue to near perfection. Conclusions for each individual training drug were attempted by comparing the results for each drug with the control group.

Therefore, the following overall conclusions were reached:

- The amphetamine animals showed partial generalisation to the caffeine cue, but did not show the expected facilitation of acquisition of the caffeine cue compared to the controls.

- The CDP and nicotine groups initially showed no generalisation to the caffeine cue at 10 mg/kg. However, they did not show the expected retardation of acquisition of the caffeine cue.

Chapter 6.0

Discussion for Study I

The aim of the experiment was to investigate if previous drug history had an effect on future drug discriminations. It was believed that subjecting animals to initial drugs with known, defined discriminative cues, might affect their subsequent responses to a different possibly relatively non-specific drug cue. The animals might respond in a different way to the caffeine cue depending upon the drug initially administered.

It might have been possible to observe differences between the "discriminating groups" and the "controls" and between the "discriminators" and the "non-discriminators" of each drug group. In order to investigate whether drug experience itself or drug discrimination history had an affect on the acquisition of a subsequent drug cue, the groups were compared and it had been planned that generalization curves would be compared when the groups had been trained to discriminate caffeine.

Amphetamine was chosen because it has a "stimulant cue", as does caffeine (Griffiths & Mumford, 1996). Previous amphetamine discrimination might have been expected to facilitate the acquisition of the caffeine cue compared to the "controls". This is because the amphetamine "discriminators" had already learnt to discriminate one stimulant drug from another. It was believed that due to the predicted partial generalisation between caffeine and amphetamine, they would acquire the caffeine cue quickly and would change cues quickly. Another advantage the amphetamine "discriminators" had over the other two groups was that they had tolerated out the rate-suppressant effects experienced after the administration of a stimulant. It was possible that the amphetamine "non-discriminators" would learn the caffeine cue

more quickly than the "controls" but slower than the amphetamine "discriminators". This was because although the animals had received amphetamine injections, they had no more experience than the "controls" in a drug discrimination paradigm. Therefore, the animals in the amphetamine "non-discriminating" group would have to learn caffeine versus saline in the drug discrimination paradigm as would the "controls". However, it was possible that because the amphetamine "non-discriminators" had already experienced a stimulant that the initial rate-suppressant effects would tolerate out more quickly than in the "controls". The "controls" might learn the slowest because they had to tolerate out the effects of caffeine and also start to learn the caffeine discrimination as well.

CDP was chosen because it is a sedative/anxiolytic, which acts at benzodiazepine receptors (Gauvin, Pierce & Holloway, 1994). As CDP is a sedative/anxiolytic, it was hypothesized that training rats to discriminate CDP might retard the acquisition of the caffeine cue due to the predicted lack of generalization to caffeine. It was believed that the "controls" would acquire the caffeine cue more quickly than the CDP "non-discriminators" who would learn faster than the "discriminators". The "controls" were believed to learn faster than the CDP "non-discriminators" because they had experience of CDP (a sedative), and would need time to readjust to the effects of a stimulant. This was because having learnt the CDP cue, the caffeine cue would be much harder to learn, hence retarding the acquisition of the caffeine cue. There would be no generalization between caffeine and CDP so, the administration of caffeine after CDP would have caused the CDP "discriminators" to select the vehicle lever. Therefore, due to the lack of drug lever selection and pressing and the

lack of generalization between caffeine and CDP, it could have been many sessions before the CDP "discriminators" responded on the drug lever after the administration of caffeine. Meanwhile, the CDP "non-discriminators" had received no discrimination training so it was unsure what effect caffeine discrimination would have on these animals' lever selections. It was predicted that if the animals showed generalization between the initial training drug cue and the caffeine cue, then the caffeine cue would be learnt quickly via facilitation and if there was no generalization between the initial training drug cue and the caffeine cue, then retardation of acquisition of the caffeine cue would be observed.

Nicotine was chosen because it acts on nicotinic receptors (Balfour, 1994). So animals trained on nicotine were being administered a drug that acted on different receptors to the other two compounds chosen. It was possible that because the caffeine cue used was relatively non-specific, the animals might respond differently to the CDP and amphetamine trained animals after the administration of caffeine. However, it was not known for sure what the effects of nicotine, either experience or drug discrimination history would have on a future discrimination of caffeine. It was possible that the nicotine "discriminators" would learn faster than the controls because they had already learnt one cue and might show generalization to the caffeine cue which should facilitate acquisition of the caffeine cue. Nevertheless, it was possible that the nicotine "discriminators" would learn the caffeine cue slower than the "controls" because they had to change the cue they attended to and that would retard the acquisition of the caffeine cue if no generalization occurred. The nicotine "non-discriminators" were used to check if any differences between the

nicotine "discriminators" and "controls" were due to discrimination training or drug experience.

Each of the three initial drug groups acted upon a different set of receptors. It was possible that the three discrimination groups would not only respond differently to the controls, but also that the "discriminators" and "non-discriminators" would behave differently within the groups. It was possible that differences in the effects of discrimination training and simple drug experience would be observed. One possible example was that the amphetamine "discriminators" would learn the caffeine cue quicker than the amphetamine "non-discriminators" because the amphetamine "discriminators" might show generalization (as indicated by the animals responding at 50% or more on the drug lever when administered caffeine initially) to the caffeine cue and hence facilitate acquisition. Whilst, the amphetamine "non-discriminators" might not show the generalization (as indicated by the animals responding at less than 50% on the drug lever when administered caffeine initially) and hence would not show the facilitation of acquisition of the caffeine cue, so the amphetamine "non-discriminators" and the "controls" would learn at the same rate. This would have shown that amphetamine discrimination training and not amphetamine drug experience had altered the acquisition rate of the caffeine cue. Therefore, by having the "discriminator" and "non-discriminator" groups, it would allow the investigation of whether the effects observed within the drug groups were due to drug discrimination history or simply drug experience.

In this study, 10 mg/kg to 20 mg/kg of caffeine was clearly not readily discriminable but this was *not* predicted. The animals took many sessions to learn the caffeine cue and only the controls learnt the cue to a very high level of accuracy. The results indicated that the animals were probably not going to learn the caffeine cue at 10 mg/kg, so the dose was initially increased to 15 mg/kg. The animals still appeared to be failing to learn the caffeine cue, so eventually the dose was increased to 20 mg/kg. Even at 20 mg/kg some of the animals only learnt the cue to slightly above chance (60%) levels e.g. the amphetamine groups. Therefore, in these animals 10 to 20 mg/kg of caffeine was poorly discriminable, but this does not readily accord with other studies that have shown reasonably rapid discrimination of caffeine at 10 to 20 mg/kg (Griffiths & Mumford, 1996; Mumford & Holtzman, 1991).

A study (McMillan *et al*, 1996) showed that initial drug stimuli can continue to have some degree of control over behaviour after future drug discrimination training. This indicates that stimuli from previous drug discriminations can combine to produce discriminative stimulus effects. The main difference between the study of McMillan *et al* (1996) and this study is that McMillan *et al* (1996) used pigeons. The advantage with pigeons is that they recognise colour, so the paradigm used by McMillan *et al* was colour dependent (which was independent of location) and not lever position dependent. This might explain why the McMillan *et al* (1996) results showed previous drug discriminations retain their stimulus control. Whilst, in this study we used rats and we kept the same lever for responses under drug after both initial training drugs and caffeine. So our paradigm was position dependent and not colour dependent. This could explain the difference between the two studies, in that

McMillan *et al* (1996) trained animals to discriminate several drugs and we failed to do so. However, it was not expected that the choice of animals used in the study would have an affect of whether drug history effects were observed or not. So, it is more likely that the main problem in this study is the poor discriminability of caffeine in these animals, rather than the fact that we used rats and not pigeons like McMillan *et al.*

The results from this study (fig 5.6) show that amphetamine (0.5 mg/kg), CDP (10 mg/kg) and nicotine (0.3 mg/kg) were all discriminable from saline. All three drugs were learnt at different rates, but to a high level of accuracy. Figure 5.7 shows that the controls learnt the caffeine cue very slowly, but to a high accuracy level circa 90%. The other groups of animals were compared to the controls to investigate differences in acquisition rate, final level of accuracy and the presence of any initial partial generalisation. The *initial* aim of this study was to see if different generalisation patterns were observed with caffeine (and other drugs) in animals with different drug histories. The dose of caffeine needed to produce full generalisation may differ, as may whether full generalisation to other stimulants known to generalise to caffeine would be observed. It would also have been interesting to carry out generalisation tests with compounds that show generalisation to CDP, amphetamine and nicotine and observe those effects. However, this stage of the study was never reached due to the caffeine cue not being learnt despite extensive training.

The results (Figure 5.7) suggest, however, that the amphetamine and caffeine cues partially generalised. The partial generalisation was observed when the animals were initially administered caffeine and they responded at about 50% all of the time on the drug lever when administered caffeine, but only about 20% of the time on the drug lever when administered saline. This is indicated by the "discriminators" responding under caffeine at about the 50% level, whilst the "controls" and amphetamine "non-discriminators" responded under caffeine at much lower levels. This ties in with other studies that have reported partial generalisation between caffeine and amphetamine (Mumford & Holtzman, 1991).

The CDP and nicotine groups showed no partial generalization to caffeine (CDP Figure 5.8; nicotine Figure 5.9). However no partial generalization was expected between nicotine or CDP and caffeine (Gauvin, Pierce & Holloway, 1994). Thus, the animals were expected to be retarded in the acquisition of the caffeine cue compared to the controls.

One suggestion for the very slow acquisition of the caffeine cue in all groups, is that the initial dose of 10 mg/kg caffeine was too low for the nicotine and CDP animals to discriminate. Therefore, as far as these animals were concerned they were effectively being trained to discriminate saline versus saline due to the minimal discriminability of caffeine at 10 mg/kg. So the animals were effectively being reinforced under "saline" for responding on both levers. Hence, the effective saline versus saline discrimination training may have "extinguished" any previously learnt drug discriminations. When the dose of caffeine was increased from 10 mg/kg to 15

mg/kg the animals may have only been receiving a barely perceivable drug cue, which was not stable over days. It may have only been when the dose was increased to 20 mg/kg that the animals received a discriminable dose of caffeine every day.

As stated above with discrimination training at 10 mg/kg of caffeine *possibly* acting as a saline versus saline discrimination, this may have "extinguished" any effect of previous drug discrimination training. If this were correct, then the "discriminators" would effectively have unlearnt their initial discrimination. Thus, this may prevent any effects of previous drug history being observed. Effectively all the groups would have the same previous drug history as the "controls". Having "unlearnt" the CDP cue could be one explanation as to why the CDP discriminative group showed no expected *retardation* in the acquisition of the caffeine cue compared to the controls. It was expected that CDP discrimination training would retard the acquisition of of the caffeine cue because the animals were having to switch from a stimulant cue to a stimulant cue, unlike the amphetamine discriminators which changes from one stimulant cue to another cue. The same explanation could be true for the nicotine groups as well explaining why no difference was observed between the nicotine discriminative group and the controls. It was possible that the nicotine discriminators would show retardation of the caffeine cue because again the animals were switching from a nicotine cue to a stimulant cue. Both groups showed no partial generalization to the caffeine cue and neither group showed the predicted retardation of acquisition of the caffeine cue.

However, the amphetamine "discriminators" showed initial partial generalization to the caffeine cue and it was very surprising that these animals never learnt the caffeine cue fully because of their initial partial generalization. This could be possibly due to the fact that prolonged chronic amphetamine treatment induced not only partial generalization to the caffeine cue but also some cross-tolerance to the drug. The effect of cross-tolerance could have confounded the results. It was assumed, that because of the partial generalization observed initially, that the amphetamine discriminators would learn the caffeine cue very quickly, However, cross-tolerance between amphetamine and caffeine may have meant that although the animals could discriminate 10 mg/kg caffeine, it was effectively a functionally lower dose. Prior amphetamine trained rats generalized partially to the caffeine cue, which we initially took to mean that 10 mg/kg of caffeine was discriminable *in all groups*. The amphetamine group could clearly discriminate 10 mg/kg, whilst the other groups perhaps could not because the amphetamine groups were already "attending" to a weak stimulant cue, and so responded to a lower dose than was needed for discrimination training in the other groups. In addition, cross-tolerance may have caused the animals not to learn the caffeine cue to a high level because the doses being administered were functionally lower than they were in the controls because of caffeine/amphetamine cross-tolerance. Hence, the amphetamine animals may have needed a much higher dose of caffeine to have learnt the caffeine discriminative cue. Therefore, the initial partial generalization possibly never increased because the doses of caffeine administered were too low due to cross-tolerance between caffeine and amphetamine. It was inferred initially that because the amphetamine groups could discriminate the caffeine cue, the other groups could as well. However, it is

now clear that inferences about caffeine's discriminability drawn from the amphetamine experienced groups probably can not be transferred to rats pre-trained under other drugs, and thus that caffeine was probably not discriminable in nicotine and CDP trained rats.

However, it was only after 18 months that all these complications became obvious. A higher dose of caffeine (e.g. 32 mg/kg) would almost definitely have been more discriminable, as shown in other studies (Griffiths & Mumford, 1996). However, the caffeine cue would have been specifically methylxanthine and not a general stimulant cue. So, although a higher caffeine dose meant that the cue would have probably been learnt more rapidly, there also would probably have been no effects of previous drug history.

It has been suggested that the initial (10 mg/kg) dose of caffeine was not discriminable at all in the CDP or nicotine groups, so the "discriminating" animals were switched from a drug, (CDP or nicotine), to a saline versus no drug discrimination (10 mg/kg caffeine) and then back to a drug (20 mg/kg caffeine) versus saline discrimination. Therefore, a higher dose of caffeine could have prevented the middle stage, *possibly* providing clearer evidence of behavioural history having some effect on subsequent drug discriminations. Hence, a fine line existed between a dose of caffeine that is sufficient to be discriminable and one that is a very specific cue for caffeine.

Therefore, in this experiment, under the experimental conditions used, there was no effect of drug history (i.e. facilitation or retardation) because the CDP and nicotine groups possibly had their previous drug history "extinguished" and the amphetamine groups showed no effect for reasons not clearly understood, but possibly due to the development of cross-tolerance during amphetamine discrimination training.

Chapter 7.0

Study II

7.1 Why changes studies

The results of the first study did not yield satisfactory results, so despite the very considerable amount of time spent on training the animals and carrying out the study, it was decided to change the line of research. This was because previous drug history had been shown to have no clear effect with caffeine and I did not want to risk the same results with another drug in a very time consuming study. Therefore, it was decided to change from 'drug history' to study the effects of 'pharmacological history'. We also decided to move away from caffeine in this study because it was shown not to be highly discriminable in the previous study and we did not want to encounter further difficulties in later studies with a poorly discriminable drug. Clozapine was chosen as the drug for the next study as it has been studied extensively in this laboratory. Considerable data on the effects and properties of clozapine was readily available in the laboratory. The 'pharmacological history' of clozapine was to be studied by investigating clozapine effects in chronic studies. The effects of chronic clozapine have not been widely studied, which is surprising because clozapine in the clinic is administered chronically. Studies in the clinic have shown that the side effects of clozapine tolerate out, however patients become more sensitized to the therapeutic effects of clozapine the longer that it is administered (Hu *et al*, 1999). Thus, it was decided to study clozapine in a tolerance paradigm.

7.2 Why study tolerance to clozapine?

Clozapine is a widely studied drug, but most of the studies carried out are on clozapine's acute effects. The effects of clozapine are known to take weeks or months to reach their maximal level in patients (Freed, 1988), but it is unknown why

it takes this long for clozapine to have an effect and very few studies have been carried out to investigate this. It has been observed in the clinic that once clozapine has an effect then as the administration of clozapine continues the patients become sensitised to its effects (Hu, Malhotkra & Pickar, 1999). This is an unusual effect of clozapine, because most drugs administered chronically eventually need higher doses to maintain the same effects e.g. Heroin. Whilst sensitisation occurs to the therapeutic effects of clozapine, then tolerance occurs to some of the side effects of clozapine e.g. sedation (Das & Fowler, 1995). It is unknown how this can happen but is assumed that the two effects are due to two different mechanisms of action.

7.3 Aims

The aim of this series of experiments was to investigate whether or not tolerance could be induced to the clozapine cue and then cross-tolerance indicted by other drugs. It was hoped that by studying clozapine tolerance assessed via a drug discrimination paradigm, it would be possible to investigate whether tolerance was induced to the clozapine cue in animals as well as in humans. Tolerance has been shown to occur to several effects in humans, in particular sedation (Das & Foweler, 1995). Studies have shown that animals tolerate to the rate suppressive effects of clozapine in a drug discrimination paradigm (Goudie & Taylor, 1998). Therefore, the aim of this study was to investigate the effects of chronically administered clozapine and to try and find out if other compounds, both clozapine-like and others, could produce the same effects i.e. olanzapine, JL13. The aim of this study was not to investigate the precise mechanisms by which chronically clozapine produces its effects. This is because the time length for the study was insufficient to carry out

such a detailed analysis. Instead, the aim of the study was to investigate if tolerance could be induced to the discriminative cue of clozapine, and if so, could any other compounds produce the same effect.

Chapter 8.0

Tolerance

8.1 Tolerance defined

Tolerance is defined as "a diminution of the initial effect of a drug as a result of repeated administration" (Young & Sannerud, 1989). As a subject experiences the effects of drug for an increasing number of times these effects decrease and require increasing amounts of the drug to be reinstated.

8.2 Cross-tolerance

Cross-tolerance occurs when treatment with one drug produces tolerance to a second drug, usually but not always from the same pharmacological class, and not necessarily with the same mechanism of action (Le & Khanna, 1989). Cross-tolerance can occur between drugs from different pharmacological classes that have very similar actions e.g. alcohol and sedative-hypnotic/anaesthetics (Kalant, 1989).

8.3 Types of tolerance

There are several types of tolerance that can occur depending on the type of experiment, the conditions and the drugs being used.

Innate tolerance – This is initial tolerance and is the difference between individuals at the start of an experiment. Any tolerance or sensitisation measured at this point cannot be reversed. This is because inherent in the state of the animals to begin with and is not due to independent variables that can be altered during the course of an experiment (Blackman, 1992).

Chronic tolerance – This is the gradual decrease in response over a few days, weeks or maybe months of drug treatment. The animals are tested after a period of administration of the drug and the response measured. As the drug is administered chronically tolerance develops and the animals' response decreases (Kalant, 1989).

Dispositional tolerance - is a decrease in the effect of the drug due to a decrease in the concentration or duration of action of the drug at the target tissue (Le & Khanna, 1989). After administration the duration of action and concentration of the drug are dependent upon absorption, distribution, metabolism, biotransformation and excretion. After chronic treatment, these processes may change and this in turn will alter the duration of action and the concentration of the drug (Le & Khanna, 1989).

Functional tolerance - is a decrease in the effect of the drug due to changes in sensitivity of the target tissue. Unlike dispositional tolerance, the concentration of the drug at the target site does not change, but instead the responsiveness of the target cell is altered (Le & Khanna, 1989).

Behavioural/Instrumental tolerance – is tolerance is a direct consequence of the efforts of the animals to regain the rewards lost as a result of drug effects. When the drug effect interferes with the gain of reinforcement, tolerance will develop. Conversely, if the drug has no negative effect or if it increases reinforcement, then no tolerance will occur. So loss of reward plays a major role in the development of

tolerance (Kalant, 1989). One example is that tolerance only occurred to amphetamine-induced stereotypy when it interfered with feeding (Kalant, 1989).

8.4 Tolerance to drug cues

Tolerance occurs to drug cues with a variety of drugs from many different classes. Drugs that are used in drug discrimination initially often produce rate-decreasing effects that are tolerated out within a few days to weeks. Tolerance to the rate suppressant effect is observed in animals learning to discriminate the drug stimulus from saline. Tolerance to drug cues is observed with many drugs including cocaine, amphetamine, morphine (Barrett, White & Caul, 1992).

Drug discrimination work has shown that once subjects have learnt a stable discrimination over a period of time it is maintained although tolerance has been shown to develop to other effects of the drug. However, if the training dose is gradually increased, tolerance does develop and lower doses of the drug lose the ability to produce a discriminative stimulus (Young & Sannerud, 1989). If discrimination training is stopped and chronic treatment carried out with a higher drug dose, re-testing at the end of the chronic treatment shows that the generalisation gradient has been shifted to the right and that tolerance has occurred (Kalant, 1989).

The development of tolerance to drug discrimination is a dynamic process that is shaped by interactions of dose, chronicity of drug treatment and the behavioural conditions under which the drug is administered, as well as the individual's own

history of pharmacological stimulus control (Young & Sannerud, 1992). It has been shown that the dose required to induce stimulus control is altered by several factors including the training dose and the subject's innate tolerance (Young & Sannerud, 1992). As tolerance occurs to the discriminative effects, then it also occurs to other effects of the training drug at the specific dose being used.

One method of demonstrating tolerance to the discriminative stimulus effects of a drug involves chronic treatment with a drug or supplemental drug dosing. The animals are trained to respond in the presence of a drug discriminative stimulus, i.e. drug versus saline training to a high level. Then the training drug is used to carry out a dose/effect curve (DEC) from 100% to 10-20% drug lever responding. The second phase involves chronic treatment e.g. twice-daily injections with a drug. At the end of chronic drug treatment, a second dose/effect curve is determined and the two DEC's compared for any shift (Young & Sannerud, 1989). Many drugs for example cocaine (Thabit, 1993), morphine (Barrett, White & Caul, 1992) studied in this way have shown tolerance or a shift in dose/response curve to the right indicating tolerance. Tolerance has developed to the discriminative stimulus of the drug during chronic drug treatment. Studies have shown that if discriminative training is not suspended during chronic treatment, then no effect of tolerance is observed. This is because during the chronic treatment phase the animals are injected with higher doses of the drug and learn to tolerate out to the drug's effects and as a consequence require more drug to produce the same internal effects. However, if discrimination training occurred as well, then the animals would tolerate out to the effects of the

higher doses of drug but still produce the same responses in the discrimination paradigm as before because they had been rewarded to do so. So, when chronic treatment is stopped, the animals show tolerance by the dose effect curve being shifted to the right compared to before chronic drug treatment (Young & Sannerud, 1989). Therefore, the development of tolerance to a drug's discriminative effects reflects an interaction between the supplemental drug treatment and the training conditions (Young & Sannerud, 1989).

8.5 Conclusion

No one explanation can be used to account fully for all cases of tolerance. There are many types and frequently two or more types that act together to produce the full effect.

Chapter 9.0

A Review of Clozapine

9.1 Introduction

Clozapine is a new antipsychotic that has been used in the clinic to treat schizophrenia. It is classed as a novel atypical antipsychotic (APD) but there is a lot of controversy about the criteria that should be used to characterize a neuroleptic as "atypical" or "novel" (Meltzer, 1996). One suggestion for the criteria for an "atypical" antipsychotics is their lack of extrapyramidal side effects (EPS). Clozapine has been shown to induce fewer EPS than typical APD's and it has been shown to be good at treating some schizophrenic patients who are resistant to normal or typical antipsychotics (Ashby & Wang, 1996).

9.2 Action of clozapine

Clozapine has reduced liability to induce tardive dyskinesia (Factor & Friedman, 1997), it also alleviates negative symptoms and cognitive defects to a much greater degree than typical antipsychotics (Factor & Friedman, 1997).

However, clozapine can induce fatal agranulocytosis. Thus, research has taken place to try and replace clozapine with compounds that have the same therapeutic benefits but do not induce fatal agranulocytosis (Goudie & Taylor, 1998).

It has been shown that both typical and atypical antipsychotics do not reach their full potential in schizophrenic patients until two or 3 weeks more of administration (Freed, 1988). When compliance with drug taking stops, there is no direct correlation between the plasma level and time to relapse for clozapine. Some patients relapse

within one week of withdrawal from medication and other patients do not show any changes in relapse for up to 2 weeks (Sams-Dodd, 1988).

9.3 Pharmacology of clozapine

Clozapine has a high affinity for many receptors and the precise ones that are needed to induce its unique pharmacological profile are unknown as of yet. Clozapine acts upon many receptors including; D_1, D_2, 5-HT_{2A}, alpha-NA, alpha-NA_2, H_1 and M_1 amongst others (Meltzer, 1994).

Dopamine receptors

Clozapine has a higher affinity for D_4 receptors than D_2 receptors, whilst typical antipsychotics do not have the same D_4/D_2 ratio (Meltzer, 1994), atypical neuroleptics have a lower affinity for D_2 receptors compared to typical neuroleptics. The action of clozapine on D_4 receptors could account for its lower frequency of EPS and its effects on negative symptoms, but it is unclear if D_4 receptors alone hold the answer to how clozapine works (Meltzer, 1994). It has been shown that D_4 receptor affinity alone cannot predict an antipsychotic action (Ashby & Wang, 1996).

5-HT receptors

Clozapine is a partial agonist at 5-HT_{1A} receptors and they may play a role in clozapine's unique action (Ashby & Wang, 1996).

Systemic administration of clozapine leads to alterations of 5-HT_{2C} receptors. It has been shown that chronic clozapine but not haloperidol causes a significant decrease

in the number of 5-HT_{2C} receptors. This interaction may partially explain clozapine's atypical profile (Ashby & Wang, 1996).

Clozapine acts at many 5-HT receptors and produces potent 5-HT_{2A} receptor blockade. It has been suggested that 5-HT_{2A} receptor antagonism may be sufficient to produce a novel antipsychotic drug (Meltzer, 1994). This may play a critical role in mediating an atypical profile, but is unlikely to be the sole receptor involved (Ashby & Wang, 1996). It has been shown that there is a significant difference in the 5-HT_{2A}/D_2 receptor ratio between typical and atypical antipsychotics (Meltzer *et al*, 1989).

Clozapine antagonises 5-HT_3 receptors but it has been suggested that this is not needed for a compound to show antipsychotic action. Clozapine has been shown not to substitute for 5-HT_3 antagonists in the drug discrimination paradigm (Wiley & Porter, 1992).

Clozapine has a high affinity of binding for 5- HT_6 and $_7$ receptors and whilst this could contribute to clozapine's pharmacology, it is not necessarily enough to explain clozapine's unique profile (Ashby & Wang, 1996).

GABA receptors

Clozapine appears to be an inhibitor for GABA uptake in synaptsomes (Fjalland 1978 as reported in Ashby & Wang, 1996).

Summary of clozapine's actions at different receptors

Clozapine acts upon many receptors and sub-types. It is possible that a combination of all of the aforementioned or some of these receptors contributes to the antipsychotic profile of both clozapine and olanzapine, and related drugs.

9.4 Drug discrimination of clozapine

Clozapine discrimination has been studied in rats (Brown & Koe, 1982), pigeons (Hoenicke *et al*, 1992) and monkeys (Carney & Bergman, 1997). Atypical compounds e.g. clozapine are reasonably discriminable, unlike typical neuroleptics e.g. haloperidol, which is not readily discriminable (Colpaert *et al*, 1976). This suggests that different neurochemical mechanisms mediate their behavioural effects (Carey & Bergman, 1997).

Clozapine discrimination is learned quite rapidly and once learnt is maintained at a relatively high level of stability (Goudie & Taylor, 1998). Clozapine at 5 mg/kg is known to initially suppress lever pressing in rats (Sanger & Perrault, 1995). However, tolerance develops to the effects of clozapine on suppression of responding (Porter & Strong, 1996).

The clozapine cue has been shown to be highly specific (Goudie & Taylor, 1998) and typical neuroleptics and non-antipsychotic drugs e.g. amphetamine, pentylenatrazol do not generalise to the clozapine cue (Goudie & Taylor, 1998). Previous studies have shown that other compounds which are clozapine-like in structure and pharmacology e.g. JL13, seroquel and olanzapine generalise to clozapine but only at doses that suppress lever pressing (Goudie & Taylor, 1998; Porter & Strong, 1996;

Bruhwyler *et al*, 1997; Carey & Bergman, 1997). Olanzapine generalises to clozapine and it acts upon D_1, D_2, D_4, M_{1-5}, alpha$_2$, H_1, H_3, 5-HT_{2A}, 5-HT_{2C}, 5-3, 5-HT_6 and 5-HT_7 receptors (Porter & Strong, 1996).

Studies have shown that CDP generalises partially to the clozapine cue (Moore *et al*, 1992). Therefore, the clozapine cue may resemble the benzodiazepine discriminative cue to an incomplete and inconsistent extent (Goudie & Taylor, 1998). Hence, clozapine may have anxiolytic actions (Rimon *et al*, 1994) and sedative actions like benzodiazepines (Edge *et al*, 1997).

The extent to which the discriminative stimulus effects of clozapine are related to its clinical efficacy still needs to be determined (Carey & Bergman, 1997).

By using clozapine drug discrimination paradigms, novel compounds may be found which are clozapine-like with reduced EPS liability and possibly the same clinical efficacy (Carey & Bergman, 1997).

9.5 Tolerance to clozapine

Cross-tolerance occurs between clozapine and JL13 and olanzapine with respect to their ability to suppress operant responding (Goudie & Taylor, unpublished).

To establish the long term effects of clozapine more work needs to be undertaken. Once patients start their treatment with clozapine they need to take the drug daily for the rest of their lives, but most studies carried out are on the very short-term effects

of clozapine. Clozapine has been shown to decrease psychopathology and improve cognitive functions but the onset of effects may be delayed in some patients (Freed, 1988). Some patients do not show improvements until three to six weeks after treatment, whilst other, more fortunate patients, respond within the first week of treatment. It has been suggested that the long-term effects of clozapine may reduce neurotoxic processes and allow neural repair processes to occur (Meltzer, 1994).

9.6 Summary of clozapine

Clozapine is classed as a novel atypical antipsychotic and is used to treat schizophrenic patients after other treatments have been tried and have failed to succeed. Clozapine is known to act at many receptors but it is unknown which receptors are necessary for it to have its unique pharmacological effect. It is believed, but not proved, that clozapine tends to act at several receptors and not only one in order to have its unique therapeutic effect. More work needs to be carried out on clozapine in the drug discrimination and other paradigms to determine its true mechanism of action and the receptors that are specifically needed to produce its unique pharmacology and therapeutic effects.

Chapter 10.0

Effects of Chronic Clozapine Treatment of Tolerance to the Discriminative Stimulus Effects of Clozapine

10.1 Introduction

Clozapine is an atypical neuroleptic that has been shown to have reasonable efficacy in treating patients that are usually resistant to other antipsychotics (Kane *et al*, 1988).

Previous studies have shown that tolerance to the discriminative cue properties of drugs, induced by chronic treatment, is greater if discrimination training is suspended during chronic treatment. This has been shown to be true for both morphine and amphetamine (Barrett, White & Caul, 1992). It is believed (Sannerud & Griffiths, 1993) that, if discrimination training is continued during the administration of chronic drug treatment, rats simply learn to discriminate a functionally lower (faded) training dose. Hence accuracy of lever selection does not decline during tolerance development, nor is the dose-response curve shifted. Alternatively, in the absence of continued discrimination training during chronic treatment, no learning about the "fading" stimulus can occur, hence the dose-response curve shifts to the right.

The aim of this study was to investigate if tolerance could develop in animals previously trained to discriminate clozapine at 5 mg/kg, after chronic administration of clozapine. This was to investigate the role of pharmacological history on clozapine's actions, as this may be relevant to either clozapine's therapeutic actions and/or tolerance to its side effects.

10.2 Methods

Subjects

A maximum of 12 female Sprague Dawley rats (bred at the Psychology Department, University of Liverpool, UK) were used in this experiment. The animals were singly housed in white Perspex cages that measured 425 mm x 266 mm x 150 mm. The floor of each cage was covered with wood shavings. The temperature of the housing room was kept at about 21° C. The rats weighed between 350 - 450g at the start of the experiment. They had free access to water at all times except during drug discrimination training and testing procedures (which were run 5-7 days/week) and their diet was standard laboratory chow (Bantin & Kingman, Humberside, UK). During the first week they were food deprived to 80% of their free feeding body weights and were allowed time to habituate to their housing. The 12 subjects were *not* experimentally naive, as they had previously been trained by another experimenter in a very brief series of clozapine drug discrimination studies with scopolamine. The animals had been previously trained to discriminate clozapine (5 mg/kg) as their training drug. Hence, the animals simply needed time to become accustomed to a new experimenter. 12 animals were used in to calculate the time/effect curve (TEC), but only 11 animals were used to calculate the dose/effect curves (DECs). This is because one of the animals was dropped from the study when she became dehydrated for no apparant reason.

There were two groups of animals used in this series of studies. One group was not experimentally naïve (as explained above) whilst the other group was experimentally naïve. The two groups responded to clozapine in the same way and so the results

were compared across studies. The groups and their drugs are shown below:

Table 10.1: Groups number and which drugs were used in each group

Group	Drug administered	Number of days between experiments
Previously trained animals	Clozapine	15
	JL13	38 (including Christmas)
	Cyproheptadine	-
Experimentally naïve animals	Olanzapine	23
	CDP	-

Apparatus

Six standard rat operant chambers (Coulbourn Instruments, USA) were used (see method section for more details, pp 54-55).

Training Procedure

The animals had been trained to press both levers and to discriminate between clozapine (5 mg/kg) and vehicle using the procedure described in the methods section.

Testing procedure for determining dose/effect curves

11 animals were used in the determination of the dose/effect curves because one of the animals had to be removed from the study due to a non-drug related illness. The paradigm used to test the animals in the operant chambers was a drug versus vehicle Fixed Ratio 30 quantal operant drug discrimination assay; a variant of the Fixed Ratio 10 assay originally developed by Colpaert *et al* (1975), as described in more detail in Goudie & Leathley (1993). Test days were usually run with at least two continuous interspersed training days to ensure that the discrimination was maintained at a high level prior to each test. Tests were not run if the group level of accuracy of lever selection fell below 85% on the prior day. On test days rats were rewarded throughout the 15 minute operant session for responding on the lever on which they first accumulated 30 responses. Therefore, on test days when a rat made a lever selection (i.e. made 30 responses on either lever) it was defined as having selected either the drug or the vehicle lever. For the group as a whole it was therefore possible to define, for each dose, the percentage of animals selecting the drug lever.

Procedure for derivation of time-effect curves

Before the clozapine generalisation curve was determined it was necessary to determine a time/effect curve first with 12 animals. The time/effect was derived initially because it was necessary to know how long clozapine had an effect on the animals, i.e. for how long the animals could discriminate clozapine. It was necessary to determine the time/effect curve in order to assess the pharmacodynamic profile of clozapine. If clozapine was a very short acting compound, i.e. if the discriminative effect wore off within a few minutes or an hour, then the relevant neurobiological

systems assumed to be involved in the development of tolerance to clozapine would probably not have been saturated for long enough to produce tolerance. If clozapine was very short acting, as many as three injections a day, or a higher dose of clozapine, would possibly have had to be given to induce tolerance. On the other hand, if clozapine had been a very long lasting agent, the drug might have accumulated during chronic treatment, and hence might have been present at high levels when generalisation curves were computed and re-computed during the assessment of tolerance, thus confounding the computation of these curves.

Once the group accuracy was consistently above 85% correct lever selection, then a time/effect curve was obtained. The animals were injected with clozapine (5 mg/kg), one sub-group (n = 5) at 0830 and the second sub-group (n = 6) at 0845 hours. Then the animals were tested at different time points throughout the day 1, 4 and 8 hours post-injection. Thus the animals were tested at 0930, 1230 and 1630 hours for group one that was injected at 0830; and at 0945, 1245 and 1645 hours for group two which was injected at 0845 hours. The action of clozapine on neural systems was measured *indirectly* by the percentage of drug lever selections made at each time interval tested.

Procedure for dose- effect (generalisation) curves

The generalisation dose/effect curve was calculated three times during this experiment. The initial dose/effect curve (DEC 1) was calculated before chronic treatment. The second dose/effect curve (DEC 2) was measured at the end of chronic treatment; whilst the third dose/effect curve (DEC 3) was calculated 16 days after the

end of the second dose effect curve.

The idea behind these test procedures for the dose-effect generalisation curves was that DEC 1 was computed (5 days after the computation of the time/effect curve) once the animals had learnt to discriminate clozapine from vehicle to a high level of accuracy. DEC 1 was designed to assess the rat's sensitivity to clozapine before any chronic treatment. Then the animals received twice daily (b.i.d) injections of clozapine of 10 mg/kg (which is twice the training dose) for ten consecutive days. Chronic clozapine was anticipated to induce tolerance to clozapine, so when DEC 2 was carried out, the DEC should have shifted to the right, indicating that tolerance had developed. Once tolerance had developed, we needed to investigate if the tolerance was spontaneously reversible, which was the rationale behind DEC 3. If the tolerance seen on computing DEC 2 was due to some change in neural sensitivity (i.e. due to pharmacodynamic tolerance), then after 16 days of no drug treatment, the relevant receptors should have reverted back to their original state, as typically seen in other drug discrimination tolerance studies (e.g. Barrett, White & Caul, 1992).

Before DEC 1 was determined, the animals had to show a group accuracy level of at least 85% correct on two successive days. Then the first of five test days was carried out. Over the five test sessions, the rats received the following doses of clozapine: 5, 2.5, 1.25, 0.625 and 0.3125 mg/kg. The doses of clozapine were counterbalanced over test days. Each test session was separated by two training days, on which the animals' lever selection accuracy had to be 85% or more. If the accuracy level of the animals on the training days fell below 85% then they were not tested for

generalisation, instead they were re-trained to discriminate clozapine until accuracy reached an acceptable level again. Once all the test sessions had been carried out, the data were analysed by counting the total number of animals at each test dose that had chosen the drug lever, then converting that value to a percentage of the total number of animals for the group as a whole. The percentage drug lever selection was plotted as a function of the dose, then a log/linear regression line based on the linear portion of the dose/effect curve was plotted, and the ED_{50} calculated. The graphs were plotted on Fig P (a graphical package for DOS) and the regression line was also plotted with Fig P.

The second phase of the experiment was begun after DEC 1 had been determined. The animals were chronically injected for ten days with clozapine (10 mg/kg b.i.d).

The dose of clozapine (twice the training dose) and the frequency of dosing (b.i.d.) were chosen to facilitate tolerance development because clozapine was shown to be a short acting compound when the time/effect curve was derived and the animals needed frequent treatment to become tolerant to its effects. The animals were injected at 0900 and 1600 hours daily for 10 days. 0900 and 1600 hours were chosen because the animals were accustomed to being injected at 0900 hr for discrimination training at 0930, so it was decided to use that time point as the constant factor between calculating DEC 1 and DEC 2. Ideally, the other daily time point should have been 2100 hours (i.e. 12 hours later), but because there was only one experimenter, it was felt that this was not practically feasible. So a compromise was reached and the animals were injected at 1600 hours seven hours after the morning

injections. On the 11-13th consecutive days after the start of chronic clozapine treatment the animals were retested and DEC 2 calculated. For this DEC the animals had no training days separating test sessions, instead all three *successive* days were test days. The doses of clozapine used to calculate DEC 2 were: 5, 2.5 and 1.25 mg/kg. A higher dose of clozapine was not used in DEC 2 because the same doses allowed a comparable shift to the right of DEC 2, rather than more generalisation to higher doses. The other reason for keeping the same dose of clozapine in DEC 2 as DEC 1, was that if no or only very little tolerance was observed with DEC 2 and higher doses used, then DEC 2 would have to have been conducted again with the same doses as those used in DEC 1. The doses of clozapine were counterbalanced between the two groups over the three test days. The animals were tested on consecutive days in order to minimise any loss of tolerance to clozapine over treatment days 11-13. The method used to compute DEC 2 was similar to DEC 1 since the animals were injected at 0850 and 0905 hours, then 30 minutes later put in the operant boxes for 15 minutes. The times of 0850 and 0905 hours were chosen because the animals were accustomed to being injected with clozapine at 0900 hours, thus the times used for the DEC 2 prevented withdrawal from clozapine due to no drug being administered at the accustomed time each morning. Once the animals had been placed in the operant boxes and their test session finished, they received a "top-up" dose of clozapine immediately after testing to ensure that during the mornings the animals had actually received the scheduled full morning dose of 10 mg/kg of clozapine, as shown in table 10.2.

Table 10.2: The doses administered to the animals for the test and the corresponding "top-up" doses in the morning.

Test dose of clozapine	Top up dose of clozapine	Total dose of clozapine
5	5	10
2.5	7.5	10
1.25	8.75	10

On days 11 and 12, the rats *also* received their usual scheduled 10 mg/kg dose of clozapine at 1600 hours to prevent withdrawal and to maintain tolerance. Once DEC 2 had been completed, the data were analysed using a log-linear least squares regression and the ED_{50} calculated in the same way as for DEC 1. The data showed that the animals did not need a longer time period of chronic treatment to induce tolerance (see Results). Therefore, the animals were simply left alone with no further drug treatment for 16 days and then DEC 3 was calculated. DEC 3 was calculated in exactly the same way as DEC 1, so each test session was separated by two training days on each of which the animals had to show 85% or more correct lever selection. The doses of clozapine used in DEC 3 were: 5, 1.25 and 0.3125 mg/kg. Only three doses of clozapine were used this time because the full generalisation range was already known for this group of rats from DEC 1. When DEC 1 had been computed the full range was not known, and it required 5 doses of clozapine for full determination of that specific DEC. For DEC 3 the doses of clozapine were again counterbalanced across the three test sessions. The data from DEC 3 were analysed,

plotted and the ED_{50} calculated in exactly the same way as for DEC 1.

Statistics

The relative potency of clozapine after the different drug treatments for the animals was compared by probit analysis using standard parallel-line bioassay techniques (Finney, 1964). This allows a comparison of the ratio of drug treatments for a given effect providing a value for relative potency. A significant relative potency difference is shown when the 95% confidence limits for the ratio does not include the value 1.0. This analysis also allows an assessment of whether the various dose/effect curves differ from parallelism (SPSS for Windows Release 6, see also Fasciano *et al*, 1997).

Drugs

Clozapine base (Sandoz, Switzerland) was administered i.p., dissolved in a few drops of 0.1 M HCl, diluted with water and buffered back with 0.1M NaOH to a pH around 5.5 and injected at a volume of 2 ml/kg. The drugs were injected 30 minutes before operant sessions.

10.3 Results

The training data for the animals learning to discriminate between clozapine (5 mg/kg) and vehicle are not shown because, as described above, the animals were trained by somebody else. However, in order to show that the animals' accuracy of lever selection was consistently high, the data showing the training just before the start of the tolerance experiment have been included.

Fig. 10.1 The graph shows sustained accurate performance of the animals before the start of the experiment.

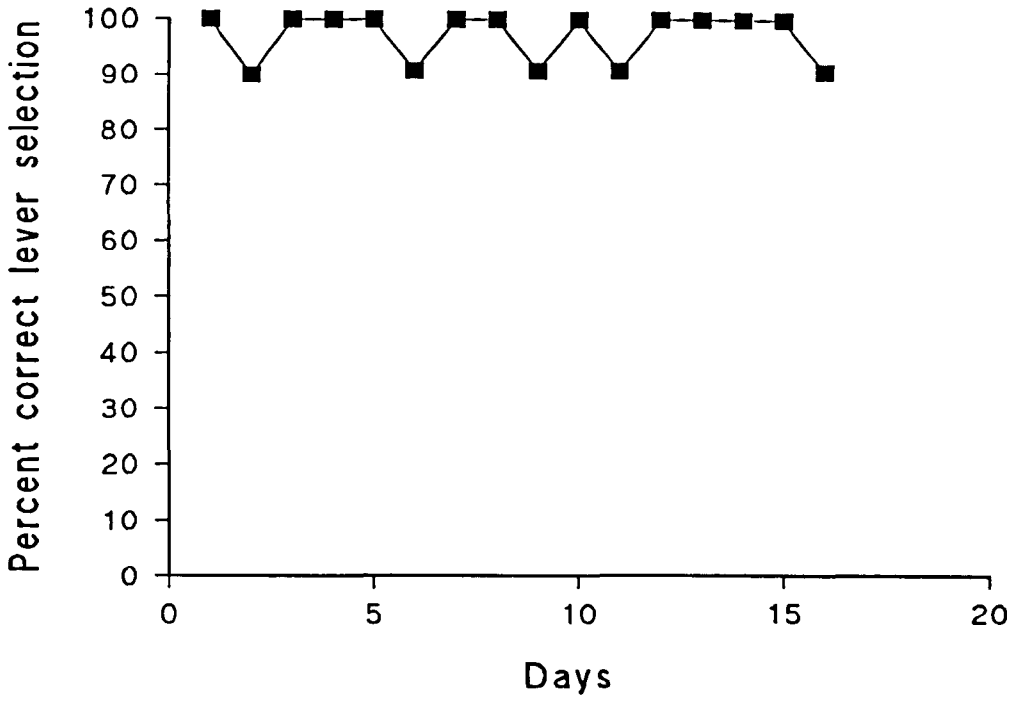

Fig. 10.1 The data show that the animals had learnt to discriminate clozapine (5 mg/kg) versus vehicle to a very high level of accuracy from the day that I started to use them. The data also show that the animals' accuracy level was stable and consistently high.

Figure 10.1 shows the data from just before the start of the tolerance experiment. The data are, however, in some respects misleading because at the start of training, not all animals made an FRF (i.e. received a reward during the training session). For the training data to be plotted only animals that actually made a lever selection were used in the calculations of percent correct lever selections, so any animals that failed to make a lever selection were not included in that day's data. On the first day of

training five animals out of eleven failed to make a selection. Over days 2-5, one rat who had received clozapine (5 mg/kg), failed to make a selection. However, after this time all the rats consistently made lever selections. It is believed that so many of the animals failed to make a lever selection initially on day 1 due to the change in experimenter rather than due to clozapine treatment. This is because on the day in question all animals received *vehicle*. Hence there was no clozapine present in the animals to suppress responding; thus the failure to respond was not due to sedative effects of clozapine.

However, once the animals had become accustomed to the different experimenter (i.e. the author) they responded consistently. Therefore, the data indicate that the animals (as a group) needed several days to acclimatise to a different experimenter. Once the animals were all responding reliably, and their response rates were stable over days (data not shown), then the time/effect curve was determined, as described previously.

Fig. 10.2 Time/effect curve of clozapine at 5 mg/kg

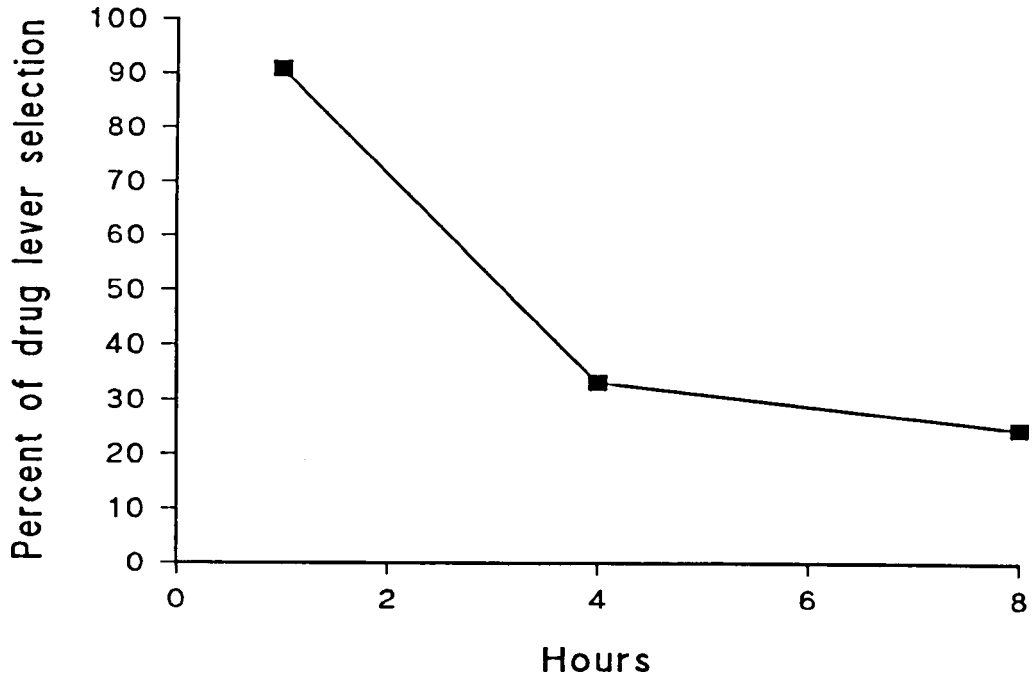

Fig. 10.2 The graph shows that after one hour most of the animals choose the drug lever. After 4 hours, drug lever selection had decreased to less than 50%, whilst after 8 hours drug lever selection had decreased further to about 30%, but there was not a dramatic or apparent significant difference between drug lever selection at 4 and 8 hours after the administration of clozapine.

The time/effect curve is an indirect predictor of the duration of action of clozapine on neural systems. It allows an indirect assessment of how long the drug remains in the animals' body. Thus, Figure 10.2 suggests that after 8 hours the animals had very little clozapine present in the body. Therefore clozapine is a relatively short acting compound after i.p. administration to rats, which is why clozapine was given at 10 mg/kg b.i.d. in the tolerance phase of the study. Clozapine was administered at 10 mg/kg during the tolerance phase twice a day, so the overall dose per day was 20

mg/kg. I felt that if the animals were given too high a dose of clozapine per day, then the animals would be too wiped out and would not tolerate out to the sedative effects of clozapine in time for them to respond on the levers in DEC 2. Other studies have shown that if a dose of drug generalises, then tolerance can be produced without discrimination training if the dose of the drug is doubled and administered twice a day (Young & Sannerud, 1989).

The subsequent stages of the experiment were to determine DEC's 1, 2 and 3. The dose/effect curve was carried out by subjecting the animals to a series of doses ranging from the training dose downwards in a logarithmic manner until the animals could barely discriminate the drug from vehicle. Hence, in this dose/effect curve the doses ranged from 0.3125 to 5 mg/kg for DEC's 1 and 3 and between 1.25 to 5 mg/kg for DEC 2. Saline was not used in the generalisation tests because during training, the response under saline on the drug lever were minimal.

Fig. 10.3 Comparison of all Dose/effect curves

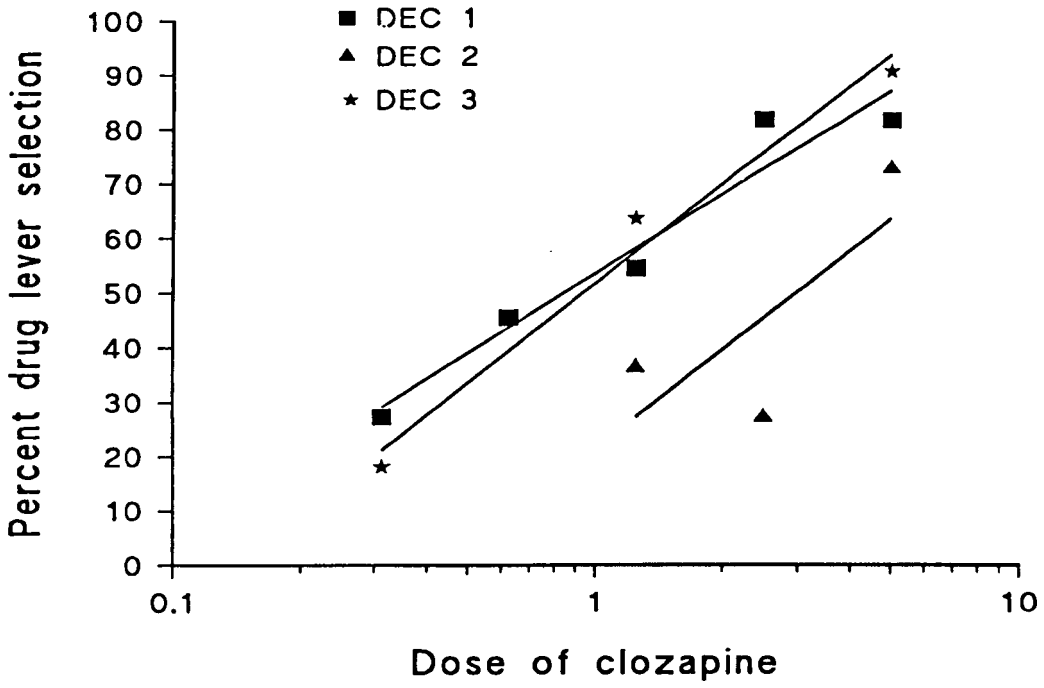

Fig. 10.3 The data show the three DEC's that the animals were subjected to; the shift to the right between DEC 1 and 2 and the shift back to the left between DEC 2 and 3.

DEC 1: The data show that the animals showed no difference in percent drug lever selections between 2.5 and 5 mg/kg of clozapine. However, they showed less drug lever selection under 1.25 mg/kg clozapine at about 50%, then at 45.5% under 0.625 mg/kg of clozapine. The lowest dose of 0.3125 mg/kg produced about 28% drug lever selection. A regression line was plotted (see figure) with all 5 points being used (See table 10.3). The regression lines provided a good fit to all the data analysed. The DECs showed that as the dose of clozapine decreased, so did the number of drug lever selections, as expected. There was no difference in response rates over DEC's,

so they are not mentioned here or later on in the thesis.

Once DEC 1 had been carried out, the animals were treated chronically as described above with clozapine (10 mg/kg, b.i.d). The animals were injected twice a day for 10 days with clozapine, and during this time received no discrimination training. The animals were weighed, injected and then placed back in their home cages until the next injection. At the end of chronic clozapine treatment, another dose/effect curve (DEC 2) was determined on days 11 to 13.

DEC 2: The chronic clozapine treatment shifted the dose/effect curve in parallel to the right (indicative of tolerance). The data (DEC 1 v 2 and DEC 2 v 3) were compared by probit analysis. The calculated probit lines were analysed for parallelism (See table 10.4).

The parallelism between the two lines shows that the same pharmacological mechanism was involved in the effects seen in both DEC 1 and DEC 2. If the shift had not been parallel then it would suggest that different mechanisms were responsible for producing the pharmacological effects seen in DEC 1 and DEC 2. A McMemar test (Siegel, 1956) was carried out on the common 2.5 mg/kg data for the two DECs and it was shown to be significantly different [$Chi^2 = 4.16$, d.f. = 1, $p = 0.05$, sig.]; confirming the conclusion derived from the probit analysis that tolerance had developed to clozapine.

The rats were then left for 16 days without further clozapine injections or discrimination training to investigate whether or not the tolerance induced by chronic clozapine would spontaneously dissipate.

DEC 3: The data show that the tolerance induced by chronic clozapine spontaneously dissipated after 16 days. The DEC reverted back to its original position. Therefore, the two lines in figure 12.3 show that the tolerance induced by chronic clozapine was spontaneously lost when the animals were left untreated. The tolerance was completely lost, as indicated by the fact that the two DECs were almost identical.

All three lines are compared to each other and the following ED_{50} values were obtained with corresponding R^2 values;

Table 10.3: ED_{50} values and R^2 values for the different DEC's:

DEC	ED_{50} value (mg/kg)	R^2 value
1	0.84	+0.94
2	2.98	+0.57
3	0.94	+0.97

The ED_{50} values of the dose/effect curves show that 10 days of chronic clozapine (10

mg/kg, b.i.d) induced tolerance a 3.5 fold shift in the DEC, but that it spontaneously dissipated completely over 16 days as the DEC reverted back to its original position. The shifts in the curves were parallel, which indicates that a common pharmacological mechanism mediated all three dose/effect curves. This would be expected because all dose/effect curves were carried out with clozapine.

The DECs were analysed by probit analysis and the following values were obtained.

Table 10.4: Comparison of parallelism of DECs and the potency ratio between the DECs.

DECs compared	Parallelism results	ED_{50} Potency ratios and 95% confidence limits
1 v 2	$X^2 = 0.032$, d.f. = 1, p = 0.839, NS	3.5 fold, lower = 0.13, upper = 0.82, Sig.
2 v 3	$X^2 = 0.12$, d.f. =1 p = 0.729, NS	3.2 fold, lower = 1.19, Upper = 43.09, Sig.
1 v 3	$X^2 = 0.594$, d.f. =1 p = 0.441, NS	1.1 fold, lower = 0.38, upper = 2.72, NS

The confidence limits did not cover 1.0 for the comparison of DEC 1 v 2 and DEC 2 v 3, so this indicates that the results are significant. Whilst the confidence limits cover 1.0 for the comparison of DEC 1 V 3 which indicates that the results are not significant (Fasciano *et al*, 1997). The significant difference in potencies show that the curves shifted (i.e. tolerance developed and the curves shifted 3.5 to the left and then 3.2 fold to the right). Thus, the results show that the three DECs can not be proved to be significantly non-parallel. The results from the probit analyses also show that there is a significant potency ratio difference between DEC 1 and 2, and between DEC 2 and 3, but not between DEC 1 and 3, as expected.

Therefore, the experiment showed that clozapine can be discriminated and also that chronic clozapine can induce tolerance to itself, and that the tolerance is spontaneously lost.

10.4 Discussion

The aim of the study was to investigate if chronic clozapine treatment (10 mg/kg, b.i.d) could induce tolerance to clozapine in rats. As was shown to be the case, then we investigated whether the tolerance produced dissipated spontaneously when the animals were left alone.

The results showed that clozapine is discriminable in rats and this agrees with previous reports (Goudie & Taylor, unpublished). The data also show that once the clozapine discrimination is learnt then the level of accuracy of lever selection is very

high and stable responding is maintained (Fig. 10.1). The level of stability remained high throughout the whole of the study (i.e. more than 85% correct lever selection).

The results also showed, in accordance with previous studies (Goudie & Taylor, 1998), that a generalisation curve for clozapine in animals trained to discriminate 5 mg/kg clozapine could be performed (Fig. 10.3). The generalisation curve showed, as expected, that as the dose of clozapine decreased the percent drug lever selection also decreased.

Once the generalisation curve had been obtained, the animals were injected with clozapine at 10 mg/kg twice a day and discrimination training was suspended. Discrimination training was suspended because it has been shown that if discrimination training is continued then no tolerance occurs (Young & Sannerud, 1989). This is due to the process of "fading".

Animals learn to attend and perform the necessary response when a drug dose is administered and this is called, by definition, discrimination training. However, if a drug is chronically administered then "fading" of the training dose may occur. This is where the animals become tolerant to the dose and hence require more drug to produce the same effect. During chronic drug treatment as time continues the stimulus level decreases or fades, so the animals end up with a weaker stimulus. Therefore, at the end of chronic drug treatment, the animals are re-tested and tolerance is shown due to a shift in the dose-response curve. However, if the animals had continued to receive discrimination training, then they would have learnt to

attend to the lower "faded" cue during the tolerance process due to the continued discrimination training. Thus, at the end of chronic drug treatment, no tolerance would have been observed because the animals would have learned to attend to the "functionally" lower stimulus. So in this experiment the discrimination training was suspended during the chronic clozapine treatment to prevent the "fading" process. The results showed that tolerance occurred and that the dose-response curve was shifted to the right in parallel (Fig. 10.3).

The tolerance due to chronic clozapine treatment to the clozapine discriminative stimulus, occurred *presumably* due to a pharmacodynamic change. The tolerance is suggested to be a result of some neuroadaptive change, and not a learnt process because when the animals were simply left for 16 days then the tolerance reversed spontaneously. If the tolerance had been due to a learnt process, then leaving the animals alone for 16 days should have had no effect at all. Thus, the third dose-response curve would not have reverted back to the original position of the first dose-response curve. This is because once animals have learnt a response simply leaving them does not allow them to unlearn the response. However, if the tolerance was due to a change in neural sensitivity, then this would be expected to be spontaneously reversible as seen in this experiment. Previous experiments (McMillan *et al*, 1996) with discrimination procedures have shown that once animals learn a particular response to a certain stimulus, then the learnt response remains and can not be unlearnt unless the animals undergo extinction training. It has been shown that once animals have learnt to discriminate a particular dose of drug, then leaving the animals with no further training has very little effect on the learnt discrimination

(McMillan *et al*, 1996). The animals can be left for weeks or even months and re-testing the animals shows that they can still discriminate the drug versus saline or vehicle (McMillan *et al*, 1996)

Pharmacodynamic tolerance could occur due to several mechanism and these include:

- changes in receptors
- loss of receptors ("downregulation")
- adaptive changes downstream from receptors (e.g. second messengers)

Neuroleptics require chronic administration to produce symptomatic relief (Wiley *et al*, 1994). The effects of neuroleptics differ depending on whether they are administered acutely or chronically. With acute administration, high doses of both atypical and typical neuroleptics produce suppression of learned schedule-controlled behaviour (Wiley *et al*, 1994), however with repeated dosing, differential effects are seen with typical and atypical neuroleptics (Wiley *et al*, 1994). It has been shown that tolerance develops completely to clozapine's initial disruption of response rate by the seventh day of chronic treatment (Wiley *et al*, 1994). Clozapine decreased response rate to start with but after two days partial recovery to pre-clozapine vehicle levels were observed. In addition, response rates on the first day of post-clozapine injection were the same as those during the pre-clozapine baseline levels. It was also shown that clozapine's effects on operant responding appear to be schedule dependent. Complete tolerance has been shown to occur to clozapine's disruption of fixed interval (FI) responding and random interval (RI) responding but only partial

attenuation of the rate disturbing effects of clozapine on fixed ratio (FR) responding occurred (Wiley *et al*, 1994). Thus, tolerance to clozapine has been shown to occur in a different paradigm to drug discrimination.

The effects of subchronic and chronic dosing regimes need to be studied, to investigate clozapine tolerance. The tolerance aspect of clozapine is important because the therapeutic effects do not tolerate out and in actual fact often increase with time (Hu, Malhotkra & Pickar, 1999), although patients usually adapt to the drug's sedative effects (Das & Fowler, 1995). It was shown that acute clozapine reduces lick rhythm dose-dependently, in contrast to lack of effects by haloperidol (Fowler & Das, 1995). Quite rapid tolerance to the number of licks was seen, the same was seen with the subchronic dosing as well. The subchronic data showed that clozapine's slowing is detected even when continuous licking was unaffected (Das & Fowler, 1995). This is the same as seen in the clinic where the sedation and therapeutic effects are disassociated (Das & Fowler, 1995). However, how the diminished lick rhythm in rats due to clozapine may be related to its therapeutic efficacy is unknown (Das & Fowler, 1995). The rhythm slowing effects of clozapine may not be related to therapeutic efficacy, but instead may be a marker for one of clozapine's side effects e.g. hypersalivation (Das & Fowler, 1995) or may be tolerance to clozapine's side effects. Ritanserin (a 5-HT_2 antagonist) had no effect compared to clozapine on any of the lick measures. Therefore, if the rhythm slowing induced by clozapine was mainly 5-HT_2 mediated, then ritanserin should have had similar effects of clozapine. So more work needs to be carried out to ascertain which receptors are involved in mediating these effects in rats.

It has been shown that classical and atypical neuroleptics do not reach their full potential for 2-3 weeks or more of administration (Sams-Dodd, 1998). After cessation of clozapine, some patients relapse within one week and others show no change for two weeks (Sams-Dodd, 1998).

Thus, this study shows that tolerance does occur to the discriminative effect of clozapine, but the precise mechanism that is involved is unknown. However, what has become clear is that more chronic studies need to be carried out because the maximal effects of clozapine take several weeks to occur.

It has been shown that the extrapyramidal side-effects (e.g. Parkisonism, tremor etc) may disappear during chronic administration but the antipsychotic effect remains (Sebens *et al*, 1995).

The precise receptors which clozapine acts upon are still unknown and require further work to be identified. Many studies that have been carried out have shown that the effects of acute and chronic clozapine differentially alter the receptors.

However, clozapine is known to act at the following receptors: $D_{1,2\ \&\ 4}$, $5\text{-}HT_{2A,\ 1A,\ 3,\ 6\ \&\ 7}$, noradrenergic-$alpa_{1\ \&\ 2}$, H_1, M_1 and GABA. So, both the discriminative stimulus effects and the production to tolerance to the discriminative stimulus of clozapine must involve either one of the aforementioned receptors or a combination of several or even all of them.

10.5 Conclusion

The study showed that chronic clozapine produced tolerance, as indicated by a parallel shift to the right of the dose-response curve to clozapine, when comparing the before and after chronic clozapine treatment dose-response curves. The precise mechanism for the induction of tolerance is unknown, the exact neural systems involved are also unknown, and this requires more work. However, one suggestion could be that clozapine chronically could lead to a change in the sensitivity of the receptors present and hence tolerance would be induced. So, tolerance could be reversed by leaving the animals alone and the sensitivity of the receptors would spontaneously revert to their normal state once chronic drug treatment ceased.

10.6 Further Work

To investigate if tolerance to clozapine, in the same paradigm and under the same conditions, could be induced by compounds that show some degree of generalisation to clozapine.

Chapter 11.0

Effects of Chronic Olanzapine Treatment on Tolerance to the Discriminative Stimulus Effects of Clozapine

11.1 Introduction

Olanzapine (LY170053) is an atypical antipsychotic (Moore *et al*, 1994).

Olanzapine has an activity profile like clozapine in many tests including measurement of a rat's tongue movement during lapping behaviour, conditioned avoidance tests, and punished responding (Moore *et al*, 1994). It has been shown to have 5-HT_2, D_1 and D_2 antagonist properties both *in vivo* and *in vitro* studies (Moore *et al*, 1993). Olanzapine has been shown to have anticholinergic activity and an affinity for D_4 receptors (Moore *et al*, 1993). Olanzapine displays a high affinity for serotonin (5-$HT_{2a/2c}$ and 5-HT_3), dopamine (D_1, D_2, D_4), acetylcholine (M_1, M_2, M_3, M_4, M_5), norephinephrine ($alpha_1$) and histamine (H_1) receptors. Hence, its binding profile is very similar to clozapine - an atypical antipsychotic (Moore *et al*, 1994).

Clozapine has been shown to induce catalepsy but only at doses higher than those needed to block a conditioned avoidance response (Moore *et al*, 1992). Olanzapine has been shown to produce the same induction of catalepsy and blockade of a conditioned avoidance response, in the same manner as clozapine (Moore *et al*, 1994). Thus, it has been suggested that olanzapine, like clozapine will produce fewer EPS than typical neuroleptics.

It has been shown that olanzapine produces clozapine-appropriate responding in rats trained to discriminate clozapine from vehicle in a two-lever drug discrimination paradigm (Moore *et al*, 1992). Clozapine has been shown to have a robust and easily

discriminable stimulus (Goas & Boston, 1978) unlike most typical neuroleptics e.g. haloperidol (Harris & Balstar, 1971). Rats can be trained to discriminate between olanzapine and vehicle easily and like clozapine olanzapine has a robust discriminative stimulus (Moore *et al*, 1992). It has been shown that clozapine can fully substitute in animals trained to discriminate between olanzapine (0.5 mg/kg) and vehicle (Porter & Strong, 1996). Generalisation between clozapine and olanzapine at the highest doses tested produced almost full suppression of responding (Porter & Strong, 1996). When the motor effects of olanzapine are studied, the lack of motor effect for clozapine correlates well with olanzapine's and clozapine's lack of EPS (Casey, 1989, Borison, 1995). Thus, the symmetrical generalisation between clozapine and olanzapine is further evidence of how similar the two compounds are in preclinical studies.

Olanzapine is a clozapine congener and acts like clozapine in many behavioural tests. It appears to have a clozapine-like atypical profile when studying several of its effects; - 1) mesolimbic selectivity, 2) blocking 5-HT_2 receptors at a lower dose that dopamine$_2$ receptors 3) inhibiting the conditioned avoidance response at doses lower than doses needed to induce catalepsy (Moore *et al*, 1997). Thus, olanzapine is very clozapine-like in behavioural tests.

It has been shown that olanzapine acts upon many receptors in several studies. The results of one study (Moore *et al*, 1993) showed that olanzapine antagonised

apomorphine-induced climbing behaviour in mice. This shows that olanzapine possess' D_1/D_2 antagonistic activity *in vivo*. Olanzapine has also been shown to antagonise 5-HT-induced head twitches at doses lower than those needed to block the climbing response (Moore *et al*, 1992). This allows further speculation of the suggestion that olanzapine is a more potent 5-HT_2 antagonist than a dopamine receptor antagonist.

It was shown that olanzapine was more potent than clozapine in blocking 5-HT and D_2 receptors. Olanzapine's ability to block these receptors supports the suggestion of it being an atypical antipsychotic (Fuller & Snoddy, 1992).

Therefore, many studies carried out investigating the effects of olanzapine compared to clozapine in behavioural tests show that they have very similar properties. Thus, olanzapine may be as good as clozapine at treating schizophrenic symptoms and may produce fewer EPS than clozapine, i.e. may be no agranulocytosis.

Consequently we investigated if olanzapine would produce cross-tolerance to the clozapine discriminative stimulus just as clozapine had previously been shown to do.

Olanzapine was the next compound compared against clozapine to investigate whether cross-tolerance would be induced or not, because olanzapine fully generalises to clozapine. I decided to start with a compound that was most likely to

induce cross-tolerance to check initially that compounds other than clozapine could induce cross-tolerance to the clozapine cue.

11.2 Methods

Subjects

A maximum of 12 female Sprague Dawley rats (bred at the Psychology Department, University of Liverpool, UK) were used in this experiment. The 12 subjects were experimentally naive. 12 animals were used to calculate the TEC, but during the tolerance phase one of the animals became ill (due to an un-operable lump) and was removed from the study. So the DEC's were calculated with 11 animals. The animals used in this study were not the same as those used in the clozapine experiment. Two groups of animals were used to conduct the tolerance experiments. The two groups used in this set of set of experiments and the particular drugs that each group was injected with are shown below.

Table 11.1: Groups number and which drugs were used in each group

Group	Drug administered	Number of days between experiments
Previously trained animals	Clozapine	15
	JL13	38 (including Christmas)
	Cyproheptadine	-
Experimentally naïve animals	Olanzapine	23
	CDP	-

Apparatus

As described in methods section.

Training Procedure

As described in the methods section.

Testing Procedure for determining dose/effect curves

As described in the clozapine chapter.

Procedure for derivation of time/effect curve

Before the clozapine generalisation curve could be determined it was necessary to determine a time/effect curve for olanzapine. It was necessary to calculate the time/effect curve in order to assess the pharmacokinetic profile of olanzapine. The animals were injected with olanzapine (2.5 mg/kg). One sub-group (n = 6) was injected at 0830 and the sub-second group (n = 6) at 0845 hours. Then the animals were tested at different time points throughout the day 1, 4 and 8 hours post-injection. Thus the animals were tested at 0930, 1230 and 1630 hours for group one which had been injected at 0830; and at 0945, 1245 and 1645 hours for group two which had been injected at 0845 hours.

Procedure for dose/effect (generalisation) curves

The method used was exactly the same as that used in the clozapine experiment except that the animals were chronically injected for ten days with olanzapine (5

mg/kg, b.i.d) instead of clozapine. The dose of olanzapine (twice that needed to produce maximum generalisation) and the frequency of dosing (b.i.d.) were chosen to facilitate tolerance development. The animals were injected at 0900 and 1600 hours daily for 10 days. In this study it was decided not to give the animals a "top-up" dose of olanzapine because once the animals had received the clozapine in the morning for the DEC, then it would have been too complicated to calculate the dose of *olanzapine* required to make the same dose of clozapine as that which the animals usually received. However, during computation of DEC 2 on days 11 and 12 the animals received their usual 5 mg/kg dose of olanzapine at 1600 hours to prevent withdrawal and maintain tolerance.

Statistics

As described in the clozapine chapter.

Drugs

Clozapine base (Sandoz, Switzerland) was administered i.p., dissolved in a few drops of 0.1 M HCl, diluted with water and buffered back with 0.1M NaOH to a pH around 5.5 and injected at a volume of 2 ml/kg. Olanzapine (Eli Lilly) was made up in exactly the same way as clozapine. The drugs were injected 30 minutes before operant sessions.

11.3 Results

The drug naive animals used in this experiment were trained to discriminate between clozapine (5 mg/kg) and vehicle.

Fig. 11.1 Animals trained to discriminate between clozapine (5 mg/kg) and vehicle

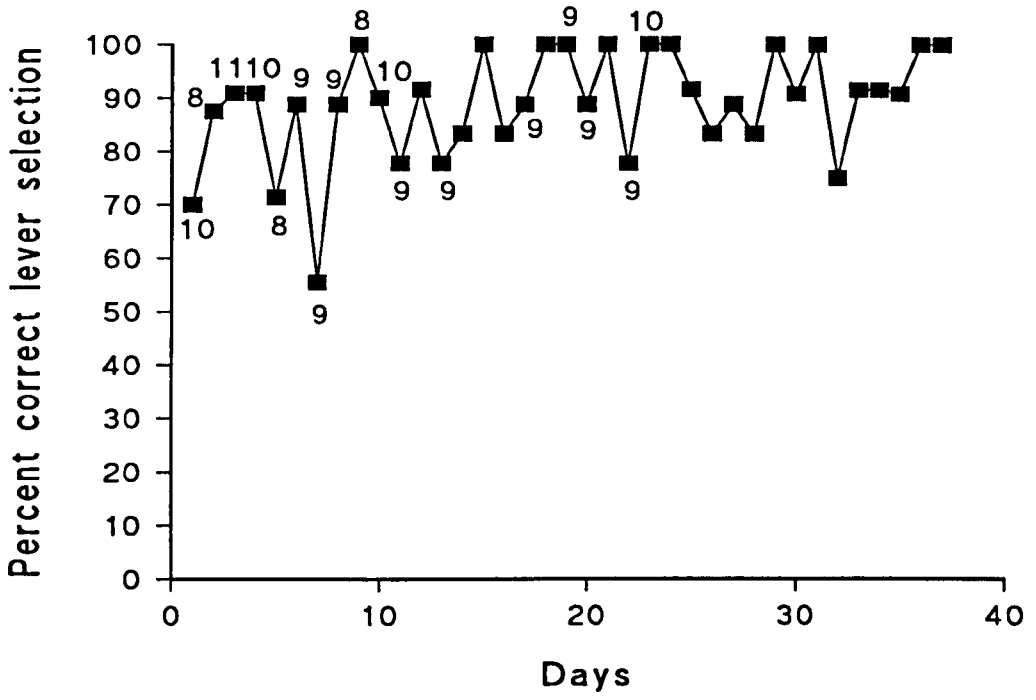

Fig. 11.1: The graph shows that the animals learnt to discriminate between clozapine (5 mg/kg) and vehicle, in under 40 sessions. The animals were required to reach a criterion of 9 correct lever selections out of 10 consecutive training sessions. The numbers above the data points represent the number of animals that made a lever selection on any one day. Where there are no numbers then all 12 animals in the group made a lever selection.

Hence, the data in figure 11.1 show that all the animals learnt to discriminate between clozapine (5 mg/kg) and vehicle reasonably quickly and to a very high level of accuracy. The animals reached perfection on several of the training sessions. Once the animals had reached a high level of accuracy for the clozapine discrimination, the next stage of the experiment was carried out.

This stage was the time effect curve (TEC) with olanzapine. The dose of olanzapine used was 2.5 mg/kg, because a previous study carried out in this laboratory (Goudie & Taylor 1998) showed that a dose of 2.5 mg/kg produced partial generalisation (38%). This dose produced maximal rate suppression so higher doses could not be tested. So this dose was used for the time effect curve. The TEC was carried out 1, 4 and 8 hours post-injection.

Fig. 11.2: Time effect curve for olanzapine (2.5 mg/kg)

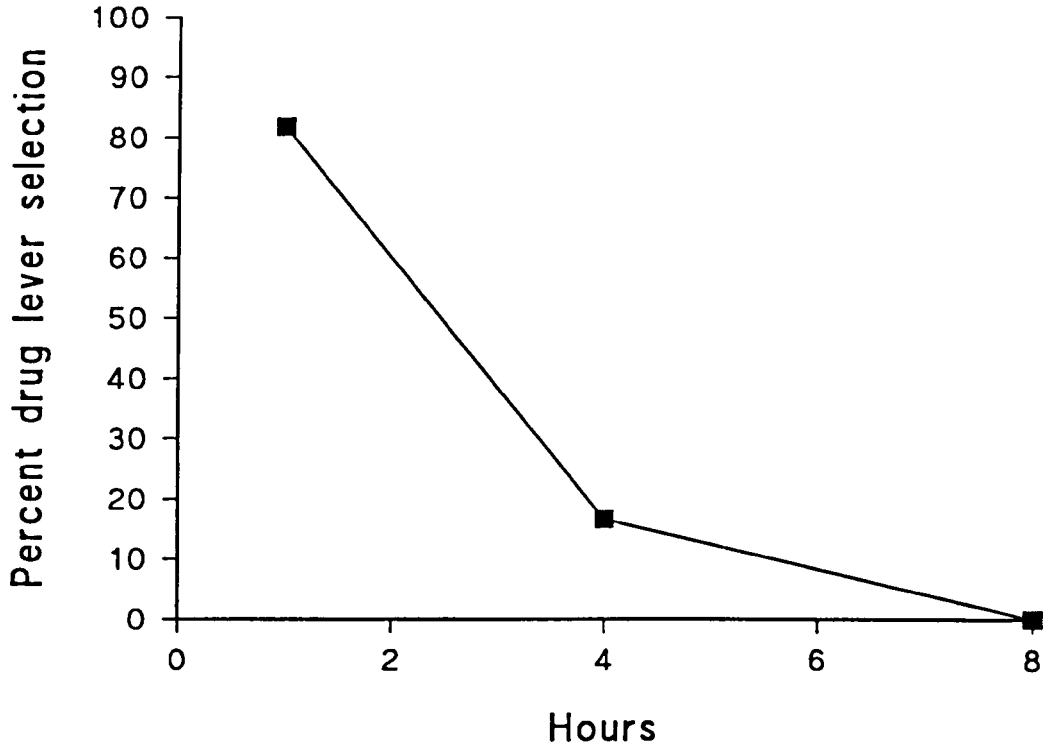

Fig. 11.2: The data showed that one hour after olanzapine (2.5 mg/kg) the animals generalised to a level of 81.8% (note this contrasts with the 38% generalisation reported by Goudie & Taylor, 1998), thus most of the animals selected the drug lever. However, the level of generalisation had decreased dramatically after 4 hours to just below 20%. After 8 hours there was no drug left in the animals' system, as indicated by the fact that all the animals choose the vehicle lever.

However, the degree of generalisation between olanzapine and clozapine is dose-dependent and no inferences can be made about the degree of generalisation of other doses of olanzapine and clozapine.

Therefore, figure 11.2 shows that as the time post-administration of olanzapine increased, the degree of generalisation between olanzapine and clozapine decreased. The graph shows that the duration of action of olanzapine was not too long, but was believed to be long enough to produce tolerance in this behavioural paradigm if given b.i.d. The time/effect curve is an indirect predictor of the duration of action of clozapine on neural systems, so it allowed an indirect assessment of how long the drug remains in the animal's body.

The time/effect curve was used to asses that the drugs studied i.e. clozapine, olanzapine showed at least partial generalisation to the clozapine cue and to also determine how long the drug remained active in the animals bodies. If no initial generalisation was observed, then the tolerance study would not have been carried out with that drug. This is because I felt it was necessary for the drug to show partial generalisation in order for cross-tolerance to be induced. Also if the drug cue was especially long lasting then only one dose a day and not two doses would have been administered during the chronic phase. Although, the TEC did not determine what exact dose of the drug to use, it did allow an indication of whether the drugs generalise to clozapine and how long the drug lasted in the animals.

The subsequent stages were carried out to investigate whether tolerance developed or not. This was carried out by subjecting the animals to a series of doses ranging from the training dose downwards in a logarithmic manner until the animals could barely

discriminate the drug from vehicle. In this dose/effect curve the doses ranged from 0.3125 to 5 mg/kg for DEC 1and 3, and between 1.25 to 5 mg/kg for DEC 2.

Fig. 11.3 Comparison of all three DEC's during olanzapine treatment

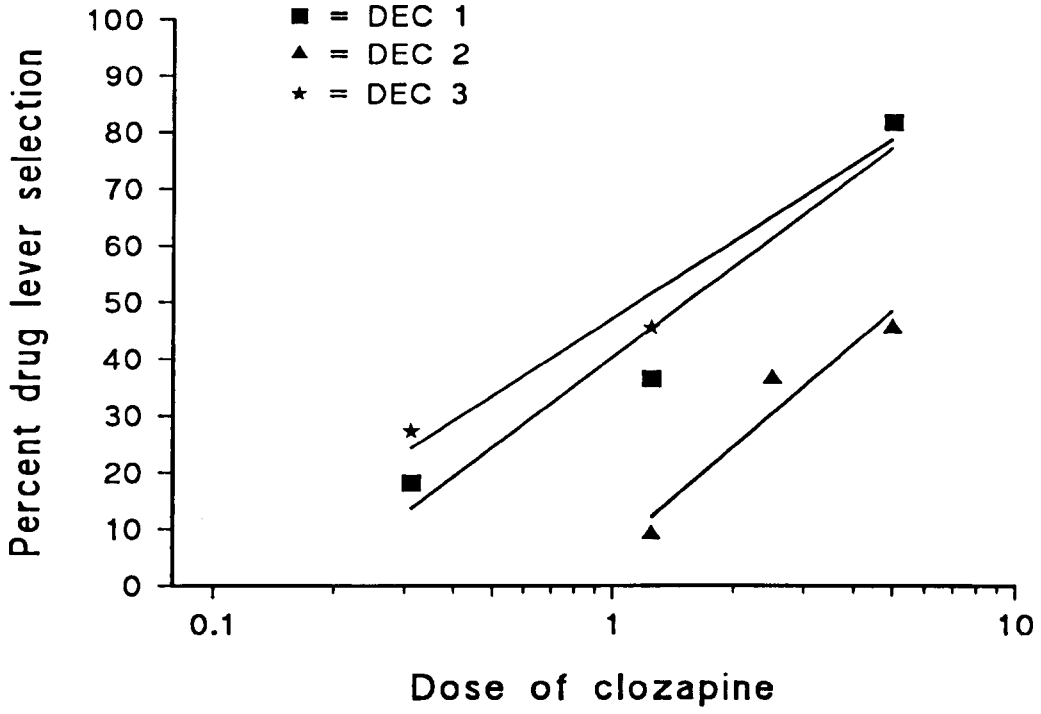

Fig. 11.3: The figure shows that chronic olanzapine induced cross-tolerance to the clozapine cue and that the cross-tolerance was spontaneously when the animals were left alone. The missing star is in exactly the same place as the square.

DEC 1: The data show that clozapine produced dose-related generalisation over the dose range 0.3125 to 5 mg/kg with a maximum of 83.3%. The animals showed 15% drug lever selection with the lowest dose of 0.3125 mg/kg, about 33% selection under the middle clozapine dose of 1.25 mg/kg, whilst under the highest dose of 5

mg/kg, the animals responded at 83.3% on the drug lever. Regression lines were plotted for all three DEC's which provided good fits to the data (See table 11.2). The DEC's show that as the dose of clozapine decreases, so does the percentage of drug lever selections, as predicted.

Once DEC 1 had been carried out, the animals were treated chronically with olanzapine (5 mg/kg, b.i.d.). The animals were injected twice a day for 10 days with olanzapine and during this time received no discrimination training. The animals were weighed, injected and then placed back in their home cages until the next injection. At the end of the chronic olanzapine treatment (5 mg/kg, b.i.d.), another dose/effect curve (DEC 2) was determined on days 11 to 13.

DEC 2: The graph shows the dose/effect curves before (DEC 1) and during (DEC 2) chronic olanzapine (5 mg/kg, b.i.d.) treatment. The chronic olanzapine treatment shifted DEC 2 to the right in parallel compared to DEC 1. The lines were analysed by probit analysis and the calculated probit lines were analysed (see table 11.3). The parallelism between the DEC's show that the same pharmacological mechanism was involved in both DEC 1 and DEC 2, which was expected because clozapine had been used to determine both DECs.

The animals were then left alone for 16 days, with no further olanzapine injections or discrimination training to investigate whether the cross-tolerance induced by chronic

olanzapine to the clozapine discriminative stimulus would spontaneously dissipate by computing DEC 3.

DEC 3: The data show that the cross-tolerance induced by chronic olanzapine was spontaneously reversible after 16 drug free days. The DEC reverted back to its original position. The lines were analysed by probit analysis. Therefore, the two plots show that the tolerance induced by chronic clozapine was spontaneously lost when the animals were untreated. The tolerance was lost completely, as indicated by the fact that the two DECs were very similar.

All three lines are compared to each other and the following ED_{50} values were obtained with corresponding R^2 values.

Table 11.2: ED_{50} values and R^2 values for the difference DEC

DEC	ED_{50} values (mg/kg)	R^2 values
1	1.52	+0.94
2	5.2	+0.92
3	1.14	+0.96

The comparison of all the dose/effect curves shows that 10 days of chronic olanzapine (5 mg/kg, b.i.d.) induced cross-tolerance to the clozapine discriminative stimulus. But the tolerance dissipated spontaneously and completely over 16 days, as the DEC reverted back to its original position. The shifts in the curves were parallel,

which indicates that a common pharmacological mechanism underlies all three dose/effect curves. This would be expected because all dose/effect curves were carried out with clozapine.

The DEC's were also analysed by probit analysis and the following values were obtained:

Table 11.3: Comparison of parallelism of DECs and the ED_{50} potency ratio between the DECs.

DEC compared	Parallelism test	ED_{50} Potency ratios and 95% Confidence limits
1 v 2	$X^2 = 0.085$, d.f. = 1, p = 0.770, NS	3.4-fold, Lower =0.012, Upper = 0.81 , sig.
2 v 3	$X^2 = 0.176$, d.f. = 1, p = 0.675, NS	4.56-fold, Lower = 1.12 Upper = 331.26, sig.
1 v 3	$X^2 = 0.136$, d.f. = 1, p = 712 , NS	1.33-fold, Lower = 0.42, Upper = 5.8, NS

The confidence limits do not cover 1.0 for the comparison of DEC 1 and 2 and DEC 2 and 3 which indicates that there is a significant difference in potencies, but the confidence limit did not cover 1.0 for the comparison between DEC 1 and 3, which indicates that the potencies are not significant. Thus the results show that the three DECs can not be proved to be significantly non-parallel. The results from the probit

analysis also show that there is a significant potency ratio difference between DEC 1 and 2, and between DEC 2 and 3 but not between DEC 1 and 3, as expected.

Consequently, the results show that animals could be trained to discriminate between clozapine (5 mg/kg) and vehicle to a high level of accuracy. In addition, once the discrimination was learnt, a high level of accuracy was maintained throughout the study. Full dose-related generalisation was produced by clozapine before chronic olanzapine treatment using the dose range of 0.3125 to 5 mg/kg. Chronic olanzapine induced cross-tolerance to the clozapine discriminative stimulus. Cross-tolerance was spontaneously lost when the animals were left alone for 16 days with no further treatment. The cross-tolerance was indicated by a parallel shift to the right in the dose effect curve after chronic olanzapine treatment. The cross-tolerance was spontaneously lost as indicated by the DEC shifting back to the left at the end of the 16 days. Therefore, in this particular behavioural paradigm, olanzapine behaved in exactly the same way as clozapine.

11.4 Discussion

The study showed that olanzapine like clozapine induced cross-tolerance to the clozapine discriminative stimulus.

Tolerance is defined as a drug-induced parallel shift to the right in the dose/effect curve (Young & Sannerud, 1989). In this experiment, the dose/effect curve moved to

the right, but more than simple tolerance was involved, because the tolerance was induced by olanzapine to the discriminative stimulus of clozapine. The definition of cross-tolerance is the resistance to a drug imparted by chronic treatment with another drug (Lê & Khanna, 1989). Cross-tolerance has been shown previously to develop between clozapine and olanzapine with respect to their rate-suppressant actions (Goudie & Taylor, unpublished). Tolerance is known to be mediated by various different mechanisms, but the mechanism involved in the observed cross-tolerance to rate suppressant actions is unclear (Goudie & Taylor, unpublished).

There are several studies showing that the discriminative stimuli of clozapine and olanzapine are very similar, in fact so much so that they can induce full generalisation to each other (Porter & Strong, 1996). Thus, the cross-tolerance shown between clozapine and olanzapine in this study is not unsurprising and both compounds act upon multiple receptors and appear to rely upon multiple systems in order to produce their therapeutic effects. As noted in the introduction to this chapter studies have shown that the receptor binding profiles of olanzapine and clozapine are very similar (Porter & Strong, 1996).

Tolerance has been shown to occur in the clinic to the side effects of olanzapine. The side effects most commonly reported were dry mouth, weight gain, increased appetite and sedation (Wood, 1998). However, the side effects tolerated out within a few

days, so there were fewer discontinuations of the drug compared to haloperidol (Wood, 1998).

There are some observations of cross-tolerance between olanzapine and clozapine. They include: 1) this experiment; whereby chronic olanzapine induced cross-tolerance to clozapine discriminative stimulus and 2) olanzapine showed cross-tolerance with clozapine' rate suppressant actions (Porter & Strong, 1996). Therefore, cross-tolerance between olanzapine and clozapine emphasise the similarities between the two compounds.

Thus, the cross-tolerance observed with olanzapine and clozapine fits in with previous studies. Nevertheless, the precise mechanism is unknown as to how cross-tolerance was induced. It is most likely that the tolerance is of a pharmacodynamic nature because the tolerance was spontaneously lost (Rang & Dale, 1994). One possible method of pharmacodynamic tolerance is the decrease in neural sensitivity due to tolerance (Rang & Dale, 1994). This is induced by prolonged exposure to drug, in this experiment chronic treatment with olanzapine (5 mg/kg, b.i.d.). So during chronic treatment, the sensitivity of the neural system decreased and this changed how the drug produced its effect. Thus, at the end of chronic olanzapine treatment there was tolerance to the clozapine cue. This experiment showed that the tolerance induced by olanzapine to the clozapine discriminative stimulus was spontaneously reversible.

The precise neural systems that may be involved are unknown. It could be one or more of the combination of systems that clozapine and olanzapine act upon. Further studies with specific receptor ligands will need to be carried out to determine the precise receptors involved. Thus, olanzapine generalises to clozapine and induces cross-tolerance to the clozapine discriminative stimulus. However, the tolerance is spontaneously reversed indicating a pharmacodynamic nature of the tolerance. So, in this paradigm olanzapine mimics clozapine.

Olanzapine has been shown to act on the following receptors:

$D_{1,2\&4}$, 5-$HT_{2A,2c,3,5\&7}$, noradrenergic $apha_2$, $H_{1\&3}$ and M_{1-5}.

However, the overlap of receptors between clozapine and olanzapine is as follows;

$D_{1,2\&4}$, 5-$HT_{2A,2C,3,6\&7}$, noradrenergic $alpha_2$, H_1 and M_1.

Therefore, since both drugs induced to tolerance to the discriminative stimulus of clozapine and this would suggest that the mechanism of action would involve receptors that both compounds acted upon. Therefore, the list of potentially active receptors has not been reduced dramatically but it could have eliminated some potential receptors.

11.5 Conclusions

The data show that the animals learnt the clozapine (5 mg/kg) versus vehicle discrimination to a high level of accuracy and once learnt the accuracy level was maintained. The results show that olanzapine produced full generalisation to the clozapine cue and that chronic olanzapine produced tolerance to the clozapine discriminative stimulus after 10 days administration. The tolerance was spontaneously lost when the animals were left alone for 16 days and then re-tested.

Chapter 12.0

Effects of Chronic JL13 Treatment on Tolerance to the Discriminative Stimulus Effects of Clozapine

12.1 Introduction

The previous study that these animals were used in showed that it was possible to induce tolerance to the clozapine stimulus by chronic clozapine treatment. The next step was to investigate whether other compounds could produce the same effect. The compound chosen to study next was JL13; which is a novel compound producing partial generalisation to clozapine (Bruhwyler *et al*, 1997). JL13 was chosen after olanzapine because although clozapine and olanzapine had both induced cross-tolerance to the discriminative stimulus of clozapine, then it was still too early to assume that any compound which induced a reasonable level of generalisation to the clozapine cue would induce cross-tolerance to the clozapine cue. JL13 has been shown to be similar to clozapine in many different paradigms (see later notes), however clozapine and olanzapine had not been compared in this paradigm. Also, whilst JL13 induces clozapine-like appropriate responding then the two compounds do not act on all the same receptors. Also, olanzapine induces full generalisation to the clozapine cue, whilst JL13 only induces partial generalisation to clozapine. Hence, differences in the level of cross-tolerance may have been observed by chronic JL13 to the clozapine cue.

JL13, (5-(4-methylpiperazin-1-yl)-8-pyrido[2,3-*b*][1,5] benzoxazepine fumarate) is a putative atypical neuroleptic in the class of pyridobenzapines and is structurally related to clozapine (Bruhwyler *et al*, 1992) produced by Therabel Research in Belgium. JL13 has very little affinity for 5-HT_{2c} receptors, but has a greater affinity for 5-HT_{2a} receptors. Like clozapine, it is classed as a "multi-receptor" antagonist

with a high affinity for several different receptors. JL13 is not only clozapine like in the drug discrimination assay (Goudie & Taylor, 1998), but also in other assays (Bruhwyler *et al*, 1995). Both JL13 (4-16 mg/kg) and clozapine (4-16 mg/kg) have shown significant dose-dependent increases in immobility time in the forced swimming test (Bruhwyler *et al*, 1997). In binding assays both clozapine and JL13 have a 5-HT_2/D_2 ratio superior to 1.12 (Bruhwyler *et al*, 1995) which appears to be an important discriminating factor between typical and atypical neuropletics. Bruhwyler *et al* (1992) showed that JL13 altered operant performance like clozapine without inducing the same side effects as clozapine which include: sialorhea, ataxia, akinesia, tremor and dystonia (Bruhwyler *et al*, 1992).

In one study JL13 was shown to induce 70% cross-generalisation to clozapine (Goudie & Taylor, 1998) when administered in exactly the same way and under exactly the same experimental conditions. Therefore, JL13 demonstrates a similar pharmacological profile to clozapine. Like clozapine, JL13 shows a separation by at least a factor of 10 between the doses that inhibit apomorphine-induced stereotypy and those that inhibit apomorphine-induced climbing or amphetamine-induced hyperactivity (Bruhwyler *et al*, 1997). Apomorphine or amphetamine-induced stereotypy is a response dependent on the integrity of the dopaminergic input to the neostriatum (Bruhwyler *et al*, 1997). Both clozapine and JL13 interfere with this neural system to a minimal level only (Bruhwyler *et al*, 1997). Apomorphine-induced climbing and amphetamine-induced hyperactivity are mediated by enhanced dopaminergic transmission in the neurones in the mesolimbic system (Bruhwyler *et*

al, 1997), which are antagonised by both clozapine and JL13 (Bruhwyler *et al*, 1997). It has been shown clozapine has a high affinity for the 5-HT_{2A} and 5-HT_{2C} receptors, whilst JL13 binds to the 5-HT_{2A} receptors, but is inactive at 5-HT_{2C} receptors (Bruhwyler *et al*, 1997). Comparison of the discriminative properties of clozapine and JL13 in squirrel monkeys showed that JL13 substituted fully for clozapine (90%) (Bruhwyler *et al*, 1997).

Hence, JL13 is a compound that displays multi receptor targets (D_4, 5-$HT2_{2A}$, alpha-1, H_1...) and has a low affinity for the D_2 receptors. It shows therefore an activity profile similar to that of clozapine, an atypical neuroleptic, whilst displaying some advantages such as the absence of sialorrhea and tremor. These results suggest that JL13 will have an atypical profile and be less likely to induce unwanted extra-pyramidal symptoms in the clinic than the currently available atypical antipsychotics (Bruhwyler *et al*, 1997).

The aim of this study was therefore to investigate if JL13 could induce cross-tolerance to the clozapine stimulus. It was predicted that JL13, like olanzapine, would induce cross-tolerance. However, if tolerance/cross-tolerance to the clozapine cue was due to a specific mechanism, then JL13 may not have produced cross-tolerance because it only partially generalises to the clozapine cue.

12.2 Methods

Subjects

A maximum of 12 female Sprague Dawley rats (bred at the Psychology Department, University of Liverpool, UK) were used in this experiment. 12 animals were used to calculate the TEC, but during the chronic phase of the study 4 animals had to be dropped from the study due to non-study related problems (two rats developed sore paws and their limbs swelled up and the other two rats developed sores at the injection sites).There were two groups of animals used in this series of experiments and these animals had been previously used to study clozapine tolerance in the drug discrimination procedure as shown in the table below:

Table 12.1: Groups number and which drugs were used in each group

Group	Drug administered	Number of days between experiments
Previously trained animals	Clozapine	15
	JL13	38 (including Christmas)
	Cyproheptadine	-
Experimentally naïve animals	Olanzapine	23
	CDP	-

Apparatus

As previously described in methods section.

Training Procedure

As previously described in method section.

Testing Procedure for determining dose/effect curves

As previously described in clozapine chapter.

Procedure for derivation of time-effect curve

It was necessary to determine the time/effect curves, in order to assess the pharmacodynamic profile of JL13 (see clozapine chapter for rationale). 10 animals were used to determine the time/effect curve.

Once the group accuracy was above 85% correct lever selection, a time/effect curve was conducted. Animals were injected with JL13 (10 mg/kg), one sub-group (n = 5) at 0830 and the second sub-group (n = 5) at 0930 hours. The animals were tested at different time points throughout the day 15 min, 30 min, 1, 4 and 8 hours post-injection. Thus the animals were tested at 0845, 0900, 0930, 1230 and 1630 hours for group one that was injected at 0830; and at 0945, 1000, 1030, 1330 and 1730 hours for group two which had been injected at 0930 hours. The duration of action on neural systems of JL13 was measured indirectly by the percentage of drug lever selections made at each time interval tested.

Procedure for dose- effect (generalisation) curve

The rationale and method used was identical to the clozapine experiment except that the animals received b.i.d. injections of JL13 (20 mg/kg, b.i.d.). The dose of JL13 (twice the highest dose for generalisation) and the frequency of dosing (b.i.d.) were chosen to facilitate tolerance development. The other difference between the clozapine and JL13 experiment was that it was decided not to give the animals a "top-up" dose of JL13 once the animals had received the clozapine in the morning for DEC 2. This is because it would have been virtually impossible to calculate the additional dose of JL13 required to make the same dose of clozapine as the animals usually received. However, on days 11 and 12 the animals *also* received their usual 20 mg/kg dose of JL13 at 1600 hours to prevent withdrawal and maintain tolerance. The data were analysed and plotted in exactly the same way as for the clozapine study. However, only 8 animals were used in the dose/effect curves, because four of the animals had to be dropped from the study due to non-drug related illnesses.

Statistics

As previously described in the clozapine chapter.

Drugs

Clozapine base (Sandoz, Switzerland) was administered i.p., dissolved in a few drops of 0.1 M HCl, diluted with water and buffered back with 0.1M NaOH to a pH around 5.5 and injected at a volume of 2 ml/kg. JL13 (Therabel) was made up in exactly the same way as clozapine The drugs were injected 30 minutes before operant sessions.

12.3 Results

The results (see clozapine chapter) showed that once the tolerance to the chronic clozapine had been lost then the animals responded normally. The animals were left for 10 days between the end of the clozapine study and this one to allow them to recuperate.

The initial part of this experiment involved a time effect curve, calculated initially at only 1, 4 and 8 hours post injection, but the results showed that in actual fact that JL13 needed to be measured at a shorter time period post injection in order to check the level of generalisation between clozapine and JL13. So two days later the animals were tested 15 and 30 minutes post injection. The time/effect curve showed, as expected, that depending on the time point studied the amount of generalisation between clozapine and JL13 altered.

Fig. 12.1: The time/effect curve for JL13

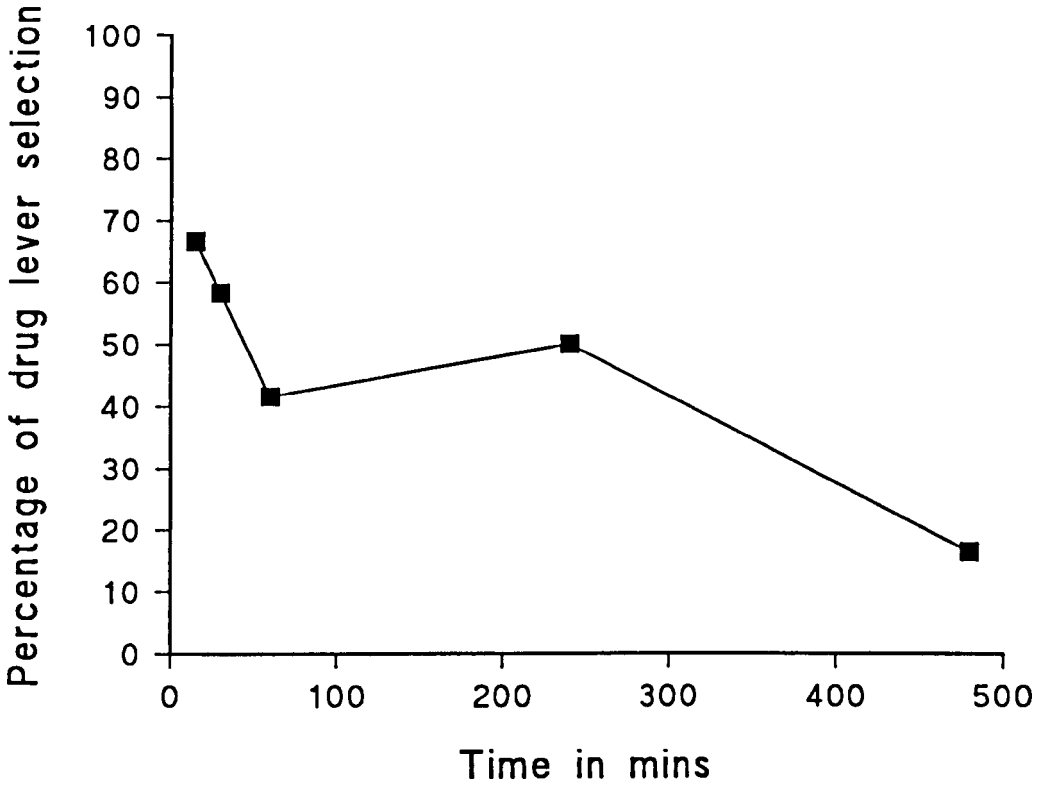

Fig. 12.1: The time/effect curve showed that at 10 mg/kg JL13 partially generalised to clozapine (66.6 % maximum), as shown by the fact that the animals chose the drug lever at this level at the start of the time/effect curve. The data show that the longer after the time point the animals were injected, then the less like clozapine, JL13 was. The data also show that JL13 was quite short acting. This is indicated by the fact that by one hour, there was only about 40% generalisation to clozapine. However, when the animals were tested at 4 hours post-injection, the level of generalisation had actually risen to above 50%. However, this level of generalisation decreased again when the animals were tested at 8 hours post-injection to about 20%.

The time/effect data show the duration of action of JL13 on neural systems, allowing an indirect assessment of how long the drug remains in the animals' body. Thus figure 15.1 suggests that JL13 is quite a short acting compound, which is why JL13 was given at 20 mg/kg b.i.d. in the tolerance phase of the study. The other advantage of carrying out a time/effect curve before the chronic study is it determines the degree of generalisation between clozapine and the test compound. If JL13 had been shown not to generalise to clozapine, there would have been no point in carrying out the chronic study. This is because it is assumed that in order for the tolerance observed with chronic clozapine to be seen with another test compound, the two drugs need to show partial if not full generalisation. If no or only little generalisation had occurred, it would have been assumed that the compound could not induce tolerance to the clozapine stimulus. Thus, the data showed that JL13 was worthy of testing because of its relatively high level of generalisation to clozapine. The disadvantage of JL13 was that it appeared to be quite short acting, and a short duration of action might actually hinder the experiment as the receptors would never be saturated for long enough to induce cross-tolerance. However, depending on which part of the time effect curve was studied the similarity of the curve to the clozapine time/effect curve varied, as shown below.

Fig. 12.2: Comparison of the clozapine and JL13 time/effect curves (Clozapine data are reproduced from chapter 10)

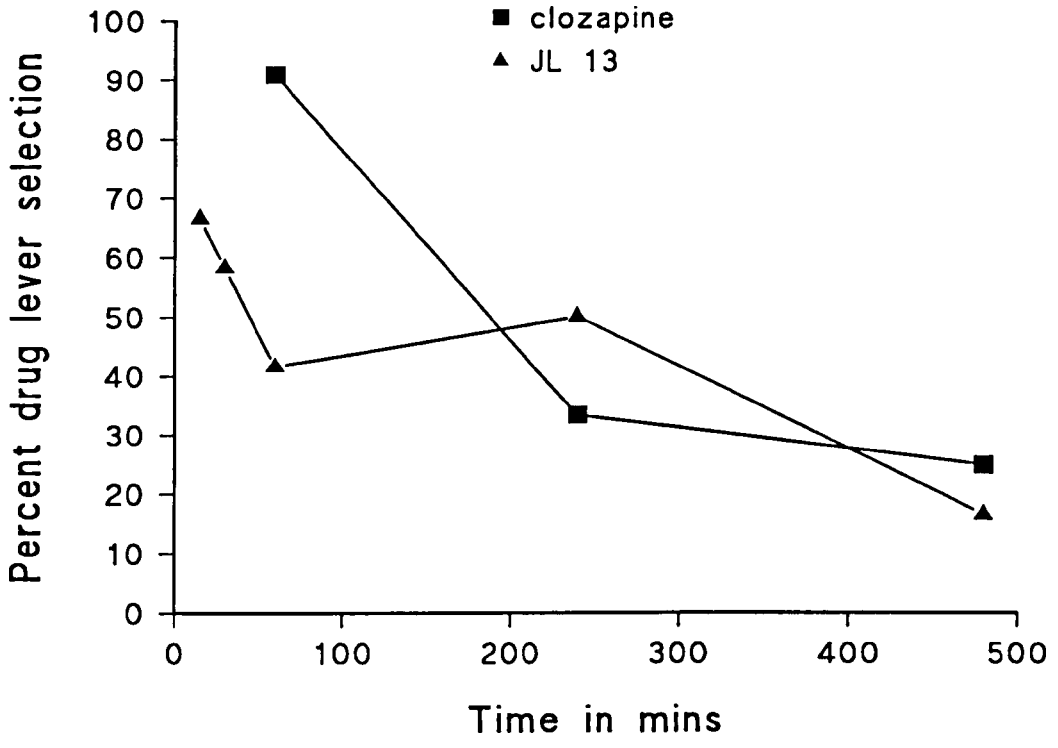

Fig. 12.2: The figure shows that initially clozapine and JL13 were not alike and that clozapine induced much more drug lever selection. However, if the points at 4 and 8 hours post injection are compared there is very little difference in the time/effect curves for clozapine and JL13. In actual fact 4 hours post-injection shows that JL13 induced slightly higher drug lever selection compared to clozapine, however the difference was minimally. The TEC showed that JL13 induced partial generalisation to the clozapine cue in these animals and that JL13 was not exceptionally long lasting.

However, the degree of generalisation between JL13 and clozapine is dose-dependent and no inferences can be made about the degree of generalisation of other doses of JL13 and clozapine.

Therefore the time/effect curve data show that JL13 does partially generalise to clozapine and that is it relatively short acting like clozapine. It was decided to double the dose for the chronic study and to administer two injections daily. The dose of JL13 was only double and administered twice daily because it was then comparable to other compounds that had been used.

The subsequent stages of the experiment were carried out. The dose/effect curve was carried out by subjecting the animals to a series of doses ranging from the training dose downwards in a logarithmic manner until the animals could barely discriminate the drug from vehicle. In DEC 1 and 3 the dose range was 5 to 0.3125 mg/kg and in DEC 2 the dose range was 5 to 1.25 mg/kg.

Fig. 12.3: Comparison of all three dose/effect curves

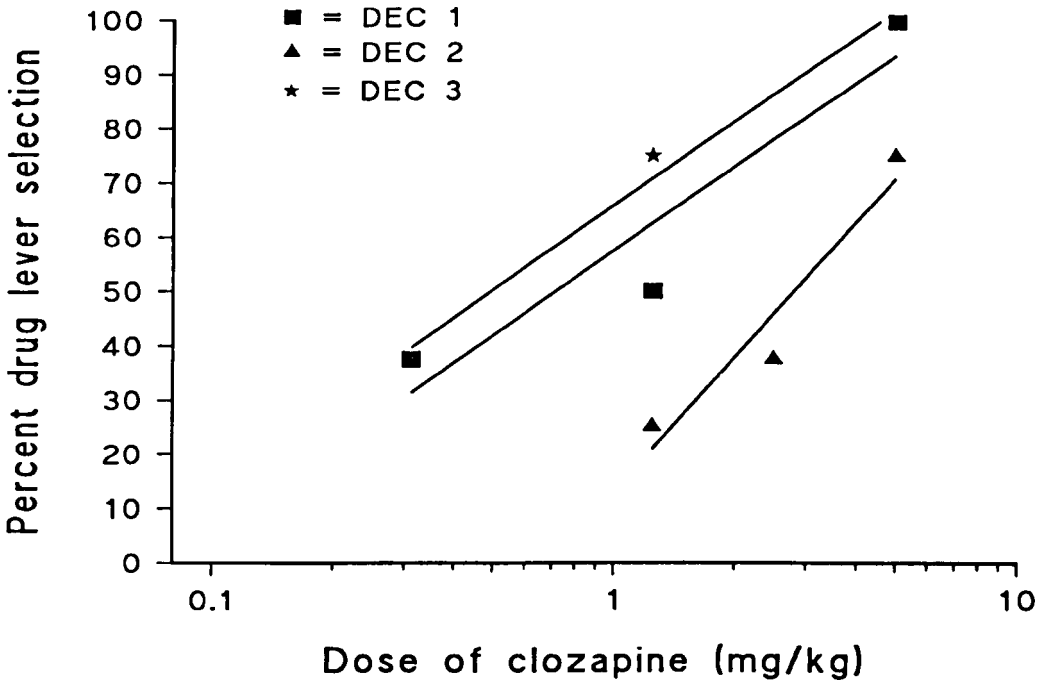

Fig. 12.3: The data show that chronic JL13 induced cross-tolerance to the clozapine cue which was spontaneously lost when they were left alone. The missing stars are hidden under the respective squares.

DEC 1:The results show that the animals decreased their drug lever selection as the dose of clozapine decreased. When the animals were tested at 5 mg/kg all the animals chose the drug lever. All the results were analysed with log/linear least squares regression analysis (see table 12.2) and the regression lines were a good fit to the data. Hence, DEC 1 showed that the animals responded as expected, and that as the dose of clozapine decreased so did drug lever selections.

Thus once DEC 1 had been calculated, the animals were treated chronically with JL13 (20 mg/kg, b.i.d.). The animals were injected twice a day for 10 days, and during this time they received no further discrimination training. The animals were weighed, injected and then placed back in their home cages until the next injection. At the end of the chronic JL13 treatment, another dose/effect curve (DEC 2) was determined on days 11-13.

DEC 2: Chronic JL13 treatment shifted the dose/effect curve to the right. The line were analysed by probit analysis and the calculated probit lines were analysed for parallelism (see table 12.3). The shift in the DECs was in parallel to the right (indicative of cross-tolerance). The parallelism shows that the same mechanism was involved in both DEC 1 and DEC 2, due to clozapine being used to determine DEC 1 and DEC 2.

The animals were left alone for 16 days with no further JL13 injections or discrimination training to investigate whether the cross-tolerance induced by chronic JL13 would spontaneously dissipate by computing DEC 3.

DEC 3: The data show that the cross-tolerance induced had been spontaneously lost after 16 drug free days. The DEC reverted back to its original position. The lines were again analysed by probit analysis (see table 12.2). Thus, a comparison of DEC 1

and 3 shows that the cross-tolerance induced by chronic JL13 was spontaneously lost when the animals were untreated. The cross-tolerance was completely lost, as indicated by the fact that the DECs were very similar.

Therefore, the results (figure 12.3) show that the animals showed dose-related generalisation to clozapine before, during and after chronic JL13. However, the difference being that during chronic JL13 treatment the doses of clozapine needed to induce generalisation were higher than in the other two stages of the experiment.

All three lines are compared to each other and the following ED_{50} values was obtained with the corresponding R^2 values as shown below.

Table 12.2: ED_{50} values and R^2 values for the different DECs

DEC	ED_{50} value	R^2 value
1	0.96	+0.89
2	2.81	+0.92
3	0.50	+0.99

The comparison of all the dose/effect curves shows that 10 days of chronic JL13 (20 mg/kg, b.i.d.) induced cross-tolerance to the clozapine discriminative stimulus, but that it spontaneously dissipated completely over 16 days as the DEC reverted back to its original position. The shifts in the curves were parallel, which indicates that a common pharmacological mechanism underlies all three dose/effect curves. This would be expected because all dose/effect curves were carried out with clozapine.

The lines were analysed by probit analysis and the following values were obtained:

Table 12.3: Comparison of parallelism of DECs and the ED_{50} potency ratio between the DECs

DEC compared	Parallelism results	ED_{50} Potency ratios and 95% confidence limits
1 v 2	$X^2 = 0.320$, d.f. = 1, p = 0.571, NS	2.92-fold shift, Lower = 0.002, Upper = 0.84, sig.
2 v 3	$X^2 = 0.036$, d.f. = 1, p = 0.849, NS	5.62-fold shift, Lower = 1.45, Upper = 1228.2, sig.
1 v 3	$X^2 = 0.194$, d.f. = 1, p = 0.659, NS	1.92-fold shift, Lower = 0.491, Upper = 7.35, NS

The results of the probit analysis calculated confidence limits which for the comparison of DEC 1 v 2 and DEC 2 v 3 do not cover 1.0, which indicates that there is a significant difference. However the confidence limits for DEC 1 v 3 do not cover 1.0 and this indicates that there is not a significant difference between these two lines as would be expected. The ED_{50} potency ratios show the degree of movement between the three lines, i.e. after the chronic administration of JL13 the curve had moved 2.92 fold to the right compared to before chronic JL13 administration. These results of the parallelism test show that the lines can not be proved to significantly non-parallel.

Therefore, the experiment showed that clozapine can be discriminated and that chronic JL13 can induced cross-tolerance to the clozapine discriminative stimulus which was spontaneously lost.

12.4 Discussion

The study showed that JL13 generalised partially (66.6%) to clozapine and induced cross-tolerance to the clozapine discriminative stimulus. Partial generalisation between clozapine and JL13 is shown by the time effect curve for JL13. It is possible that full generalisation may have been achieved if a higher dose of JL13 had been used in the TEC. Although the response rates (data not shown) of some of the animals were lower than when administered vehicle. Also other studies have shown

that only partial generalisation occurs between JL13 and clozapine (Bruhwyler *et al*, 1997). The induction of cross-tolerance is shown by the parallel, significant shift to the right in the DEC after chronic JL13 treatment. Tolerance was reversible, shown by the fact that the DEC moved significantly back to the left. Therefore, the data show that JL13 resembles clozapine in this aspect of its psychopharmacological profile.

The initial part of this experiment was the time/effect curve (TEC). JL13 was quite short acting and the initial reasonably high level of generalisation to clozapine wore off quite quickly. However, once the initial decrease in effect had occurred, the remainder of the drug was around for quite a few hours. As already stated, the idea behind the TEC was 1) to check the initial generalisation, and 2) to check that the drug was not exceptionally long lasting in the animal. The by-products that were created by the drug being metabolised were of no consequence. Whilst the dose of JL13 did not induce possible maximal generalisation then there was sufficient to say that the JL13 and the clozapine were similar.

During chronic JL13 treatment the animals received no further discrimination training. Discrimination training was suspended because previous studies (Sannerud & Griffiths, 1993) have shown that if discrimination training is continued, then no tolerance occurs. This is due a process of fading (see clozapine chapter). The chronic treatment of JL13 had no adverse effects on the animals at all. Chronic JL13 induced

cross-tolerance to the clozapine discriminative stimulus, which was both significant and parallel.

This reversibility of tolerance suggests that the cross-tolerance was of a pharmacodynamic nature. Rang & Dale (1994) have shown that when receptors or neural systems are made less sensitive to a stimulus, if the receptors or neural systems are subsequently left alone with no further treatment, they spontaneously revert back to their original sensitivity. However, the precise mechanism of the tolerance observed is unknown. It is possible that, since both clozapine and JL13 act on multiple receptors, tolerance could be due to actions on several receptors. The actual receptors have not been identified and much more work is necessary before any suggestions can be made about the involvement of specific receptors.

Therefore, the results show that JL13 induced cross-tolerance to the clozapine discriminative stimulus that was spontaneously reversible. The precise mechanism of the tolerance is unknown, although it is suggested to be pharmacodynamic in nature and possibly involving multiple receptors. Hence, JL13 acts in a similar manner as clozapine in this behavioural test.

JL13 has actions at the following receptors:

$D_{2,\&4}$, 5-HT_{2A}, noradrenergic $alpha_1$ and H_1, whilst clozapine is known to act at many more receptors. Therefore, the overlapping receptors are;

D2, 5-HT_{2A}, noradrenergic $alpha_1$ and H_1.

JL13 is a more selective compound compared to clozapine, but it still produces effects like clozapine i.e. partial generalisation, cross-tolerance to clozapine's discriminative stimulus. Therefore, receptors that are acted upon by both compounds are potentially the most likely to be used in the production of cross-tolerance to the discriminative stimulus of clozapine. Hence, JL13 has allowed the potential number of involved receptors to be narrowed even further than olanzapine.

12.5 Conclusions

The data showed that JL13 induced partial generalisation to the clozapine discriminative stimulus. Chronic JL13 also induced cross-tolerance to the clozapine discriminative stimulus, as shown by the shift to the right in the DEC. The cross-tolerance was spontaneously lost, as shown by the fact that when leaving the animals for 16 days with no further drug treatment or discrimination training and re-testing of the animals, then the DEC had reverted back to its original position.

Chapter 13.0

Effects of Chronic Cyproheptadine Treatment on Cross-tolerance to the Discriminative Stimulus Effects of Clozapine

13.1 Introduction

Cyproheptadine like clozapine, is a multi-receptor antagonist and both have actions at 5-HT_2, H_1 and M_1 receptors. It has been shown that cyproheptadine generalises to clozapine possibly due to its common receptor actions (Brown & Koe, 1982). However, unlike clozapine, cyproheptadine does not act at lots of different receptors, instead it has a more selective pharmacological profile than clozapine.

In pigeons, cyproheptadine has been shown to produce discriminative effects similar to clozapine (Hoenicke *et al*, 1992). It was shown that compounds which had antagonistic actions at both 5-HT_{2A} and 5-HT_{2C} receptors produced discriminative stimulus effects similar to clozapine e.g. cyproheptadine, metergolide etc. However, 5-HT receptor antagonists that were selective for 5-HT_{2A} receptors compared to 5-HT_{2c} receptors did not produce substantial clozapine-appropriate responding. Other 5-HT receptor compounds that did not produce high levels of clozapine-appropriate responding included; ondansetron (5-HT_3 antagonist), quipazine (5-HT_{2C} agonist). Therefore, the results suggest that the blockade of both 5-HT_{2A} and/or 5-HT_{2C} receptors is needed for the mediation of clozapine discriminative stimulus properties. The precise role of 5-HT_{2C} receptor in the clozapine discriminative stimulus is unknown. The blockade of 5-HT_{2A} receptors does not appear to be sufficient to produce the clozapine discriminative stimulus alone in pigeons. Therefore, these data suggests that the 5-HT_{2C} antagonist effect *in vivo* may be important in clozapine's unique pharmacological profile as an atypical neuroleptic (Steinpreis *et al*, 1996).

So, several studies have shown that clozapine and cyproheptadine act in similar ways and it has been shown that cyproheptadine generalises to clozapine (Brown & Koe, 1982). So, it was decided to investigate the effects of cyproheptadine on the clozapine discriminative stimulus in this behavioural paradigm.

The rational behind using cyproheptadine as the next compound in the sequence, is that whilst it generalises partially to clozapine in the drug discrimination paradigm, it acts upon fewer receptors than the previously tested compounds. So it allowed the potential number of receptors involved in mediating cross-tolerance to the clozapine cue to be reduced. The use of cyproheptadine, if it induced cross-tolerance to the clozapine cue, would be that as shown in other studies then cyproheptadine is similar to clozapine. It could be suggested that the more clozapine-like cyproheptadine is then the more chance it has of acting like an atypical antipsychotic in the clinic at the correct dose.

13.2 Methods

Subjects

A maximum of 12 female Sprague Dawley rats (bred at the Psychology Department, University of Liverpool, UK) were used in this experiment. The 12 subjects were *not* experimentally naïve, as they had previously been used in the clozapine and JL13 tolerance studies. 12 animals were used to calculate the TEC, but only 8 animals were used in the tolerance phase (see JL13 chapter for the reasons why the 4 animals were dropped from the study). This is because the TEC for cyproheptadine was

carried out before the JL13 study, but due to problems with high doses of the drug not dissolving, then the JL13 study was carried out whilst advice was received from the manufacturers on solubility. At the end of the clozapine and more importantly the JL13 studies the animals had been shown to have lost all their tolerance to the clozapine discriminative stimulus.

Two groups of animals were used in this series of experiments and the groups and their drugs are shown below:

Table 13.1: Groups number and which drugs were used in each group

Group	Drug administered	Number of days between experiments
Previously trained animals	Clozapine	15
	JL13	38 (including Christmas)
	Cyproheptadine	-
Experimentally naïve animals	Olanzapine	23
	CDP	-

Apparatus

As described in the methods section.

Training Procedure

As described in the methods section.

Testing Procedure for determining dose/effect curves

As described in the clozapine chapter.

Procedure for derivation of time-effect curve

It was necessary to calculate the time/effect curve in order to assess the pharmacodynamic profile of cyproheptadine (see clozapine chapter for rationale). 11 animals were used to calculate the time/effect curve. The animals were injected with cyproheptadine (40 mg/kg), one group (n = 5) at 0830 and the second group (n = 6) at 0845 hours. Then the animals were tested at different time points throughout the day 1, 4, 8 and 16 hours post-injection. So the animals were tested at 0930, 1230 and 1630 hours for group one that were injected at 0830, and at 0945, 1245 and 1645 hours for group two which had been injected at 0845 hours. In order to establish the generalisation level between cyproheptadine and clozapine 16 hours post-injection of cyproheptadine, the animals were injected at 1630 and 1645, then run the next day at 0830 and 0845 respectively. The duration of action of cyproheptadine was measured by the percentage drug lever selection made at each time interval tested.

Procedure for dose- effect (generalisation) curves

The method used was the same as in the clozapine chapter except that the animals were chronically injected for ten days with cyproheptadine once a day (40 mg/kg). Also only 8 animals were used to determine the dose/effect curve because of four of the animals in between the time/effect curve and determination of the dose/effect curves were dropped from the study due to non-drug related illnesses. The dose of

cyproheptadine of 40 mg/kg was chosen despite the fact that in the rest of these tolerance and cross-tolerance studies the dose usually used was twice that which produced maximum generalisation. There were two reasons for this; 1) the dose of 40 mg/kg of cyproheptadine was long lasting and was believed to be sufficiently long lasting (lasted longer than 8 hours) to produce cross-tolerance during the chronic period; and 2) the drug failed to dissolve at higher doses than 40 mg/kg. The animals were injected at 0900 daily only for 10 days. After chronic cyproheptadine treatment, the animals were left alone for one day with no injections or discrimination training, in order to allow any residual cyproheptadine to dissipate, then DEC 2 was carried out. On the 12-14th consecutive days after the start of chronic cyproheptadine (40 mg/kg) treatment the animals were re-tested and DEC 2 calculated. In previous experiments, on days 11 and 12 the rats had also received their usual dose of drug at 1600 hours to prevent withdrawal and maintain tolerance, but as cyproheptadine was so long lasting that this was decided not to be necessary in this specific study.

Statistics

As described in the clozapine chapter.

Drugs

Clozapine base (Sandoz, Switzerland) was administered i.p., dissolved in a few drops of 0.1 M HCl, diluted with water and buffered back with 0.1M NaOH to a pH around 5.5 and injected at a volume of 2 ml/kg. Cyproheptadine base (Sigma, UK) was administered i.p., dissolved in distilled water and buffered back with 0.1N NaOH to a

pH around 5.5 and injected at a volume of 2 ml/kg. The drugs were administered 30 minutes before operant sessions.

13.3 Results

The animals were re-trained to discriminate clozapine (5 mg/kg) versus saline after resting after the JL13 study, for 5 days on which their accuracy was at least 85% on any one day. Once the animals were all responding reliably and their response rates were stable over days, the time/effect curve was determined.

Fig. 13.1 Time/effect curve of cyproheptadine for 40 mg/kg

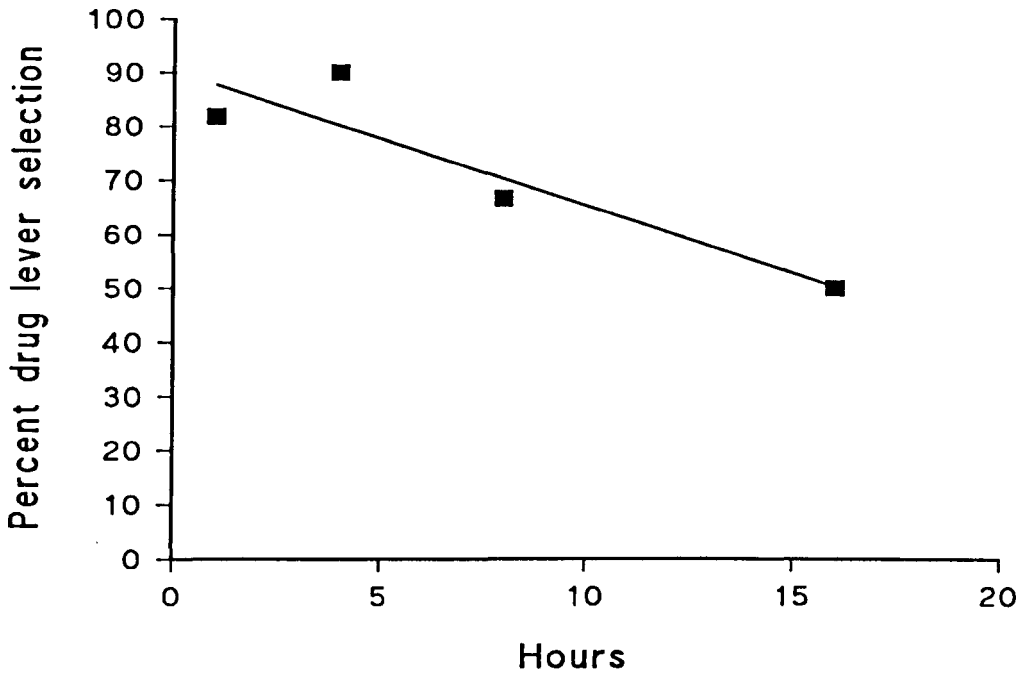

Fig. 13.1 The graph shows that one hour post injection, most of the animals choose the drug lever. After 4 hours drug lever selection had increased very slightly and after 8 hours drug lever selection was still above 50%. It was only after 16 hours drug lever selection had decreased to only 50%.

However, the degree of generalisation between cyproheptadine and clozapine is dose-dependent and no inferences can be made about the degree of generalisation of other doses of cyproheptadine and clozapine.

The time/effect curve is a predictor of the duration of action of cyproheptadine. It allows an indirect assessment of how long the drug remains in the animals' body.

Thus, figure 13.1 shows clearly that cyproheptadine is very long acting and that even after 16 hours, the animals showed 50% drug lever selection. It is because cyproheptadine so is long acting, that it was decided to only administer the compound once a day and not b.i.d. and to also use 40 mg/kg and not double the dose used for the TEC for chronic treatment.

The subsequent stages of the experiment were to determine if cyproheptadine could induce cross-tolerance and if so, would it be lost spontaneously. The dose/effect curve was carried out by subjecting the animals to a series of clozapine doses ranging from the training dose downwards in a logarithmic manner. Hence, for DEC 1 and 3 the doses ranged from 0.3125 to 5 mg/kg and for DEC 2 the doses ranged from 1.25 to 5 mg/kg.

Fig. 13.2 Comparison of all three dose/effect curves

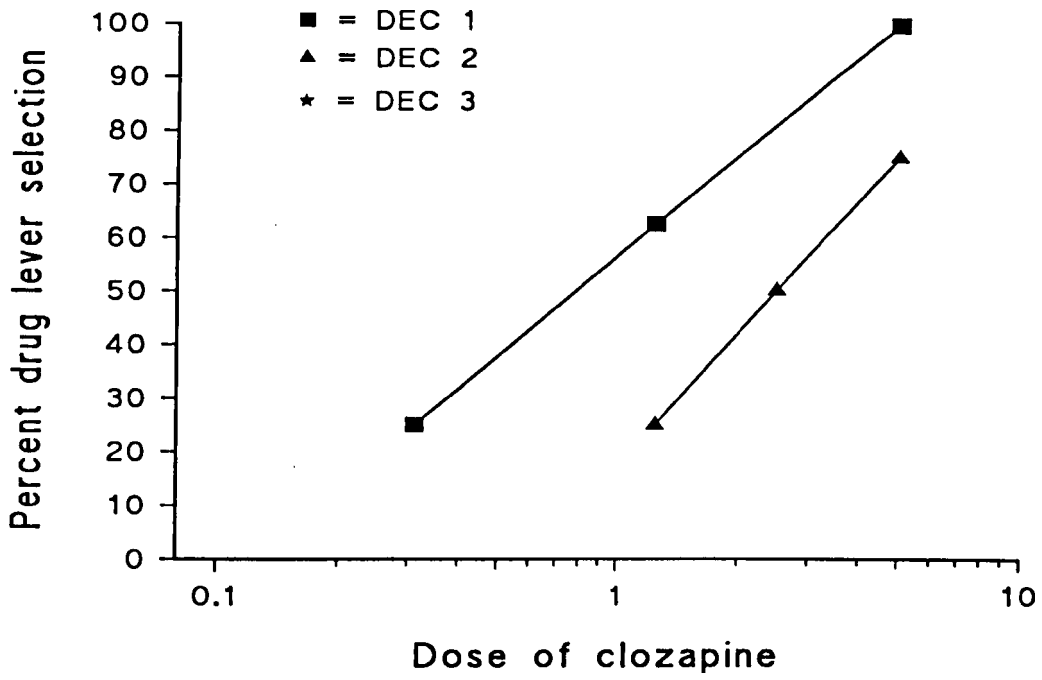

Fig. 13.2 The data show that chronic cyproheptadine induced cross-tolerance to the clozapine cue that was spontaneously reversed when the animals were left alone. The missing stars are all located under the squares.

DEC 1: The data show that the animals decreased their drug lever selection as the dose of clozapine decreased. The animals showed 100% drug lever selection when the training dose of 5 mg/kg of clozapine was administered. When a dose of 1.25 mg/kg was administered about 72% of the animals responded on the drug lever; whilst once the dose decreased to 0.3125 mg/kg about 27% of the animals responded on the drug lever. A regression line was plotted for all 3 lines and the regression line provided a perfect fit to all the data (see table 13.2).

Once DEC 1 had been carried out, the animals were treated chronically with cyproheptadine (40 mg/kg). The animals were injected once a day for 10 days with cyproheptadine, and during this time, they received no discrimination training. The animals were weighed, injected and then placed back in their home cages until the next day to be injected. At the end of the chronic cyproheptadine (40 mg/kg a day) treatment regime, another dose/effect curve (DEC 2) was carried out. The second dose/effect curve was carried out on days 12-14, on day 11, the animals were left alone and received no cyproheptadine or clozapine, to ensure they were drug free when computing DEC 2.

DEC 2: The data show that chronic cyproheptadine shifted the dose/effect curve to the right. The lines were analysed by probit analysis and the calculated probit lines were analysed for parallelism (See table 13.3).

The shifts in the DECs were parallel to the right (indicative of tolerance). The parallelism shows that the same mechanism was involved in both DEC 1 and DEC 2 due to clozapine being used to determine DEC 1 and DEC 2.

The rats were then left alone for 16 days without any further cyproheptadine injections or discrimination training to investigate by computing DEC 3 if the tolerance induced by chronic cyproheptadine would spontaneously dissipate.

DEC 3: The data show that the tolerance induced by chronic cyproheptadine had spontaneously dissipated after 16 days. The DEC reverted back to its original position in fact all the points for DEC 1 and 3 line up exactly. So it appears that there is only DEC in the graph. The lines were analysed by probit analysis (See table 13.3). The data, DEC 1 v 3, show that the tolerance induced was spontaneously completely lost when the animals were untreated for 16 days.

All three lines are compared to each other and the following ED_{50} values were obtained with corresponding R^2 values as shown below.

Table 13.2: ED_{50} values and R^2 values for the different DECs

DEC	ED_{50} value	R^2 value
1	0.78	+1.0
2	2.5	+1.0
3	0.78	+1.0

A comparison of all the dose/effect curves shows that 10 days of chronic cyproheptadine (40 mg/kg) induced cross-tolerance to the clozapine discriminative stimulus. The tolerance spontaneously dissipated completely over 16 days as the DEC reverted back exactly to its original position. The shifts in the cures were

parallel, which indicates that a common pharmacological mechanism underlies all three dose/effect curves. This would be expected because all dose/effect curves were carried out with clozapine.

The lines were analysed by probit analysis and the following values were obtained.

Table 13.3: Comparison of parallelism of DECs and the relative ED_{50} potency ratio between the DECs

DEC compared	Parallelism results	ED_{50} potency ratio and 95% confidence limits
1 v 2	X^2 =1.0, d.f. = 1, p = 1.0, NS	3.2-fold shift, Upper = 0.009, Lower = 0.86, sig.
2 v 3	X^2 = 1.0, d.f. = 1, p = 1.0, NS	3.2-fold shift, Upper = 1.15, Lower = 109.0, sig.
1v 3	X^2 = 1.0, d.f. = 1, p = 1.0, NS	1.0-fold shift, Upper = 0.35, Lower = 2.8, NS

Thus, the results of the parallelism tests show that the three DECs can not be proved significantly non-parallel. The results from the probit analysis show that there is a

significant potency ratio difference between DEC 1 and 2, and between DEC 2 and 3, but not between DEC 1 and 3, as expected. This is shown by the confidence limits not covering 1.0 for DEC 1 v 2 and DEC 2 v 3, which indicates a significant difference, but for DEC 1 v 3 then the confidence limits did cover 1.0 so there was not a significant difference.

Therefore, the experiment showed that clozapine can be discriminated and that chronic cyproheptadine induced cross-tolerance to the clozapine stimulus, and that the cross-tolerance spontaneously lost completely.

13.4 Discussion

The results show that, like olanzapine and JL13, cyproheptadine also induced cross-tolerance to the discriminative stimulus of clozapine after chronic treatment. Thus these data show that cyproheptadine shows cross-tolerance to clozapine's discriminative stimulus and *possibly* might act as an atypical neuroleptic. This observation has been suggested in previous studies i.e. drug discrimination studies and some clinical studies. Simply because two compounds show partial generalisation to the discriminative stimulus effects, then it is not sufficient to assume that the same drugs will induce cross-tolerance to each others discriminative stimulus. This is because the discriminative stimulus of the two compounds may be sufficiently similar to induce generalisation but their mechanism of action may be different. If the mechanism of action of the two compounds is different then cross-

tolerance to the discriminative stimulus would not be induced. Hence, this paradigm suggests that clozapine and cyproheptadine have similar mechanisms of action at commonly shared receptors.

The initial study of the TEC was carried out to investigate the degree of generalisation between clozapine and cyproheptadine and cyproheptadine's duration of action. The time/effect curve showed that cyproheptadine is a long lasting drug and that even after 16 hours, half the animals still chose the drug lever, indicating high levels of the drug to be still present. Thus, it was decided to administer drug to the animals only once a day with cyproheptadine for 10 days and also to leave a single day rest period in between the chronic cyproheptadine injections and the calculation of DEC 2. The rest day was to allow the accumulated levels of cyproheptadine to decrease sufficiently so that the testing clozapine would not be confounded by residual cyproheptadine. It was decided not to inject the animals with a 'top-up' dose of cyproheptadine at 4 p.m. when computing DEC 2 as in the other studies, because cyproheptadine might have accumulated and possibly confounded the effects of clozapine on the next day. Hence, the time/effect curve indicated that initially there was a high level of similarity between cyproheptadine and clozapine with respect to their discriminative stimulus effects. Therefore, it was decided to carry on with the experiment and to administer cyproheptadine chronically.

Hence, once DEC 1 had been calculated, the animals were treated chronically for 10 days once a day with cyproheptadine (40 mg/kg). The animals suffered no adverse

side effects from the dose or frequency of injections. The animals did not receive discrimination training during the chronic drug administration period, in case "fading" occurred (See notes in clozapine chapter). The comparison of DEC 1 to DEC 2 showed that cyproheptadine had induced cross-tolerance to the clozapine discriminative stimulus. If the shift between the curves had not been parallel, then it would have indicated that different mechanisms had been responsible for producing the two dose/effect curves.

The animals were left alone for 16 days with no further drug treatment or drug discrimination training to investigate if the cross-tolerance induced would spontaneously disappear. The results showed that the DEC reverted back exactly to its original position after 16 days, which indicates that the cross-tolerance was spontaneously lost.

Thus, the results indicate that cyproheptadine in this particular behavioural paradigm acts like an atypical neuroleptic such as olanzapine, clozapine and JL13. There have been suggestions that cyproheptadine might act as an atypical antipsychotic (Silver *et al*, 1989). However, more work needs to be carried out in this area to fully investigate such effects such as whether or not cyproheptadine acts as an antipsychotic. More controlled studies need to be carried out on the clinic to investigate fully whether cyproheptadine could help some patients in conjunction with other drugs or alone, or whether it is only in animals studies that cyproheptadine appears to have antipsychotic properties.

There have been studies (Heonicke *et al*, 1992; Steinpreis *et al*, 1996) that have compared cyproheptadine and clozapine with respect to their actions on receptors. The results in this study indicate that the cross-tolerance is due to pharmacodynamic mechanisms, because the tolerance was lost by simply leaving the animals alone. This indicates that the cross-tolerance is not due to learnt phenomenon, whereby the animals have learnt to tolerate a higher dose of the drug after repeated exposure to the drug and then when administered a lower dose of the drug make fewer response than before the repeated exposure, but instead is due to a spontaneous change of receptors or neural systems. The precise receptors or neural system is unknown and has not been determined in this set of experiments. However, the cross-tolerance will depend upon receptors that the drugs have been shown to act upon.

Another study (Meltzer *et al*, 1996) was carried out on the effects of clozapine withdrawal in humans in the clinic. The results showed that when the patients were withdrawn from clozapine with no substitute neuroleptic, then psychotic symptoms re-appeared. If an additional neuroleptic was administered then the positive symptoms of schizophrenia were prevented. Cyproheptadine (a non-selective 5-HT receptor antagonist as described above) was shown to increase the antipsychotic effect of neuroleptics in most patients who relapsed after the withdrawal from clozapine and it also helped to reduce EPS in other patients (Meltzer *et al*, 1996). In some patients cyproheptadine *may* have atypical neuroleptic actions, and this could possibly explain why clozapine and cyproheptadine generalise and in this present

study why cyproheptadine induced reversible tolerance to clozapine's discriminative stimulus.

One study (Silver *et al*, 1989) suggested that negative symptoms of schizophrenia were improved by 5-HT_2 receptor blockers such as cyproheptadine. It has been shown that many chronic schizophrenic patients on neuroleptics respond poorly to the medication with respect to negative symptoms. It has been suggested that there is a link between chronic schizophrenia and abnormalities of serotonergic function. Cyproheptadine has been reported to have psychoactive properties in depression and anorexia nervosa (Gold *et al*, 1980). The results of this study showed that cyproheptadine might be of therapeutic benefit in some chronic schizophrenic patients. The results showed that the negative symptoms were improved significantly, but the EPS effects were not reduced (Silver *et al*, 1989). However, when the effects of cyproheptadine were investigated more closely in a later study it was shown that cyproheptadine had no effects on negative symptoms (Silver *et al*, 1991), and in actual fact cyproheptadine actually exacerbated some of the positive symptoms of schizophrenia. The second study also showed that cyproheptadine also increased suspiciousness and uncooperativeness. The increase in such symptoms ties in with the suggestion that they are mediated by serotonin systems (Silver *et al*, 1991). However, cyproheptadine was shown to have an antiparkinsonian action which is suggested to be via an interaction between serotonin and dopamine systems (Ceulemans *et al*, 1984). Therefore, in the clinic cyproheptadine appears not to be

like clozapine, whereas the results of this study suggest that cyproheptadine is clozapine-like in drug discrimination assays.

Therefore, the data again provide no clear conclusions about which particular receptors are responsible for the induction of cross-tolerance between clozapine and cyproheptadine in this behavioural paradigm. However, it is suggested that the cross-tolerance is pharmacodynamic in nature, because the tolerance was spontaneously reversed when the animals were left alone. It is also suggested that multiple receptors are involved in the cross-tolerance observed because all the compounds that induced cross-tolerance to the clozapine discriminative stimulus so far acted upon multiple receptors.

The precise receptors involved in inducing cross-tolerance to the clozapine cue can not be determined at this stage. Than cyproheptadine is a more selectively acting compound than other compounds tested in this paradigm i.e. olanzapine, JL13. Cyproheptadine is known to act at 5-HT, M_1 and H_1 receptors.

The overlapping receptors between clozapine and cyproheptadine are:

$5\text{-}HT_{2A\ \&\ 2C}$, noradrenergic $alpha_1$ and H_1.

This shows that clozapine acts upon all of the receptors that cyproheptadine acts upon as well as many others which cyproheptadine has no effect on. Therefore, it is reasonable to suggest that cross-tolerance to the discriminative stimulus of clozapine

involves these receptors, and also that mediation of these receptors alone is sufficient to induce cross-tolerance. This does not mean that other receptors are not involved in the inducement of cross-tolerance, but that these receptors do appear to be involved. Hence, by using cyproheptadine then the potentially active receptors involved in inducing cross-tolerance to the discriminative stimulus of clozapine has been reduced again.

13.5 Conclusions

The animals were trained to discriminate clozapine (5 mg/kg) from vehicle and once the discrimination was learnt to a high level of accuracy then that level of accuracy was constant. The study showed that chronic cyproheptadine induced cross-tolerance to the clozapine discriminative stimulus. The shift in the DEC's was parallel and significantly to the right. The animals were then left alone for 16 days and the cross-tolerance spontaneously disappeared. Thus cyproheptadine induced cross-tolerance to the clozapine discriminative stimulus. In this behavioural paradigm cyproheptadine behaved like clozapine, although previous work has shown that cyproheptadine is not necessarily like clozapine in the clinic. Although further studies are possibly required on this topic.

13.6 Further work

It would be interesting to investigate if cross-tolerance could be induced to clozapine's discriminative stimulus with compounds that are specific for one receptor

type e.g. CDP. Cross tolerance may show other compounds can act like clozapine that do not necessarily generalise to clozapine. Also, cross-tolerance may look at different actions and aspects of the clozapine that standard generalisation tests do not investigate.

Chapter 14.0

Effects of Chronic Chlordiazepoxide on Tolerance to the Discriminative Stimulus Effects of Clozapine

14.1 Introduction

Chlordiazepoxide (CDP) was another drug that was studied in the cross-tolerance studies on the clozapine discriminative stimulus. CDP is not an antipsychotic, but instead is a benzodiazepine that is widely used in the clinic to treat anxiety and has sedative properties.

Benzodiazepines have been shown to produce weak dose-related partial generalisation in animals trained to discriminate clozapine (Franklin & Tang, 1994). However, there are discrepancies about the degree of generalisation of benzodiazepines in clozapine trained rats. One study showed about 70% generalisation to clozapine with 10 mg/kg CDP (Moore *et al*, 1992). Whereas, another study showed that diazepam (another benzodiazepine) produced a maximum generalisation level of 28% in clozapine trained rats (Franklin & Tang, 1994).

There is evidence to suggest that the generalisation between clozapine and benzodiazepines is asymmetrical, because clozapine does not generalise in rats trained to discriminate diazepam, thus, the cue properties of clozapine and CDP maybe similar but not identical (Moore *et al*, 1992). Therefore, the data suggest that the clozapine cue resembles the benzodiazepine cue, albeit to an incomplete and inconsistent extent. There is evidence to suggest that in rat models of anxiety, clozapine is usually less efficacious than benzodiazepines (Bevenga & Leander, 1995). Clinical findings have also suggested that clozapine may have anxiolytic

actions (Goudie & Taylor, 1998) and it may potentiate sedative actions of benzodiazepines (Goudie & Taylor, 1998).

CDP produced weak dose-related generalisation to clozapine when two groups of rats were compared (Goudie & Taylor, unpublished). One group was trained on 5 mg/kg clozapine and the other group was trained on a lower dose of 2 mg/kg clozapine. When the two groups were tested with 20 mg/kg CDP, data showed that the 5 mg/kg trained animals showed partial generalisation, while the animals trained on 2 mg/kg clozapine failed to make a lever selection. This suggests that under 20 mg/kg CDP the high dose of clozapine regimen led to cross-tolerance to the rate suppressant action or the sedative effects of CDP compared to the low dose group. This could suggest that on the high dose of clozapine the animals felt the sedative effects initially but they were tolerated out during the course of the experiment. However, the animals under the low dose of clozapine never really felt the sedative effects of clozapine so never became tolerated out to them, so when administered CDP could not display cross-tolerance. In the same study, it was shown that in the high and low dose trained rats, CDP caused rate suppression and that the dose/effect curve was shifted in parallel to the right by approximately 2 fold (Goudie & Taylor, unpublished).

It has been shown that the weak benzodiazepine-like actions of clozapine do not directly involve the benzodiazepine or $GABA_A$ receptor, because clozapine does not bind to it. Instead, clozapine acts as a functional *antagonist* at some $GABA_A$ receptor

subtypes (Korpi *et al*, 1995). However, this effect is clearly difficult to reconcile with benzodiazepine-like behavioural actions of clozapine. Squire & Saederup (1991; 1997) have observed that many antipsychotics are $GABA_A$ antagonists and they hypothesised that clozapine and other similar compounds preferentially block inhibitory GABAergic interneurone located on GABA cells. This effect of clozapine leads to the disinhibition of GABA and thus mediating sedation and probably the weak generalisation to clozapine induced by weak generalisation to clozapine induced by benzodiazepine. However, on the other hand, antagonist actions on *other* $GABA_A$ receptors may be associated with the ability of clozapine to induce convulsions (Squire & Saederup, 1991).

Thus it was decided to investigate whether chronic CDP would induce cross-tolerance to the clozapine discriminative stimulus. CDP was used as a negative control to investigate whether or not the effects observed with the other compounds were because of actions at multiple receptors or because of tolerance to side effects such as sedation. CDP did not generalise to the clozapine cue, so if cross-tolerance was induced it would not have been due to common actions at receptors. Instead, it would have indicated that cross-tolerance was due to either chronic administration of second drug or that cross-tolerance was due to tolerance to side effects such as sedation.

14.2 Methods

Subjects

11 female Sprague Dawley rats (bred at the Psychology Department, University of Liverpool, UK) were used in this experiment. The 11 subjects were not experimentally naive and had previously been used in the olanzapine cross-tolerance study.

Two groups of animals were used in this series of experiments and the groups and the drugs administered are shown in the table below:

Table 14.1: Groups number and which drugs were used in each group

Group	Drug administered	Number of days between experiments
Previously trained animals	Clozapine	15
	JL13	38 (including Christmas)
	Cyproheptadine	-
Experimentally naïve animals	Olanzapine	23
	CDP	-

Apparatus

As described in method section.

Training Procedure

As described in the method section.

Testing Procedure for determining dose/effect curve

As described in the clozapine chapter.

Procedure for derivation of time-effect curve

The time/effect curve was carried out in order to assess the duration of action of CDP and to investigate if CDP would produce generalisation to the clozapine stimulus. 11 animals were used to determine the time/effect curve of CDP. The animals were injected with CDP (20 mg/kg), one sub-group (n = 5) at 0830 and the second sub-group (n = 6) at 0845 hours. Then the animals were tested at different time points throughout the day 30 minutes, 1, 4 and 8 hours post-injection. Thus animals were tested at 0900, 0930, 1230 and 1630 hours for sub- group one that was injected at 0830; and at 0915, 0945, 1245 and 1645 hours for sub-group two which had been injected at 0845 hours. The duration of action of CDP was measured by the percentage drug lever selection made at each time interval tested.

Procedure for dose- effect (generalisation) curves

The dose/effect curve was calculated twice during this experiment. Chronic CDP was suggested to possibly induce tolerance to clozapine. 11 animals were used to determine the dose/effect curves because one of the animals had to be dropped from the study due to a non-drug related illness. The animals were chronically injected for

ten days with CDP (40 mg/kg). The high dose of CDP (twice the dose used for the time/effect curve) and the frequency of dosing (b.i.d.) were chosen to facilitate tolerance development. The animals were injected at 0900 and 1600 hours for 10 days. The data showed that CDP failed to induce cross-tolerance to the discriminative stimulus of clozapine (see Results), so DEC 3 was obviously not carried out, as there was no tolerance to spontaneously disappear.

Statistics

As described in the clozapine chapter.

Drugs

Clozapine base (Sandoz, Switzerland) was administered i.p., dissolved in a few drops of 0.1M HCl, diluted with water and buffered back with 0.1M NaOH to a pH around 5.5 and injected at a volume of 2 ml/kg. Chlordiazepoxide (CDP) was administered i.p., dissolved in distilled water and buffered back with 0.1N NaOH to a pH around 4.0 and injected at a volume of 2 ml/kg. The drugs were injected 30 minutes before operant sessions.

14.3 Results

The animals were left for 23 days from the end of the olanzapine study and the start of the CDP study. The fear of residual tolerance from the chronic olanzapine we believe was unfounded because the cross-tolerance to olanzapine was spontaneously and completely lost by the end of the olanzapine study (see chapter 11). Once all the

animals were responding reliably and the discrimination was stable, the time/effect curve was determined, as described above. The animals were responding 100% of the time on the saline under vehicle and at least 90% on the drug lever under clozapine.

Fig. 14.1 Time/effect curve for CDP at 20 mg/kg

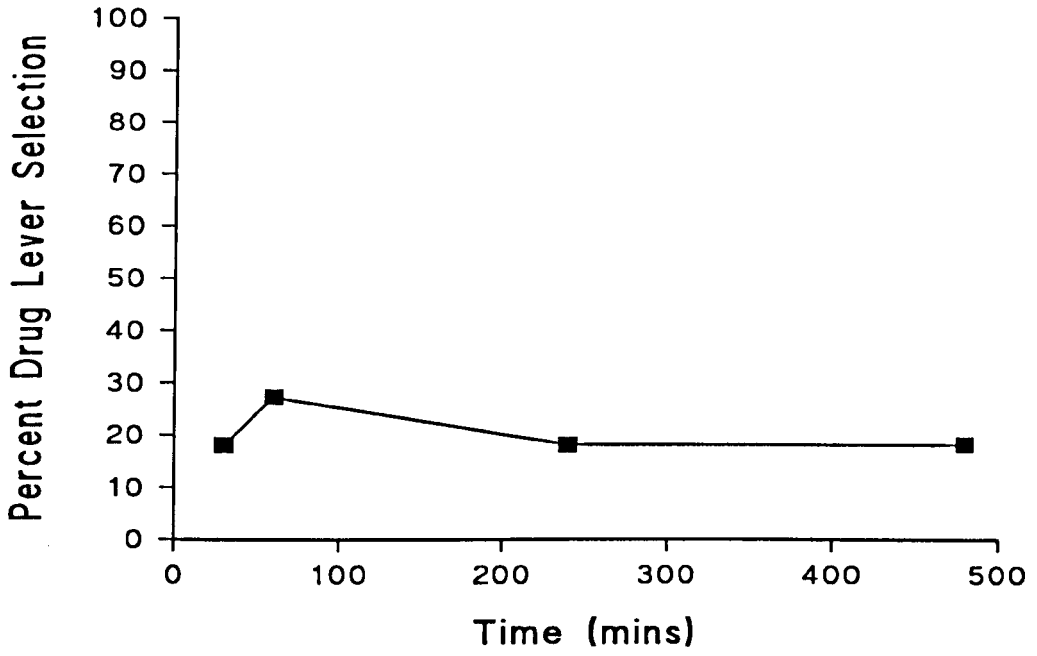

Fig. 14.1: The graph shows that after 30 minutes there was only about 20% generalisation between clozapine and CDP. However, after 1 hour there was about 30% generalisation and this tailed off to about 20% again for both 4 and 8 hours post-injection.

The time/effect curve allows a measurement of the degree of generalisation between clozapine and CDP. This is because the cross-tolerance investigated is between the stimulus cue generated by the two compounds. The results of the time/effect curve

show that there is minimal generalisation between CDP and clozapine, but that this level did not alter over time. Figure 14.1 indicates that CDP possibly shows very low partial generalisation to clozapine, but this could be due to chance. On training days, the animals responded 100% of the time on the saline lever under vehicle. Therefore, as the animals learnt to recognise the absence of drug it was possible that 20% generalisation represent minimal generalisation rather than random responding. It also shows the specificity of the clozapine cue at 5 mg/kg, because CDP showed minimal or no generalisation. These data show that for compounds to generalise to the clozapine cue they must act upon multiple receptors. The other compounds i.e. clozapine, JL13 all acted upon multiple receptor systems and all showed generalisation to the clozapine cue, whereas CDP acts on a single receptor system and showed either no or minimal generalisation. This indicates that for compounds to generalise to the clozapine cue, they must act on more than the benzodiazepine site alone.

However, the degree of generalisation between CDP and clozapine is dose-dependent and no inferences can be made about the degree of generalisation of other doses of CDP and clozapine. A higher dose of CDP was not tested because it is generally accepted that 20 mg/kg is a behaviourally active dose.

The TEC effectively showed that CDP did not generalise to clozapine reliably at any time at the dose used in this study. CDP had shown either no or minimal generalisation to clozapine, so I decided to treat CDP exactly the same as clozapine,

JL13 and olanzapine, and to administer CDP at 40 mg/kg b.i.d. in the tolerance phase of the study to try to facilitate production of tolerance.

The subsequent stages of the experiment were to investigate if CDP could induce cross-tolerance to the clozapine cue and if so could it be spontaneously reversed. The dose/effect curve was carried out by subjecting the animals to a series of doses from the training dose downwards in a logarithmic manner. Hence, in DEC 1 the doses ranged from 0.3125 to 5 mg/kg and in DEC 2 the doses ranged from 1.25 to 5mg/kg.

Fig. 14.2 Comparison of Dose/effect curves

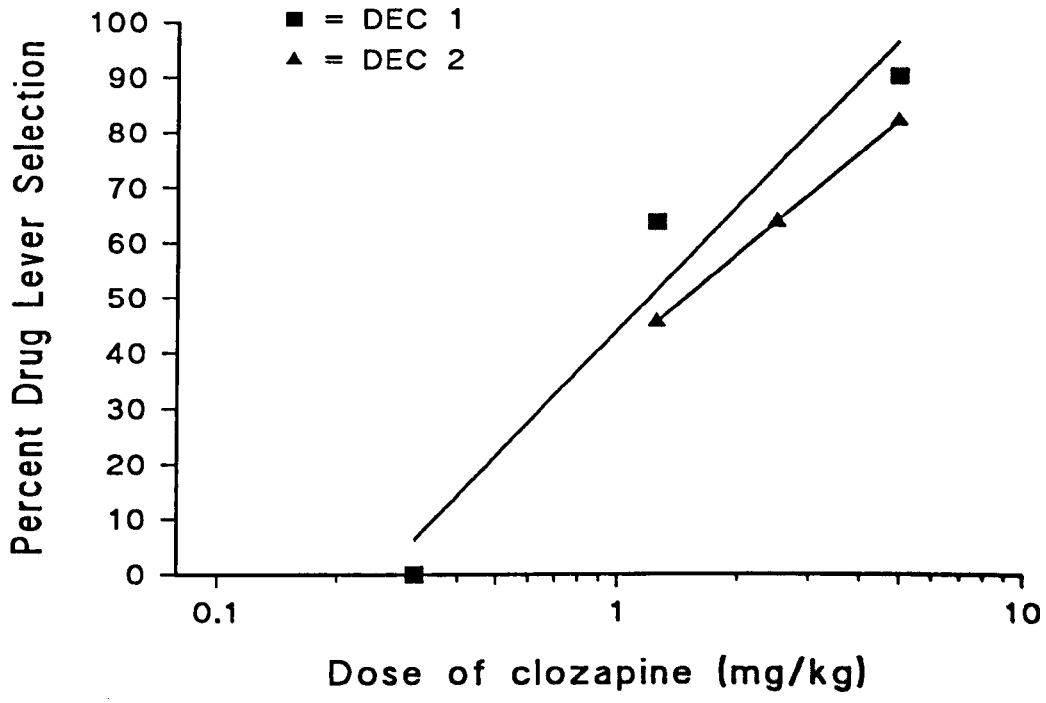

Fig. 14.2: The data show that CDP did not induce tolerance to the clozapine cue.

DEC 1: The data show that the animals produced dose-related generalisation to the clozapine stimulus as expected. As the dose of drug decreased so did drug lever selection. When the dose was 5 mg/kg, 90% of the animals responded on the drug lever, decreasing to about 63% when the dose was 1.25 mg/kg. The last dose of 0.3125 mg/kg produced vehicle lever selection only. A regression line was plotted for both DECs and ED_{50} values calculated (See table 14.2). Hence, DEC 1 showed that the animals responded as expected, and that as the dose of clozapine decreased so did drug lever selection.

Once DEC 1 had been carried out, the animals were treated chronically with CDP (40 mg/kg, b.i.d.). The animals were injected twice a day for 10 days with CDP, and during this time, they received no discrimination training. The animals were weighed, injected and then placed back in their home cages until the next time to be injected. At the end of the chronic CDP (40 mg/kg, b.i.d.) treatment, another dose/effect curve (DEC 2) was determined on days 11 to 13. The animals were also administered a "top-up" dose of CDP (40 mg/kg) each afternoon during the computation of DEC 2.

DEC 2: The data show that chronic CDP treatment clearly had no effect at DEC 2 and no tolerance was induced. The data were analysed by probit analysis (see table 14.3).

DEC 3 was not carried out because there was obviously no cross-tolerance to be lost and therefore no need to carry out this investigation.

In order to compare the ED_{50} values easily the data are presented below.

Table 14.2 A comparison of the ED_{50} values (mg/kg) for DEC 1 and DEC 2

DEC	ED_{50}	R^2 value
1	1.2	+0.95
2	1.48	+0.99

The table shows that there was no calculable or observable shift in the DEC's from before to during chronic CDP treatment. This is shown by there not being a change in the ED_{50} value between DEC 1 and 2.

The data were analysed by probit analysis and the following data were obtained:

Table 14.3 A comparison of the relative ED_{50} potency's and parallelism for DEC 1 and DEC 2

DEC compared	Parallelism Test	ED_{50} Potency ratios and 95% confidence limits
1 v 2	$X^2 = 1.0$, d.f. = 1, p = 1.0, NS	1.23-fold, Lower = 0.27, Upper = 1.8, NS

The data in table 14.3 show that the parallelism results indicate that the curves were not significantly non-parallel, hence can be considered as being parallel to each other. Also in the potency ratio test, the confidence limits for DEC 1 v 2 cover 1.0 which indicates that there is no significant difference between the lines which would be expected due to the lack of tolerance induced by CDP to the clozapine cue.

14.4 Discussion

The results (see olanzapine chapter) show that there was no residual tolerance left over from the olanzapine study. The animals were left for several weeks after the end of the olanzapine study before the CDP time/effect curve was carried out.

The time/effect curve showed that, unlike the other compounds tested in this paradigm, CDP failed to produce more than minimal generalisation to the clozapine discriminative stimulus. The generalisation level between the two compounds was about 30% at the most, which agrees with some studies that have also shown very little generalisation between clozapine and CDP (Taylor, Ph.D. Thesis, 1999), but contrasts with other studies which have shown much higher levels of generalisation (Moore *et al*, 1992). All the studies mention previously (this study, Taylor, Ph.D. and Moore *et al*) all trained animals to discriminate clozapine (5 mg/kg) from vehicle in a drug discrimination paradigm. The difference between Moore *et al* (1992) and the other studies is that they tested CDP at 10 mg/kg and the other two mentioned studies tested CDP at 20 mg/kg. The other difference as already mentioned between Moore

et al (1992) and the other two studies, is that Moore's group showed 70% generalisation between CDP and clozapine, whilst this study and Taylor (Ph.D., 1999) showed 30 and 40% generalisation between CDP and clozapine respectively. There are two suggestions that can be made about the difference in the data, 1) a lower dose of CDP i.e. 10 mg/kg and not 20 mg/kg is a more general cue and thus "feels" more clozapine like to the animals, or 2) the cue in Moore's animals for clozapine was not as specific as the cue was in both my animals and in the animals used by Taylor. The lack of specificity for the clozapine cue in Moore's animals could have been due to fewer training sessions or a less stringent criterion to be reached for accuracy. However, the data show that there is clear controversy about the level of generalisation between CDP and clozapine.

The results of this study showed that at a high dose CDP failed to induce even high partial generalise to clozapine and also failed to induce cross-tolerance to the clozapine discriminative stimulus, unlike the other compounds that were tested in this paradigm. After the animals had been treated for 10 days with CDP (40 mg/kg, b.i.d., i.p.) and the dose/effect curve was recomputed there was no shift between DEC 1 and DEC 2. The fact that the curve did not shift indicates clearly that there was no cross-tolerance between the discriminative cues of clozapine and CDP. This is despite evidence that a subset of $GABA_A$ receptors may be involved in the antipsychotic effects of clozapine (Squires & Saederup, 1997).

CDP acts upon GABA receptors as does clozapine, However, no cross-tolerance was induced to the discriminative stimulus of clozapine by CDP. Hence, this suggests that the GABA receptors do not play a role in the production of cross-tolerance to the discriminative stimulus of clozapine.

14.5 Conclusions

This experiment has shown that the animals in this study only showed minimal generalisation between CDP and clozapine. In addition, this study has shown that chronic CDP at a very high behaviourally active dose given b.i.d. failed to induce cross-tolerance to the clozapine discriminative cue, unlike the clozapine congeners and cyproheptadine that were tested previously.

CDP was administered the cross-tolerance paradigm to investigate if there was a pharmacological specificity to the compounds which would induce tolerance to the clozapine discriminative stimulus. If CDP had generalised fully to the clozapine discriminative stimulus then a different compound would have been chosen. The fact that CDP only partially generalised to the clozapine cue, made it ideal. If it had fully generalised then a different compound may have been chosen.

Thus, these data show a degree of pharmacological specificity to the tolerance and cross-tolerance effects reported in earlier chapters. Daily (b.i.d.) administration of a high dose of chlordiazepoxide failed to induce cross-tolerance to the clozapine stimulus. It would therefore appear that in order to induce tolerance and cross-

tolerance to the clozapine stimulus, it is necessary to administered a clozapine-like drug which generalises to clozapine in the clozapine discrimination assay.

Chapter 15.0

Discussion of Clozapine Studies

The tolerance studies have shown that chronic clozapine administered induced tolerance to the clozapine discriminative stimulus. This was shown because the DEC was shifted to the right when comparing DEC 1 and 2. The tolerance induced is possibly a pharmacodynamic phenomena, because the tolerance was spontaneously lost when the animals were left alone for 16 days with no further treatment. It is believed that if the tolerance were due to a learnt process, then simply leaving the animals for 16 days would not have reversed the tolerance. Previous studies have shown that once animals have learnt to attend to a particular cue, then even without further training, the cue remains learnt for many months. The animals can be left for many months both with no discrimination training and by training the animals to discriminate another drug from a different pharmacological class and the cue will be retained (McMillan *et al*, 1996). Studies in pigeons have shown that both leaving the animals or training the animals to discriminate another class of drug has no effect on the discriminability of the initial drug (McMillan *et al*, 1996). Thus, the data indicate that the tolerance induced to the discriminative stimulus of clozapine is most likely due to pharmacodynamic mechanisms. The precise receptor or neural systems that are involved are unknown and further work needs to be carried out to determine these.

The effects of other known atypical antipsychotics and novel agents were investigated. The compounds that are under discussion are JL13 and olanzapine. Both compounds were investigated in the same behavioral paradigm as clozapine. Both drugs induced cross-tolerance to the clozapine discriminative stimulus, again indicated by the fact that DEC 2 had shifted to the right compared to DEC 1. It was

also shown in this study that both JL13 and olanzapine generalized substantially to the clozapine discriminative stimulus. Previous studies have shown that both olanzapine and JL13 have similar pharmacological profiles to clozapine (Porter & Strong, 1996; Bruhwyler *et al*, 1993). Thus, it was not unduly surprising that JL13 and olanzapine induced cross-tolerance to the discriminative stimulus of clozapine. Olanzapine and JL13 induced cross-tolerance within 10 days of administration, which was significant and the lines were parallel. It was predicted that the lines would be parallel because clozapine had been used to calculate the dose/effect curves. The tolerance induced by both JL13 and olanzapine was spontaneously reversible when the animals were left for 16 days with no further treatment. Thus, again the data suggest that the tolerance induced to the clozapine discriminative stimulus be of a pharmacodynamic nature. Previous data have shown that both olanzapine and JL13 also act upon multiple receptors (Porter & Strong, 1996; Bruhwyler *et al*, 1997). Hence, the precise mechanism of tolerance to the clozapine discriminative stimulus can not be further determined other than to suggest that it may involve actions on multiple receptor systems.

The next compound tested was cyproheptadine. This compound that has been suggested to have some antipsychotic properties, but that depends upon the behavioral test studied (Silver *et al*, 1989). Cyproheptadine acted like the previously discussed antipsychotic drugs and induced cross-tolerance to the clozapine discriminative stimulus. Cyproheptadine induced cross-tolerance within ten days of administration, which was shown to be significant. DEC 1 and 2 were parallel to each other, as observed with the previously reported antipsychotic compounds

clozapine, olanzapine and JL13. Again, the parallel lines were expected because clozapine had been used to determine the shift between DEC 1 and 2. It would also indicate that the same mechanism was used in the dose-dependent effects of clozapine both before and after chronic treatment with cyproheptadine. The tolerance produced with cyproheptadine was spontaneously reversible, when the animals were left alone for 16 days with no further treatment DEC 3 reverted back to the same position as DEC 1. Hence, the data again suggest that the mechanism which induced tolerance, was of a pharmacodynamic nature. Cyproheptadine although not a recognized anti-psychotic drug, has been shown to have some properties, which are similar to clozapine. One of the main similarities between the two compounds is the fact that cyproheptadine and clozapine both act upon multiple receptor sites (Browne & Koe, 1982). The exact mechanism by which the tolerance and cross-tolerance to the clozapine stimulus was induced is not possible to determine. However it would appear to require actions at multiple receptor systems. Cyproheptadine is known to act at 5-HT, M_1 and H_1 receptors. So these receptors appear involved in the mediation of cross-tolerance to the discriminative stimulus of clozapine. However the role of other receptors can not be ruled out until work has been carried out with specific receptor agonists and antagonists.

The final compound to be tested in this series of experiments was CDP. CDP is a known anxiolytic that acts upon the $GABA_A$/chloride ionophore complex (Gauvin, Pierce & Holloway, 1994). Although, it is accepted that CDP acts upon other receptor systems as well, including noradrenergic and serotonergic pathways (Gauvin, Pierce & Holloway, 1994). Since CDP acted upon more than one receptor

type and it had anxiolytic properties as does clozapine (Goudie & Taylor, 1998) it was possible that CDP would induce cross-tolerance to clozapine's discriminative stimulus. The results showed that CDP failed to induce cross-tolerance after 10 days chronic treatment. Thus, CDP and clozapine showed no cross-tolerance in this study under these experimental conditions. Despite the fact that the dose of CDP of 40 mg/kg is a very high known behaviorally active dose.

Olanzapine was chosen because it has been shown to fully generalize to clozapine and it has also been shown to act like clozapine in behavioural tests. The other main factor for choosing olanzapine after studying clozapine in the cross-tolerance paradigm, is that olanzapine acts as an atypical antipsychotic in the clinic as does clozapine. So after clozapine, then olanzapine had the next best chance of inducing cross-tolerance to the clozapine discriminative stimulus cue.

JL13 was chosen as the next compound because it has been shown to partially generalize to the clozapine cue and to also act clozapine-like in some behavioural tests. It is presumed the JL13 like clozapine will act as an atypical antipsychotic in the clinic. However, although clozapine and JL13 have similar actions, then JL13 acts at fewer receptors than clozapine does, which could explain why JL13 only partially generalizes to the clozapine cue. It was predicted that unless cross-tolerance was due to a very specific mechanism that required most of the receptors that clozapine acts upon to be activated, then chronic JL13 would induce at least some if not complete cross-tolerance to the clozapine cue.

Cyproheptadine was chosen as the next compound because it has been shown to have clozapine-like properties in some behavioural tests. However, studies carried out with cyproheptadine in the clinic have shown, so far, that cyproheptadine does not have atypical neuroleptic properties. Cyproheptadine acts at fewer receptors than the previously tested compounds, so if it did not induce cross-tolerance, then it could be concluded that in order to induce cross-tolerance to the clozapine cue that the compound needed to act at more receptors. Also, if cyproheptadine did induce cross-tolerance to the clozapine cue, then the receptors that cyproheptadine acts upon could potentially be the necessary ones to induce cross-tolerance to the clozapine cue. It was unsure whether cyproheptadine would induce cross-tolerance to the clozapine cue, but it was necessary to try more selective compounds in the paradigm. It was possible that chronic cyproheptadine would induce cross-tolerance to the clozapine cue, but that the dose-effect curve would not have shifted as much to the right as observed with the previously tested compounds.

CDP was chosen as the last compound to study as a way of checking that there was come specificity to the compounds that would induce cross-tolerance to the clozapine cue. CDP was not expected to show high levels of, if any, generalization to the clozapine cue. Hence, CDP was predicted to act as a negative control in the study. If CDP had induced cross-tolerance to the clozapine cue after not generalizing, then it would have shown that there was no specificity in the receptors involved or in the drugs that could induce cross-tolerance to the clozapine cue.

Therefore, the overall conclusions show that clozapine, olanzapine, JL13, and cyproheptadine all induced tolerance to the discriminative stimulus effect of clozapine after 10 days of chronic administration. All four compounds also showed that the tolerance induced was spontaneously reversed by leaving the animals alone for 16 days with no drug treatment of discrimination training. However, CDP failed induce cross-tolerance to clozapine's discriminative stimulus.

The cross-tolerance paradigm showed that olanzapine, JL13 and cyproheptadine all are clozapine-like in another behavioural paradigm. This is not particularly surprising for olanzapine and JL13 because they have been shown to act as atypical neuroleptics like clozapine in many behavioural tests already. It was also more surprising for cyproheptadine to have acted like clozapine because some studies have shown that it does not antipsychotic properties in the clinic.

The difference in using the cross-tolerance paradigm compared to the drug discrimination paradigm is that the cross-tolerance paradigm allows the animals to directly compare the effects of olanzapine, etc. with the effects of JL13. This is because the animals have to respond on the levers depending how they "feel". Also, the cross-tolerance paradigm allows the animals to tolerate out to the effects of the chronic drug i.e. olanzapine, etc. but then also requires the animals to take that learnt knowledge and apply it to a different drug when clozapine in administered in the DEC phases.

It was decided to use the cross-tolerance paradigm rather than the drug discrimination paradigm, because clozapine has been extensively researched in the drug discrimination paradigm, but not in the cross-tolerance paradigm. Also the cross-tolerance paradigm may have been a more sensitive technique *or* it may have been a less specific technique for comparing novel compounds and scrutinizing them against clozapine for novel atypical neuroleptics.

By using the cross-tolerance paradigm rather than the drug discrimination paradigm, then it has shown that there is another behavioural test with which to screen novel compounds for their clozapine-like properties. The study also suggests that perhaps more work should be carried out on the properties of cyproheptadine.

The precise mechanism by which the tolerance is induced to clozapine is unknown and the aim of this study was never to determine this in detail, but instead was to determine if tolerance could be induced to the discriminative stimulus effects of clozapine. Further studies would have to be carried out to determine the precise mechanism(s) underlying the induction of tolerance. In order to determine the precise mechanism of action of cross-tolerance between clozapine and its congeners, then specific receptors would need to be targeted and the effects studied both with agonists and antagonists and mixtures of such drugs. Nevertheless, it can be concluded tentatively that tolerance to the clozapine cue appears to be induced *only* by drugs acting concurrently at multiple receptors, although the precise receptors, involved are not clear.

In the chapters of the individual drug's effects on the discriminative stimulus of clozapine (Chapters 11-14), then the receptors that clozapine and the specific drug shared actions at were compared. However, when all the drugs are compared together, to investigate which receptors all the compounds which induced cross-tolerance to the clozapine discriminative stimulus acted upon, then the following receptors were left:

5-HT_{2A} and H_1.

Hence, there are three possible conclusions to be made;

1) cross-tolerance to the discriminative stimulus of clozapine is induced by these receptors alone,

2) the aforementioned receptors play an important role in mediating cross-tolerance to the discriminative stimulus of clozapine. However, other receptors play a role as well and the additional receptors depend upon which other receptors that the compound in question acts upon,

3) each drug induced cross-tolerance to the discriminative stimulus of clozapine by the involvement of different receptors to end up with the same apparent end results, with many different ways of ending up there.

Thus, the assay developed may be regarded as one demonstrating neuroadaptations (mediating tolerance) which may be relevant either to: a) The tolerance that is seen to side-effects of clozapine (e.g. sedation); or b) The progressive enhancement of clozapine's therapeutic actions with chronic treatment, if the relevant

neuroadaptations were such as to facilitate clozapine's antipsychotic actions, although this conclusion is clearly very speculative.

Chapter 16.0

Overall Conclusions

16.1 Study I

The aim of the first study was to investigate the effects of drug history on caffeine discrimination in rats in a two lever operant task. The first study involved amphetamine, CDP, nicotine and caffeine. Animals were split into one of seven groups. Three groups were trained to discriminate either amphetamine (0.5 mg/kg), CDP (10 mg/kg) or nicotine (0.3 mg/kg). These were called the "discriminating" groups (n=12). Three groups received yoked injections of amphetamine, CDP or nicotine at the same doses. These were called the "non-discriminating" groups (n=12) and the final group received daily injections of saline. These were called the "control" group (n=13). The results showed that the "discriminating groups" learnt to discriminate their respective drugs of amphetamine, CDP or nicotine to a relatively high level of accuracy in approximately 100 sessions. The second phase of the experiment was to train all the individual groups to discriminate between caffeine (10 to 20 mg/kg) and saline. The aim behind the experiment was to investigate differences in the caffeine with respect to their previous drug experience. However, the results showed that none of the animals learnt to discriminate caffeine at 10 mg/kg, so the dose was increased to 15 mg/kg caffeine. The data showed that even at 15 mg/kg caffeine, none of the animals learnt to discriminate the caffeine cue reliably. Therefore, eventually the dose of caffeine was increased to 20 mg/kg. At this dose, the animals in the "control" group learnt the caffeine cue to a "reasonably" high level of accuracy.

Amphetamine trained animals showed partial generalization to the caffeine cue at 10 mg/kg, which is in agreement with other studies (Chait & Johanson, 1988). However,

these animals did not show the expected facilitation of acquisition of the caffeine cue. CDP and nicotine groups showed no partial generalization to the caffeine cue at 10 mg/kg. Also neither of these groups showed the expected retardation of acquisition of the caffeine cue relative to the controls.

Therefore, the problem must probably be due to the very slow rate of acquisition of the caffeine cue due to its low discriminability. The poor discriminability of caffeine was not predictable because other studies have shown the doses of caffeine used to be discriminable (Mumford & Holtzman, 1991). However, in amphetamine trained animals, caffeine is discriminable, as shown by the partial generalization. Maybe the prior amphetamine experience enhanced the salience of the caffeine cue, so inferences about the discriminability of the caffeine cue from the amphetamine animals can not be transferred directly to the other training groups. One tentative explanation for the behaviour of the amphetamine animals may be that chronic amphetamine either via training or non-contingent injections, induced a degree of cross-tolerance to the caffeine cue. So, it is possible that this may have prevented us seeing the expected facilitation of caffeine acquisition even though the drugs initially generalized. This explanation is post-hoc and therefore unsatisfactory. However, it is not easy to come up with a clearer explanation for the data.

It is suggested that the data deviate from the expected outcomes due to the low discriminability of caffeine at 10 mg/kg which may lead to; 1) Extinction of the previously learned CDP and nicotine discrimination because the animals were

reinforced on both levers under "saline" and 2) retardation of the subsequent acquisition of the caffeine cue.

16.2 Conclusions from Study I

Thus, the overall conclusion of study I is that under these experimental conditions, there was no effect of drug history because the CDP and nicotine groups possibly had their previous drug history extinguished and the amphetamine groups showed no effect for reasons not clearly understood, but possibly due to the development of cross-tolerance during discrimination training.

16.3 Study II

Animals were trained to discriminate clozapine (5 mg/kg) from vehicle. Once a high level of accuracy was achieved, a time effect curve of clozapine (5 mg/kg) was carried out. Then a dose effect curve (DEC 1) was calculated at a range of doses from 0.3125 to 5 mg/kg. The animals were then administered clozapine (10 mg/kg) twice a day for 10 days and another dose effect curve (DEC 2) carried out with a dose range of 1.25 to 5 mg/kg. At the end of DEC 2, the animals were left alone for 16 days with no drug treatment or discrimination training. At the end of this, another dose effect curve (DEC 3) was carried out. The results showed that DEC's 1 and 3 were similar but that DEC 2 had significantly shifted to the right from DEC 1. After the animals were left alone for 16 days, then DEC 3 had shifted significantly to the left compared to DEC 2. Essentially the same experiment was carried out with olanzapine (5 mg/kg, b.i.d.), JL13 (20 mg/kg, b.i.d.), cyproheptadine (40 mg/kg, daily) and CDP (40 mg/kg, b.i.d.) administered chronically instead of clozapine. The

results showed that clozapine, olanzapine, cyproheptadine and JL13 all induced tolerance or cross-tolerance to the clozapine discriminative stimulus which was spontaneously reversible. CDP at a high dose did *not* induce cross-tolerance to the clozapine discriminative stimulus.

16.4 Conclusions from Study II

Therefore, the overall conclusions for study II are that cross-tolerance to the clozapine discriminative stimulus occurs only with compounds that are clozapine-like, in that they act upon multiple receptors and *may* have antipsychotic properties.

16.5 Overall conclusions

The data show that previous drug experience had no clear effect on future drug discrimination for complex reasons, as discussed above. However, pharmacological history did have an effect and only clozapine-like compounds induced cross-tolerance to the clozapine discriminative stimulus.

16.6 Further work

It seems pointless trying to take study I any further because the animals failed to learn the caffeine cue at the doses studied to any great extent and therefore no differences between drug history could be studied. If the dose of caffeine had been pushed up any higher the dose would have no longer been a "general" non-specific cue, but instead would have developed into a specific methylxanthine cue. It would be interesting to try the same study with a different set of drugs and maybe if the second drug was more discriminable then clearer effects might well have been

obtained. It would have been interesting to try study I with clozapine instead of caffeine. Clozapine has a compound discriminative cue at all doses, unlike caffeine that is a general compound cue at specific doses. So by using clozapine as the second compound after initial single receptor compounds, then it could have allowed differences to be observed.

It would be interesting to take study II further and investigate if cross-tolerance could be induced with compounds that act at specific receptors i.e. 5-HT receptor antagonists, dopamine receptor agonists and antagonists or mixtures of such drugs i.e. two specific receptor compounds together at varying concentration, to try to determine the specific receptors needed to induce cross-tolerance to clozapine's discriminative stimulus which may be related to the use of clozapine in the clinic.

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