EFFECTS OF MORPHINE AND OTHER DRUGS WITH ANTAGONIST AND MIXED AGONIST-ANTAGONIST PROPERTIES UPON BEHAVIOUR MAINTAINED BY A SCHEDULE OF INTERRESPONSE TIME REINFORCEMENT

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CURRICULUM STUDIORUM

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ABSTRACT

Effects of morphine and other drugs with antagonist and mixed agonist-antagonist properties upon behaviour maintained by a schedule of interresponse time reinforcement.

The separate effects of graded doses of morphine, naloxone, dl-cyclazocine and d-amphetamine on responding maintained by a DRL schedule of food presentation were examined in rats. Morphine did not alter response rates at doses of 1 - 5.6 mg/kg; at 10 mg/kg, a 57% decrease in responding was observed and behaviour was even more severely depressed by 30 mg/kg of morphine. Naloxone did not affect responding at doses ranging from 0.1 to 10 mg/kg. dl-Cyclazocine at doses of 3 and 5.6 mg/kg induced substantial increases in responding not observed when the dose was increased to 10 mg/kg. Cyclazocine, as well as morphine, produced dose-dependent decreases in the number of reinforcements per session. d-Amphetamine exerted a biphasic effect on responding; small doses increased response rates (0.3 to 3 mg/kg) and responding was suppressed by the drug at a dose of 10 mg/kg. Behaviourally-active doses of d-amphetamine caused a dose-dependent reduction in the number of reinforcements per session. Naloxone at otherwise inactive doses (1 - 10 mg/kg) was

found, in separate experiments, to antagonize the rate-decreasing effects of morphine and to reduce the rate-increasing effects of d-amphetamine. Naloxone (1 mg/kg), however, failed to alter the rate-increasing effects of dl-cyclazocine but slightly modified the latter drug's rate-disruptive activity.

In additional experiments, morphine was given daily for 25 consecutive sessions at a dose of 30 mg/kg 5 min preceding each test session. Responding was suppressed throughout this period and the dose of morphine given before each session was reduced to 10 mg/kg for 35 further sessions. Tolerance to the rate-decreasing effects of morphine was demonstrated; naloxone given in conjunction with morphine (10 mg/kg) in morphine-tolerant rats restored to control values the number of reinforcements per session without causing significant change in overall rates of responding. Few experiments have dealt previously with the development of tolerance to the behavioural effects of morphine under comparable dose regimens, time-course relationships or behavioural testing procedures. Systematic analyses of these interrelated variables are needed since the present experiments show that the schedule employed to maintain responding itself exerts significant effects.

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INTRODUCTION

Over the past decades, several attempts have been made towards the development of new methods in the treatment of opiate dependence. Existing programs such as the Synanon therapeutic community and the methadone maintainance scheme have shown limited therapeutic value, thereby enhancing the search for more appropriate treatment approaches.

One of the most promising forms of opiate dependence management now being considered by researchers and clinicians involves the use of narcotic antagonist drugs. Ever since Martin and his co-workers (1966) first suggested the substitution of antagonistic substances to methadone in long-term treatment of heroin addicts, researchers have recognized and even expanded on the possible clinical usefulness of these compounds. Narcotic antagonists have been suggested as possible prophylactic agents in both the development of opiate dependence and tolerance (Fink, 1970; Fink, Zaks, Resnick and Freedman, 1971) and the relapsing of ex-addicts following detoxification (Wikler, 1971). The expanding clinical applications of the narcotic antagonists have stimulated researchers to investigate within

an operant conditioning framework the full behavioural actions of these compounds.

Since the middle 50's, the extensive use of operant conditioning techniques as preclinical tools in the assessment of drug-behaviour interactions has led to the finding that ongoing patterns of responding engendered by different schedule contingencies is an important determinant of drug action (Kelleher and Morse, 1968). Since schedule-controlled behaviour has such fundamental significance for drug action, the investigation of the behavioural effects of a drug using various operant baseline performances is essential in behavioural pharmacology. Such an endeavor allows for the specification of the whole spectrum of behavioural actions of a particular drug.

Most psychoactive drugs have been extensively studied on various operant behaviours and with different species. Although many studies on the effects of morphine on schedule-maintained behaviour have been performed, few investigations have been done on the behavioural effects of other narcotic analgesic drugs and the narcotic antagonists or mixed-acting narcotic antagonists. A recent series of experiments performed by Holtzman (1974a, 1974b, 1974c) and Holtzman and Jewett (1972b,

1973) dealt with the individual effects of morphine, cyclazocine, naloxone and other narcotic antagonists upon continuous avoidance behaviour in the rat. The effects of naloxone as a narcotic antagonist were also tested in combination with (1) morphine, (2) cyclazocine, a mixed-acting narcotic antagonist, and finally, (3) d-amphetamine, a non-opioid psychoactive drug. The present research was designed to replicate and to extend some of Holtzman's studies in the rat by using a different schedule of reinforcement, namely, a Differential Reinforcement of Low Rates (DRL) operant schedule. Under a DRL schedule, a response is reinforced only if preceded by a pause of a specified duration timed from the previous response (Ferster and Skinner, 1957). Until very recently, the pattern of responding engendered by DRL schedules of reinforcement had not been used as a baseline to assess the behavioural effects of any narcotic drug. Ford and Balster (1976) investigated morphine and morphine withdrawal effects upon DRL performance. These researchers did not however assess the effects of other narcotic drugs upon DRL behaviour. To the extent that limited work has been done on the action of narcotic analgesic drugs using such a behavioural baseline, this research is somewhat exploratory in nature.

Several drug-behaviour manipulations were carried out in the present work. Statement of the specific aims of drug treatments follow the review of literature presented in Chapter I. The survey of literature involves a brief exposé on the main theoretical principles of behavioural pharmacology, followed by an evaluation of DRL behaviour as a pre-clinical tool in the assessment of drug-behaviour interactions and finally, an analysis of the behavioural effects of various narcotic and narcotic antagonist drugs. Chapter II provides a description of the experimental and statistical procedures used in this dissertation. Results are presented in Chapter III and discussed in Chapter IV.

CHAPTER I

Review of literature

Most reviews on drug-behaviour interactions agree that a behavioural drug response can be considered as a joint function between the substance's biochemical activity and the prevailing environmental conditions under which the drug was administered (Dews, 1958; Kelleher and Morse, 1968; Sidman, 1959). Particular factors have been found to be of extreme significance in the determination of the specificity of the behavioural effects of drugs. Before elaborating on some of these significant variables in drug-behaviour manipulations, a brief exposé on the rationale of operant conditioning techniques and their use in behavioural pharmacology will be presented.

Operant conditioning techniques

One of the fundamental requirements in the evaluation of drug behaviour interactions is a reproducible sample of behaviour upon which drug-correlated changes can be measured. The operant conditioning methods developed by B.F. Skinner nearly forty years ago provide accurate, sensitive, and reproducible techniques for controlling the behaviour of the individual subject (Skinner, 1938). The methods are based upon the follow-

ing principle: the characteristics of behaviour are, to a large extent, determined by its consequences. Behaviour controlled by its consequences is termed "operant behaviour" and the process of manipulating behaviour by means of its consequences or reinforcers is known as "operant conditioning". The schedule of reinforcement involves the precise way in which reinforcers are programmed to maintain operant behaviour (Skinner, 1938; Ferster and Skinner, 1957).

With these methods, reproducibility has been achieved: (a) by first choosing for assessment and manipulation, a response congenial to the behavioural repertoire of the organism and one that can be readily repeated (e.g., bar-pressing, key-pecking, etc.); (b) by selecting a reinforcing stimulus that is appropriate to the organism (e.g., food, water, etc.); (c) by limiting the experimental setting and finally; (d) by utilizing motivational levels such as hunger, thirst, pain, etc., that are powerful enough to abrogate many irrelevant factors (Skinner, 1938). When an organism is placed under these rigid experimental controls, a pattern of responding ensues that is highly stable. A "steady-state behaviour", defined as, "one in which the behaviour in question does not change its characteristics

over a period of time" results (Sidman, 1960).

Operant conditioning has been successfully used with birds and various mammals from the rat to human subjects (Weiss and Laties, 1967). Since the use of human experimental subjects in pharmacological studies is usually precluded on ethnical grounds, for most drugs, comparative behavioural pharmacology is inevitable. According to Weiss and Laties (1967), the most effective way to a truly comparative behavioural pharmacology is to emphasize behavioural principles that extend across a broad range of species. To the extent that operant conditioning principles are applicable to humans, the reliability in the generalization of drug-behaviour interactions derived from animals using operant techniques is greatly improved. Briefly, the steady-state behaviour generated by an operant schedule of reinforcement can act as a sensitive and reliable baseline upon which the action of various pharmacological agents can be evaluated (Sidman, 1960).

With the extensive use of operant conditioning methods in behavioural pharmacology, researchers have discovered that other factors unrelated to the drug's particular pharmacological properties were influencing a behavioural drug response. Chief determinants of drug action will be examined in the following section.

Determinants of drug action

The effects of pharmacological agents upon operant behaviour depend on a number of variables, with schedule-controlled behaviour being of utmost importance. The large body of research from which this fundamental generalization emerged has been extensively surveyed elsewhere (Kelleher and Morse, 1968). The present review will thus be limited to a concise analysis of the research that has largely contributed towards the elucidation of the nature of this schedule-drug interaction.

In an early study, Dews (1955) was the first to recognize the importance of schedule-controlled behaviour in the determination of a drug response. He reported that a barbiturate, pentobarbital, tested at doses of 0.25 to 5.6 mg, exerted differential effects on operant behaviour maintained by two different schedules of reinforcement. Pigeons responding under a fixed-ratio (FR 30) schedule of food presentation showed increments in key-pecking at dose levels of 0.5 to 4 mg, and substantial decreases at higher doses. On the other hand, rates engendered by a fixed-interval (FI 5 min) schedule were enhanced at very low doses (0.25 to 0.5 mg) and gradually reduced with increasing dose levels of the barbiturate (1 to 4 mg). The effect of a particu-

lar dose of pentobarbital varied from an increment to a decrement in response rates depending on the current schedule contingencies. Similar findings have been reported by other researchers using a multiple FI FR schedule (Morse and Herrnstein, 1956). The importance of schedule contingencies in the determination of drug effects is not restricted to the barbiturates, but extends across the major classes of psychoactive drugs. For instance, amphetamines (Dews, 1958; Kelleher and Morse, 1964; Smith, 1964) and tranquillizers (Cook and Kelleher, 1961; Kelleher and Morse, 1964) were also reported to exhibit differential effects depending upon the ongoing rate of responding under control conditions used in the assessment of their behavioural action.

In an attempt to further clarify the nature of the dependency of drugs upon schedule-maintained performance, Dews (1958b) elaborated another experiment looking at the effects of methamphetamine upon food-reinforced responding engendered by several schedules in pigeons, namely variable-interval (VI 1 min) fixed-interval (FI 15 min), and fixed-ratio (FR 50 or FR 900) schedules of food presentation. In the ratio schedules, the organism must emit a specified number of responses before the occurrence of the reinforcement, while the interval contingencies require that a specified interval of time elapsed before

reinforcement can occur. It is widely known that operant schedules of reinforcement fashion a great variety of temporal and serial patterns of steady-state behaviour (Ferster and Skinner, 1957; Kelleher and Morse, 1968). Under an FI schedule, ongoing behaviour is generally characterized by an initial period of no responding followed by a gradual increase in the response rate as the end of the interval approaches. On the other hand, variable-interval schedules typically generate moderate, even rates of responding. Fixed-ratio schedules engender high overall rates of responding, the exact patterning of behaviour depending however, on the parameter value of the schedule. As the schedule parameter is increased, the latency of the initial response increases yielding a post-reinforcement pause followed by sustained high terminal rates of responding.

In his study using the aforementioned schedules, Dews (1958b) observed that behaviour controlled by the FR900 and FI 15 min schedules, which yielded low pre-drug response rates, was facilitated by methamphetamine, while the high control rates maintained under both the VI and FR 50 components, were lowered by the psychomotor stimulant drug. Different schedules that yielded comparable baseline performances were similarly modified by amphetamines. From his observations, Dews (1958b) con-

cluded that the behavioural action of amphetamine was not dependent on the type of maintenance schedule per se, but on the rate of ongoing behaviour maintained by such a schedule. In other words, amphetamine's influence on behaviour is a function of the pre-drug baseline rate of responding.

It was previously stated that characteristic patterns of responding are generated by different schedule contingencies. In some schedules of reinforcement, the frequency of occurrence of responses within a single temporal segment may vary from near-zero values at one point to as many as one or more responses per second at another point (Ferster and Skinner, 1957). If a distribution of interresponse times (IRTs)[1] was plotted from these different components of a timing segment, the values of IRTs would differ greatly. High response rates generally yield short IRTs while low rates of responding mostly generate long IRTs.

By sampling different temporal periods of an FI segment, Smith (1964) studied the effects of amphetamine

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[1] The following definition, proposed by Morse (1966), is implied whenever the term interresponse time is is used in the present paper: "The elapsed time between the initiation of the response (R_N -1) and the next response (R_N) will be considered as a measurable property of the response R_N and called its interresponse time."

upon local FI rates of responding, i.e., the first and last minutes of the 5-min FI component. As expected from Dew's rate-dependency hypothesis, effective doses of amphetamine markedly increased low rates of responding or shortened the long IRTs characteristic of the first minute, while lowering the high rates of responding or short IRTs typical of the fifth minute. Besides confirming the rate-dependency phenomenon of amphetamine action, these data suggest that changes in the temporal distribution of responses can provide another useful index in the specification of drug-behaviour relationships.

The dependency of amphetamine action upon the rate and patterning of operant behaviour has consistently been reproduced using a variety of procedures and reinforcers in the pigeon, rat and monkey (Kelleher and Morse, 1968). For a detailed review on the rate-dependency of the behavioural effects of amphetamine, Dews and Wenger's (1977) recent paper is most complete. Abundant research extensively reviewed by Kelleher and Morse (1968), suggest that the behavioural effects of many psychoactive agents such as barbiturates (Dews, 1955; Dews, 1958a; Weiss and Laties, 1964), minor tranquillizers (Kelleher, Fry, Deegan, and Cook, 1961; Smith, 1964), anti-depressants (Smith, 1964), narcotic analgesics (McMillan, 1973a, 1973b; Thompson, Thrombley, Luke, and Lott, 1970), and hallucinogens (Appel,1971) are also dependent or at least partially dependent upon the rates and patterns

of schedule-maintained responding. Furthermore, the qualitative action of most drugs on behaviour has been found to be relatively independent of the reinforcing event used or the animal species studied, as long as comparable patterns of responding were generated under these different environmental conditions. Such experimental findings have led Kelleher and Morse (1968) to suggest that the effects of drugs depend more on the overt characteristics of the behaviour examined than upon the character of the reinforcing event or the nature of the motivational state supporting the behaviour. Different schedules of reinforcement engender different patterns and rates of operant responding which in turn are selectively influenced by drugs. Hence, the dependency of the effects of drugs on schedule-controlled behaviour occur because ongoing behaviour is a primary determinant of drug action (Kelleher and Morse, 1968).

These laboratory findings parallel human clinical and experimental observations that various psychoactive drugs exert differential effects depending upon the behavioural state of the individual at the time of drug administration (Freedman, Kaplan and Sadock, 1975; Hill, Belleville, and Wikler, 1957). For instance, tricyclic antidepressants such as imipramine, exert their mood-elevating and overall stimulating effects merely in

patients showing signs of endogenous depression. It has relatively little euphoriant action in normal persons (Freedman et al., 1975).

One last important determinant of drug action relates to the particular pharmacological properties of a given compound, namely its time and dose-effect relations. Several authors (Iversen and Iversen, 1975; Kelleher and Morse, 1968; Sidman, 1959) have reported that most drugs given at minimally effective dose levels exert inconsistent effects on behaviour, while at sufficiently large doses, it will invariably depress any behaviour. Between these two points, the drug will stimulate, disorganize or disrupt ongoing behaviour, but on many instances, the effective dose range is narrow and easily missed unless a wide range of doses is employed. Only by studying the effects of pharmacological agents over a broad range of dose levels will the behavioural pharmacologist be able to take into account the full spectrum of behavioural actions of a drug. Dose effect curves are thus essential in the study of drug-behaviour interactions.

In summary, the rate and patterning of schedule-controlled behaviour are critical variables outranking both the animal species and reinforcing event in the determination of drug action. Evidently, the type and

dose level of the pharmacological substance examined are also of fundamental importance in drug-behaviour manipulations.

Since steady-state behaviour is a basic determinant of drug action, full understanding of the characteristics of the behavioural performances employed in the evaluation of drug-behaviour relationships becomes imperative. As previously stated in the Introduction, the particular behavioural baseline used in the present work involves a DRL schedule of reinforcement also known as a schedule of interresponse time reinforcement. The next part of the review purports to analyze the distinctive features of a DRL maintained behaviour and second, to evaluate in the light of past research on drugs and DRL performance, its sensitivity as a pre-clinical tool in the assessment of the pharmacological and behavioural actions of drugs.

Drug effects on DRL-maintained behaviour

The DRL schedule can be described as follows: a response is reinforced only if preceded by a pause of specified duration timed from the preceding response (Ferster and Skinner, 1957). Behaviour engendered by a DRL schedule is characterized by a relatively low and stable rate of responding where the organism is expected to space its responses a certain minimal interval of time

to be reinforced (Ferster and Skinner, 1957). If the period of time seperating successive responses is less than the required waiting interval, no reinforcement will be given and the timing process will start again from the last emitted response. The interresponse time (IRT) distribution under a DRL schedule provides, by plotting the relative frequencies of IRTs, a useful measure of any drug-induced changes in the timing process.

In his study comparing the effects of dl-amphetamine and alcohol on water-reinforced DRL performance in rats, Sidman (1955) was the first to show the sensitivity of DRL behavioural baseline to the effects of psychoactive drugs. Administration of dl-amphetamine at large doses (1.5 - 3.0 mg/kg) generated a marked increment in the overall DRL responding as well as a shift of the modal IRT distribution towards the shorter IRT intervals, indicating that the animals tended to press too soon. On the other hand, ethyl alcohol given intraperitoneally at a dose of 1.0 mg/kg produced no alteration in the distribution of IRT intervals with the exception of a slight flattening of the curve as a consequence of a marked decline in the total response output. Lengthy periods of no responding contributed to the decrement in the overall response rate.

Using rats trained under a DRL 20 sec schedule of water reinforcement, Laties and Weiss (1962) reported similar findings. A small dose of alcohol (.25 g/kg) had rate-increasing effects while higher dose levels (0.5 - 1.0 g/kg) exerted suppressive effects on DRL responding without however disrupting the timing behaviour of the animals. The stimulating effect of small doses of alcohol may be comparable to the initial excitatory effects experienced by humans under the influence of alcohol. In fact, the effects of alcohol on DRL performance is not specific to rodents since humans under analogous operant conditions generated similar results (Laties and Weiss, 1962).

Sidman's (1955) finding that amphetamine disrupted spaced-responding by shortening waiting periods has consistently been reproduced (Ando, 1975; Kelleher et al., 1961; Sanger, Key and Blackman, 1974; Segal, 1962). Using animals trained on a modified DRL schedule with a limited hold (LH) contingency, which limits the upper limit of available reinforcements, Kelleher et al., (1961) reported marked increases in DRL responding after oral administration of d-amphetamine (0.3 - 3.0 mg/kg); an effect which peaked at a dose level of 0.75 mg/kg but still remained above control at 3.0 mg/kg of the drug. Such increases in DRL response rate disrupted the

efficiency in spacing the responses, resulting in dose-related decreases in reinforcement rates. Further research consistently reproduced the inverted U-relationship between doses of amphetamine and the rate of bar-pressing in rodents using a multiple DRL (Smith and Clark, 1975) and concurrent VI DRL (Segal, 1962) schedules of food reinforcement. Methylphenidate at doses of 2.5 to 10.0 mg/kg was also found to have comparable effects on DRL performance maintained by an operant schedule of water reinforcement (Stretch and Dalrymple, 1968).

Ando (1975) showed that other psychomotor stimulants such as methamphetamine, pipradol given subcutaneously, exerted similar actions on food-reinforced DRL performance, namely, rate-enhancing effects with concomitant decreases in the number of reinforcements. A shift of the IRT distribution towards shorter intervals was also noted.

The action of psychomotor stimulant drugs on DRL-maintained behaviour in rats is thus highly consistent from one research to the next despite some differences in the contingencies of reinforcement, in the type of appetitive reinforcer, the route of drug administration used, and finally in the dosage range studied. These results are also in agreement with the contention that amphetamine has rate-dependent effects, low pre-drug response rates being increased by amphetamine while

high baseline rates are depressed (Dews, 1958b). It was previously stated that the DRL schedule of reinforcement because of its requirement that responses be seperated a specified interval of time favors a low rate of responding, and thus shows the expected alterations of response rate under the influence of stimulants.

In view of the highly homogeneous and stable action of psychomotor stimulants on DRL performance in rats, it is surprising to find that such uniformity in data fails to appear with other infrahuman species. In monkeys, the effects of amphetamines are highly inconsistent. Researchers observed both a decrease in total DRL response rates with a shortening of the mean IRT intervals (Hodos, Ross, and Brady, 1962; Weiss and Laties, 1967) and a lengthening of the waiting times between successive responses (Weiss and Laties, 1967).

Human studies dealing with the action of amphetamine on a baseline behaviour similar to DRL-controlled responding also yielded variable results. In 1958, Dews and Morse examined the effects of a single dose of d-amphetamine (5.0 mg per os) against a behavioural baseline maintained under a concurrent FR DRL schedule of money reinforcement. In keeping with the results obtained in animal studies the average distribution of IRT's was significantly moved to the left without affect-

ing the efficiency of the behaviour as reflected by the unchanged number of reinforcements from control performance. Slight but statistically insignificant increases in the total response output were observed under the influence of the drug. Negative results were however reported by Weiss and Laties (1964a) regarding the effects of 10 mg per os of amphetamine on human DRL behaviour.

The failure of human studies to consistently reproduce amphetamine action analogous to those observed in rodents challenges the degree of cross-species generalization obtainable with this kind of work. Weiss and Laties (1967) stated some possible reasons why psychomotor stimulants as compared to other drugs such as alcohol yield conflicting cross-species results. Researchers working with amphetamine experienced many reservations in giving to humans high dose levels which may induce severe side-effects. Thus, dose-response curve of the drug can hardly be done, limiting the observations of researchers to a single or few drug dosage levels that are minimally effective. In humans, amphetamine has been found to display its peak effects several hours after the administration of the drug (Burns, House, and Miller, 1962). With human subjects, it is hardly feasible to extend an experimental session over a period of time which would be sufficiently long to permit full

observation of the drug effects. Dews and Morse (1958) also suggest that it is improbable that nickel reinforcers in man bears comparable motivational incentive as food or water in a severely deprived animal. Further work needs to be done before elaborating any conclusions on the degree of generality of interspecies studies on amphetamine behavioural action.

The effects of tranquillizing drugs on DRL ongoing pattern of behaviour have also been investigated but not as extensively as the psychomotor stimulants. The DRL-controlled behavioural baseline has proven to be differentially sensitive to the two main classes of tranquillizers: the minor ones producing a general stimulatory effect while the major tranquillizers or neuroleptics induced an overall depressant action.

The administration of minor tranquillizers in animals trained under a DRL schedule of food reinforcement surprisingly yielded data analogous to results obtained with stimulants (Richelle, Xhenseval, Fontaine, and Thone, 1962; Sanger and Blackman, 1975). Richelle et al., (1962) discovered that although rats under chlordiazepoxide showed signs of depression in their general behaviour such as impaired coordination of movements, somnolence, etc., their conditioned behaviour was altered

in a completely opposite way. Increases in DRL responding and a shortening of the mean IRT intervals were observed for small doses of chlordiazepoxide (1.2 - 5.0 mg/os). Chlordiazepoxide tested at a more extensive dose range of 1.0 to 16 mg/kg was found to generate similar effects (Sanger and Blackman, 1975; Sanger et al., 1974). Like amphetamines, higher dose levels of the drug resulted in the depression of the overall response rates. Another minor tranquillizer, meprobamate displayed amphetamine-like effects on DRL-maintained behaviour, including the displacement of the modal IRT towards the lower IRT values (Kelleher et al., 1961).

The inverted U-relationship between drug dosage of chlordiazepoxide and the total response output was however not found by some authors (Ando, 1975; Smith and Clark, 1975). Using a multiple DRL schedule of food reinforcement where each parameter values of 10 sec, 20 sec, and 60 sec intervals were paired to a different auditory stimulus signalling their onset, Smith and Clark (1975) discovered that the administration of chlordiazepoxide at doses up to 40 mg/kg generated no significant effects on DRL performance in rats. In another research (Ando, 1975), chlordiazepoxide and diazepam were observed to affect DRL behaviour in a manner totally opposite to previous reported data. Instead of a shift of

the IRT curve towards the left, an increased dispersion in the relative frequencies of the IRT distribution was noted under the influence of diazepam (1 and 2 mg/kg) and chlordiazepoxide (16 mg/kg). An increment in the total behavioural output was not observed.

In an attempt to reconcile these seemingly incongruous results, a prime consideration of the generally recognized fact that different situational conditions control different patterns of operant behaviour and that drugs differentially modify these ongoing patterns of behaviour is warranted (Kelleher and Morse, 1968). A careful review of Smith and Clark's (1975) paper revealed that the apparatus used comprised a licking tube and a running wheel designed to measure the intercurrent behaviours, an addition which may introduce confounding variables that would reduce the degree of comparability of their study with previous ones. Moreover, the onset of the different parameter values of the multiple schedule they used was controlled by external stimuli, which were shown to be significant factors in the determination of certain drug effects on operant behaviour (Heise and Lilie, 1971; Laties and Weiss, 1966). As for Ando's study, the experimental design lacked clarity and controls. The animals were exposed to an increasing dosage

levels of a drug till "marked changes were observed in the IRT histogram." No replication of the observations were done eventhough a small number of animals were used. Considering that an animal's first exposure to a drug is often variable and unpredictable, one can doubt the reliability of his data.

The analogous effects of minor tranquillizers and stimulants on behaviour maintained by a DRL schedule are unexpected, since these drugs are widely known to exert different and even opposite effects on the general behaviour of both humans and animals. This paradoxical finding has lead researchers to suggest that DRL maintained behaviour lacks sensitivity to the differential effects of drugs, thus questioning the usefulness of DRL ongoing behaviour as a mean to assess behavioural effects of psychoactive drugs (Morrison and Stephenson, 1974). The analysis of individual features of DRL-maintained behaviour revealed to be particularly useful in the assessment of the differential effects of benzodiazepines and amphetamines. In a study comparing the effects of d-amphetamine and chlordiazepoxide, Sanger et al., (1974) have shown that the effects of stimulants and minor tranquillizers on behaviour maintained under a DRL schedule may be differentiated in terms of their effects on bursts of responding, that is, the number of

responses occurring within 1.5 sec of a previous one. Chlordiazepoxide was found to increase the percentage of these response bursts following unreinforced responses while d-amphetamine had no consistent effect on this measure. This effect occurred at all doses regardless of the drug effect on the overall response rate (Sanger and Blackman, 1975). Both drugs also differed in their relative potency; d-amphetamine-induced changes being more intensive and occurring at markedly smaller dose levels. In addition, tolerance did not develop to the rate-increasing effects of meprobamate and chlordiazepoxide on DRL performance (Richelle et al., 1962), while it did to the amphetamine rate-enhancing action (Schuster and Zimmerman, 1961).

The action of major tranquillizers such as chlorpromazine and prochlorperazine on spaced-responding in rats has been fully investigated, yielding highly consistent results between different studies despite some procedural and schedule differences (Ando, 1975; Kelleher et al., 1961; Sanger and Blackman, 1975; Smith and Clark, 1975). A dose-related decrease in DRL responding as well as an increment in the average IRT were observed under the effect of phenothiazines tested at various dose levels (Ando, 1975; Kelleher et al., 1961). This finding supports previous reports showing that

chlorpromazine can depress rates of operant responding maintained under different schedules of reinforcement (Clark, 1969). Despite their common depressant action on the nervous system, the major and minor tranquillizers are quite different in their structural aspects and in their general behavioural effects in humans (Iversen and Iversen, 1975). These qualitative differences are being reflected in their differential action on DRL steady-state behaviour.

One class of compounds that share strong chemical similarities with the phenothiazines, but exerts entirely antithetic mood-modifying effects are the tricyclic anti-depressants. Besides being structural analogue, it seems that these two pharmacological agents are similar when it comes to their effects on conditioned operant behaviour. Anti-depressants like the neuroleptics produced dose-related decrements in food-reinforced behaviour maintained under various schedules of reinforcement (Cook and Kelleher, 1962; Iversen and Iversen, 1975). Ando (1975) showed that this preceding observation is also appropriate when describing the effects of imipramine upon DRL performance.

The effects of barbiturates, hallucinogens and cannabinols were also examined upon DRL-maintained behaviour. As previously reported for the above-mentioned psycho-

active agents, they were also found to affect DRL performance in a unique manner (Appel, 1971; Frankenheim, 1974; Kelleher et al., 1961).

Few studies have utilized the DRL baseline as a mean for assessing the behavioural action of drug antagonism, but the relatively few that did, yielded interesting results (Stretch and Dalrymple, 1968; Fontaine and Richelle, 1967). Reserpine given at appropriate daily doses over a period of about ten days consistently induced suppression in lever-pressing animals under a DRL schedule of water reinforcement (Sidman, 1956; Stretch and Dalrymple, 1968). Methylphenidate at doses of 2.5, 5.0 and especially 10.0 mg/kg reinstated reserpine-suppressed responding to a degree proportional to the psychomotor stimulant rate-enhancing effect (Stretch and Dalrymple, 1968). In this same study, the effects of methylphenidate alone and in combination with pentobarbital were also assessed. Both of these drugs given alone at doses of 2.5 and 5.0 mg/kg were found to elevate DRL responding in rats. Combinations of the drugs at the same doses produced rate increments greater than those exhibited by either drug acting alone. Similarly, both drugs administered at a dose level of 10.0 mg/kg reduced the response frequency below control values and below

those of each drug given seperately.

Fontaine and Richelle (1967) examined the effects of tremorine alone and in combination with either one of two cholinergic-blocking agents, atropine and scopolamine. The dose-related decline in the total number of emitted DRL responses under the cholinomimetic drug, i.e., tremorine was fully antagonized by both cholinergic-blocking agents. Judging by the results of these two studies, the ongoing behaviour maintained under a DRL schedule of reinforcement is quite sensitive to drug combinations and seems most promising for future research in the study of drug antagonism.

To summarize, past research has amply illustrated the differential sensitivity of DRL performance to the individual effect of several major classes of psychoactive drugs. Some antagonistic interactions resulting from drug mixtures were also clearly demonstrated using such a behavioural baseline. In the present research, the DRL behaviour will be used to assess the individual and combined effects of some drugs belonging to the pharmacologic group of narcotic analgesic drugs. Apart from one recent publication (Ford and Balster, 1976), the behavioural actions of narcotic analgesic drugs upon DRL-maintained behaviour have not previously been

evaluated. Ford and Balster (1976) studied the effects of acute and chronic administration of morphine and its withdrawal upon DRL performance in the rats. They did not however, measure the effects of other narcotic analgesic drugs upon timing behaviour. Because little research presently exists on the specific action of narcotic analgesic drugs upon DRL responding, the effects of such drugs on operant behaviours maintained by other schedules of reinforcement will be examined in the following section.

Behavioural effects of narcotic analgesic drugs and narcotic antagonists

"Opioids" or "opiates" involve drugs that are naturally occurring alkaloids of the opium poppy of which morphine is the main example, and the many related synthetic drugs (Goth, 1976). Morphine exerts several effects besides its analgesic activity. It produces mixed effects on the central nervous system; depressing some functions of the brain (e.g., respiration) and stimulating others. It also produces a number of psychological effects of which its ability to induce a state of euphoric well-being, an effect strongly contributing to its status as a drug of abuse.

The substitution in any opioid of an allyl or cyclopromethyl radical have resulted in the creation of a number

of narcotic compounds commonly known as the narcotic antagonists. This class of substances vary in their capacity to block morphine effects. Antagonists such as cyclazocine and nalorphine can reverse some of morphine-induced effects such as its respiratory depression, although they retain its analgesic activity. These mixed-acting narcotic antagonists exert some effects similar to those of morphine (agonist) and block some of morphine's activities (antagonist). Other narcotic antagonist such as naloxone and naltrexone are almost completely devoid of agonistic properties (Goth, 1976).

The most extensive series of experiments on the behavioural effects of narcotic analgesic drugs and narcotic antagonists, when given alone or in combination, were done in the pigeon using a multiple FI FR schedule of food reinforcement (Dysktra, McMillan, and Harris, 1974; McMillan and Harris, 1973; McMillan and Morse, 1967; McMillan, Wolf, and Carchman, 1970); in the rat using an electric-shock avoidance schedule (Holtzman, 1974a, 1974b, 1974c; Holtzman and Jewett, 1972a, 1972b, 1973), and finally, in the monkey using both of the aforementioned schedules of reinforcement (Downs and Woods, 1976; Goldberg, Morse, and Goldberg, 1976; Holtzman, 1976; McKearney, 1974). In the studies using a multiple FI FR schedule of food reinforcement

(Downs and Woods, 1976; Goldberg et al., 1976; McMillan and Morse, 1967), it was generally found that acutely-administered morphine exerts dose-related decreases in responding under both the FI and FR components and in the FI quarter-life value (i.e., the percentage of the interval taken for the first quarter of the responses to occur). While behavioural stimulation occasionally occurred following low doses of morphine, this effect was slight and inconsistent. Comparable results were obtained with rats trained under a variety of operant schedules of food reinforcement (Ford and Balster, 1976; Thompson et al., 1971; Tsou, 1966). For example, Ford and Balster (1976) reported that acute administration of morphine produced small statistically non-significant increments in DRL responding at low doses (1.8 - 5.6 mg/kg) and decreases in the frequency of emitted responses at higher dose levels (10 - 30 mg/kg).

In another study looking at the effects of morphine in rats responding under a continuous avoidance schedule, morphine surprisingly generated a dose-effect curve similar to that of a psychomotor stimulant (Holtzman and Jewett, 1972a). Following acute morphine treatment, large dose-related increments in avoidance behaviour were observed at doses of 0.3 to 8.0 mg/kg. A higher dose only disrupted avoidance responding as evidenced by a

change in the temporal patterning of responses and the increasing number of shocks received by the animals. Work done in the squirrel monkey using similar schedule parameters yielded qualitatively analogous results (Holtzman, 1976a). The considerable divergence in morphine action upon shock-maintained behaviour when compared to food-reinforced performance have led researchers to investigate the extent to which the nature of the maintaining event may be important in determining the behavioural actions of morphine.

In his study, McKearney (1974) compared the effects of morphine, d-amphetamine and chlorpromazine upon comparable patterns of behaviour generated by FI schedules of either food or shock presentation. It was found that d-amphetamine and to a lesser extent, chlorpromazine effects were relatively independent of the character of the reinforcing stimulus. In contrast, morphine was reported to increase FI responding in the shock maintained baseline, but to only decrease response rates at the same dosage (0.1 - 0.3 mg/kg) under the FI schedule of food presentation. From his observations, McKearney (1974) concluded that the differential effects of morphine on behaviour reinforced by different environmental events apparently indicated a dependence of the narcotic analgesic drug on

the nature of maintaining stimulus.

A more recent experiment (Byrd, 1976) using similar schedule parameters and animal species failed to reproduce the behavioural stimulation in shock-maintained responding observed by McKearney (1974) following acute morphine administration. Since these two studies were comparable in most respects, i.e., ongoing rates and patterns of behaviour, shock intensity and duration, species, doses tested, etc., it is difficult to explain their contradictory results. Further research is needed before more conclusive statements regarding the interaction between morphine and the reinforcing stimulus can be elaborated.

Tolerance to a drug is the diminished responsiveness of an organism after repeated exposure to the compound; or conversely, a gradual increase in the dose of a drug required to elicit an effect similar to the effect of an initial dose. Tolerance can occur with or without the development of physical dependence, which is an abnormal state that becomes manifest by a number of characteristic autonomic and behavioural signs, the withdrawal or abstinence syndrome, upon abrupt termination of chronic drug treatment or may be precipitated by administration of a specific antagonist substance (Seevers and Deneau, 1963). Both tolerance and physical dependence develop with chronic

administration of morphine.

Tolerance development to morphine-induced behavioural depression in rats was reported following chronic daily administration of morphine at doses as low as 7.5 mg/kg (Rhodus, Elsmore and Manning, 1974) to as large a dose as 600 mg/kg/day (Ford and Balster, 1976). In the first study, morphine was given for fifteen consecutive days at a daily dose of 7.5 mg/kg to rats lever-pressing for either food or saccharine solution under a multiple FI FI schedule. Chronic morphine treatment quickly reduced the drug's initial rate-decreasing effects, but pre-drug levels of responding were however, not reached for most animals. In Ford and Balster's (1976) experiment, physical dependence on morphine was induced by twice daily injections at an initial dose of 40 mg/kg/day which was increased by 80 mg/kg/day until 600 mg/kg/day was attained; this dose regimen was then continued for 14 consecutive days. During chronic administration of morphine, DRL responding was more variable and generally decreased. However, some tolerance to the rate-reducing effects of morphine was apparent during the last five days of the chronic morphine regime, when mean response rates were near or within the control baseline. Cessation of morphine treatment produced a marked decrease in responding early in withdrawal (tested at 22.5 h) followed by res-

ponse rate increases during the period of 70.5 to 118.5 h after chronic administration of morphine was discontinued. Disruption in FI rate of responding following morphine withdrawal or nalorphine-precipitated abstinence was also noted in morphine-dependent rats (Babbini, Gaiardi and Bartoletti, 1976). These experiments suggest that operant behaviour is a sensitive indicator of the behavioural changes associated with morphine tolerance and the morphine withdrawal syndrome.

In summary, morphine at certain doses can act to increase or decrease schedule-controlled responding in the pigeon, rat and monkey. The occurrence and magnitude of these different effects of morphine depend on the schedule contingencies, the species studied and possibly on the type of reinforcing stimulus. The influence of this last factor in the determination of the behavioural effects of morphine remains to be elucidated. Tolerance and withdrawal phenomena associated with chronic morphine administration were also clearly illustrated with operant behavioural measures.

The use of operant baseline performances as a means to assess the individual effects of various narcotic antagonists generated data qualitatively similar to those described for acute administered morphine. In a series of experiments examining the effects of narcotic antagonists

upon continuous avoidance behaviour in the rat, Holtzman (1974a, 1974b, 1974c) showed that cyclazocine, pentazocine and nalorphine produced substantial increases in responding over wide ranges of doses. Large dose levels of the antagonists with the exception of nalorphine decreased the overall avoidance rates at or below pre-drug control values. Similar biphasic effects of cyclazocine were reported upon avoidance behaviour of squirrel monkeys (Holtzman, 1976), and the FI food-maintained responding of pigeons (McMillan and Harris, 1972; McMillan and Morse, 1967). The last authors have also replicated pentazocine biphasic action in pigeons trained to key-press for food under a multiple FI FR schedule of reinforcement. These results suggest that the behavioural effects of these mixed-acting narcotic antagonists are not species specific nor do they depend on the nature of the reinforcing stimulus.

The behavioural action of naloxone, a narcotic antagonist with no agonist activity except at very large doses, was also assessed, using schedule contingencies analogous to those employed in previously reported studies. Naloxone diverged from the other mixed-acting narcotic antagonist drugs, by its relative lack of activity upon behaviour maintained by either shock-delivery or termination of shock in both the squirrel monkey and the rat (Byrd, 1976;

Holtzman, 1974b, 1974c, 1976a; Holtzman and Jewett, 1973). However, on the pattern of responding maintained by a multiple FI FR schedule of food reinforcement in the pigeon, McMillan, Wolf and Carchman (1970) noted that naloxone exhibited behavioural effects characteristic of other narcotic antagonists, i.e., an increment in FI responding at low doses (3.0 - 16.0 mg/kg) while higher dose levels (32 and 64 mg/kg) decreased the rate of responding under both FI and FR components. Using an identical schedule of reinforcement in pigeons and monkeys, other researchers (Downs and Woods, 1976; Goldberg et al., 1976) were not successful at reproducing consistent rate-enhancing effects with low doses of naloxone. Large doses of the drug which often induced observable behavioural disturbances generally depressed operant behaviour, as previously reported by McMillan et al., (1970). In conclusion, it appears that non-toxic doses of naloxone exert no consistent, systematic effects on learned behaviour.

Schedule-controlled behaviour has also been used to investigate the interactions between narcotic analgesic drugs and narcotic antagonists. Since the present dissertation deals specifically with the antagonistic effects of naloxone, the survey of drug-antagonism studies will be restricted to this narcotic antagonist.

In a typical study of morphine and naloxone inter-

actions upon shock-maintained behaviour in squirrel monkeys, Byrd (1976) observed reversal of morphine rate-suppressive effects and a shift of the morphine dose-effect curve to the right when appropriate doses of naloxone (.01 - 1.0 mg/kg) were administered in combination with morphine. The magnitude of this antagonism was a function of the dosage level of naloxone. Irrespective of the schedule conditions used and the species studied, naloxone invariably acted as a strong antagonist of morphine behavioural depression in several experiments (Downs and Woods, 1974; Goldberg et al., 1974; Holtzman, 1976a, 1976b; McMillan et al., 1970). Furthermore, the stimulatory effects of morphine upon the learned behaviour of some pigeons and monkeys were also blocked by concomitant administration of naloxone (McMillan, 1974; McMillan et al., 1970). Briefly, naloxone appears to be a potent antagonist of both the stimulant and depressant components of morphine action upon operant behaviour.

As previously mentioned, mixed-acting narcotic antagonists such as cyclazocine and pentazocine exert morphine-like effects upon schedule-controlled performances. Like morphine, these compounds are also quite sensitive to the antagonistic actions of naloxone. For instance, naloxone (at doses of 8.0 - 16 mg/kg in rats and 1.0 mg/kg in monkeys) was reported to strongly antagonize the

stimulant and depressant effects of cyclazocine upon avoidance behaviour in animals (Holtzman, 1974, 1976; Holtzman and Jewett, 1974) and of cyclazocine-induced hallucinations and euphoria in man (Jasinski, Martin, and Haertzen, 1967). However, the pigeon appears to differ in this respect. Naloxone was found to be ineffective against cyclazocine suppression of FI behaviour maintained by food presentation (McMillan et al., 1970). Whether the dissimilarities are a result of species differences or the different schedules used remains to be determined.

Naloxone's potent antagonistic properties do not appear to be limited to drugs of the opioid class. When tested in combination with amphetamine, naloxone blocked amphetamine effects upon avoidance behaviour in rats, by producing a downward shift in the amphetamine dose-effect curve (Holtzman, 1974a). This type of drug antagonism differs from naloxone's competitive antagonism of opioid drugs which is usually characterized by a parallel shift of the narcotic dose-response curve to the right. To the extent that the effect produced by the combination of naloxone and amphetamine is significantly different from the sum of their individual effects, Holtzman (1974c) acknowledges the existence of a particular interaction or antagonism between these two drugs. However, the nature of or the mechanism of action underlying this antagonism

remains unknown.

Summary and statement of problems

The review of literature began by emphasizing the usefulness of operant conditioning methods in the study of drug-behaviour relationships, with particular attention being given to those variables that have proven most significant in the determination of drug action. Outcome research on the behavioural actions of several psychoactive agents upon a schedule of DRL reinforcement was then considered and preceded the final section on the behavioural effects of narcotic analgesic and narcotic antagonist drugs. To recapitulate, the critical review suggested the following conclusions:

1. The steady-state behaviour engendered in the application of operant conditioning principles provides accurate, sensitive, and reproducible baselines upon which the action of various pharmacological agents can be evaluated.

2. The behavioural effects of drugs are highly dependent on schedule-maintained behaviour; the rate and patterning of operant responding being primary determinants of drug action.

3. Operant behaviour generated by a schedule of interresponse time reinforcement (DRL) has proven to be quite sensitive to the differential effects of several major classes of psychoactive drugs.

4. Morphine, a prototypical narcotic analgesic drug, can act to increase or decrease the occurrence of schedule-controlled responding in the pigeon, rat and monkey. Tolerance and the withdrawal syndrome associated with chronic morphine administration

are readily detected by schedule-controlled behavioural baselines.

5. The mixed-acting narcotic antagonists, such as cyclazocine, exert morphine-like biphasic effects upon conditioned avoidance behaviour in the rat and monkey, and on the food-reinforced key-pecking behaviour of pigeons; whereas, naloxone, a relatively pure narcotic antagonist is behaviourally inactive at non-toxic doses.

6. Naloxone is a strong competitive antagonist of morphine-induced changes in operant behaviour, and of cyclazocine stimulant and depressant effects on conditioned behaviour in rats and monkeys. It, however, fails to antagonize cyclazocine effects upon food-maintained behaviour in the pigeon.

7. Naloxone antagonism is not limited to drugs of the opioid class, since it also modifies amphetamine effects upon avoidance behaviour in rats.

Having summarized the major concepts and experimental findings which serve as a framework for this dissertation, a statement of the problems to be investigated follows.

Since schedule-controlled behaviour is a fundamental determinant of drug action, the investigation of the whole spectrum of behavioural effects of a drug necessarily involves the observation of its activity upon various operant baseline performances. With the exception of morphine, behaviour engendered by a schedule of interresponse time reinforcement (DRL) has never been used in the assessment of the behavioural effects of narcotic analgesic drugs and narcotic antagonist drugs. Most behavioural studies on the individual and combined effects of opioid agents

were conducted using electric-shock avoidance behaviour in rodents (Holtzman, 1974a, 1974b, 1974c). The present research was designed to extend some of Holtzman's drug-behaviour manipulations in the rat using a DRL schedule of reinforcement in which low rates of responding could be maintained by food-presentation for purposes of comparison with Holtzman's work involving electric-shock avoidance behaviour.

In this dissertation, the acute effects of morphine, naloxone, dl-cyclazocine and a psychomotor stimulant drug, d-amphetamine, upon DRL behaviour were studied across appropriate ranges of doses. Further experiments involved testing combinations of doses of morphine and naloxone, dl-cyclazocine-naloxone, and d-amphetamine-naloxone upon the same behavioural baseline.

Kelleher and Morse (1968) have strongly advocated that the behavioural state of an organism is a critical determinant of drug action. If animals are rendered behaviourally tolerant to morphine through repeated exposure to it, it seems legitimate to assume that such treatment will modify the ongoing state of the organism. Consequently, comparisons of morphine antagonism by naloxone in animals (a) in a non-tolerant state, and (b) in a state of morphine tolerance are needed. Thus, in another experiment, induction of tolerance to the behavioural

effects of morphine was studied, followed by further tests with the morphine antagonist, naloxone.

CHAPTER II

Methodology

Subjects

The animals were 11 experimentally naive male and female hooded rats of the Royal Victoria Hospital strain weighing 300-400 g (males) and 195-265 g (females) when allowed free access to food and water. They were deprived of food until they reached 80% of their free-feeding weight, and subsequently maintained at this weight by adjusted feedings after each experimental session. Each rat was caged individually in a temperature-and humidity-controlled colony room illuminated according to a 12-hour light-dark cycle. Water was always available in the home cages, but not in the experimental chambers.

Apparatus

Behaviour was studied in commercially-manufactured operant test chambers (Grason-Stadler, E3125C) situated within sound-attenuating, ventilated isolation cubicles. Each experimental space measured 29.8 cm by 26 cm by 25.4 cm. Two walls were constructed of sheet metal; the back, top and door were made of clear plastic. A Gerbrands rat lever, 5 cm wide, was located 1.5 cm to the right of the center of one of the metal walls of the chamber, 9.2 cm above the grid floor, extending 1.6 cm into the experimental space. When a rat pressed the lever with

a force of 15 g or more, a response was recorded and an audible click occurred. A pellet dispenser (Ralph Gerbrands Co., Arlington, Mass.) delivered standard 45 mg Noyes food pellets into a recessed opening located above the grid floor to the left of the response lever. A shielded 10 W houselight illuminated the experimental space and low-level masking noise (75 db) was continuously present during all sessions; a red cue light situated above the response lever was illuminated for 0.3 sec whenever a food pellet was delivered.

Response contingencies were automatically programmed by timers, stepping switches and relays located in an adjoining room. Responses and food-pellet presentations were tabulated by electro-magnetic digital counters (Sodeco); session length was recorded by an elapsed time meter.

Procedure

Preliminary training After the establishment of the deprivation schedule, each animal was hand-shaped to press the lever, i.e., each approximation to a bar press was successively reinforced until the rat emitted the operant response. Thereafter, each rat was allowed to lever-press for a maximum of 100 reinforcements under a continuous schedule of reinforcement (CRF). The length of the CRF training varied between animals but on the average, three

sessions were sufficient for the rats to reach the criterion of 100 reinforcements.

Subsequently, the animals were exposed to the DRL schedule of reinforcement. From the introduction of the DRL schedule to the completion of the experiments, daily sessions lasted for either one hour or until the subjects had obtained a maximum of 100 food-pellets, whichever came first. The subjects first worked under a DRL 20 sec schedule modified by the addition of a limited-hold (LH) set at 30 sec. Such a schedule required that successive responses be spaced at least 20 sec apart but not more than 50 sec to be successfully reinforced. Once the animals had succeeded to obtain the maximum available reinforcements under DRL 20 LH 30, the limited hold contingency was lowered to 10 sec.

Stabilization of performance Under the DRL 20 LH 10 schedule, a response was reinforced if it followed the preceding given response with a time interval of at least 20 sec but not exceeding 30 sec. A reinforced response started the timing cycle again. Lever-presses that occurred beyond the limits of the LH period were not reinforced and also reset the timing cycle. The animals were trained on the DRL 20 LH 10 schedule of reinforcement for about 25 consecutive sessions to achieve a stable baseline performance before any drug experiments were conducted. During stabilization, the animals were

injected on two occasions with 1 ml/kg of saline, 5 min before the sessions, in order for them to adapt to the injection procedure.

Drug Experiments Morphine sulphate, naloxone hydrochloride and d-amphetamine sulphate were dissolved and diluted with 0.9% saline solution, using sterile procedures. All doses are expressed in terms of the salts. dl-Cyclazocine, as a free base, was dissolved in a vehicle consisting of 3 parts of 8.5% lactic acid and 2 parts 1.ON NaOH (pH4-5). Drug solutions were diluted to a concentration that allowed each dose studied to be injected in a volume of 0.1 ml/100 g body weight. Equivalent volumes of 0.9% saline solution, and/or vehicle for cyclazocine doses, served as control injections. Saline and drug injections were made intraperitoneally (i.p.). When mixtures of two drugs were studied, one drug was injected i.p. on one side of the animal and the other drug was injected i.p. on the other side of the abdominal midline. All injections were given immediately before the animal was placed in the test chamber, 5 min before the session began. Animals were tested at the same time each day, seven days per week. Unless otherwise stated, at least two non-drug days, one of which chosen at random was preceded by a control (saline) injection, intervened

between individual drug test sessions. Drug treatments were not administered unless response and reinforcement rates were typical of baseline performance.

Separate effects of morphine, naloxone, dl-cyclazocine, and d-amphetamine. Four female rats were used to assess the effects of naloxone at doses of 0.1, 0.3, 1, 3, 5.6 and 10 mg/kg on DRL performance. Each dose of naloxone was given twice to each animal on separate occasions; the testing sequence for individual doses was randomized. Subsequently, the same animals were used to determine the effects of acute doses of morphine (1, 3, 5.6, 10 and 30 mg/kg), using identical testing procedures with the exception that each dose of morphine was tested on three separate occasions for each animal. Three male rats received dl-cyclazocine at doses of 0.01, 0.1, 0.3, 1.0, 3.0, 5.6, and 10 mg/kg. Each dose was tested on three separate occasions per animal in a random sequence; at least two control sessions, one of which was preceded by a control (vehicle) injection, intervened between drug test sessions. Three additional male rats, following identical testing procedures, were used to determine the effects of d-amphetamine at doses of 0.1, 0.3, 1, 3, 5.6 and 10 mg/kg; individual doses were given twice to each animal on separate occasions, using a random sequence.

Combined effects of morphine and naloxone in non-tolerant and in morphine-tolerant animals The female rats, used previously to assess the separate effects of morphine and naloxone, participated in two further experiments. First, each animal received on two separate occasions each of the following dose combinations of the two drugs: 3 mg/kg morphine and 0.1 mg/kg naloxone; 3 mg/kg morphine and 1 mg/kg naloxone; 10 mg/kg morphine and 0.1 mg/kg naloxone; 10 mg/kg morphine and 1 mg/kg naloxone; 30 mg/kg morphine and 0.1 mg/kg naloxone, and 30 mg/kg morphine and 1 mg/kg naloxone. As before, drug test sessions were followed by not less than two control sessions of which one, chosen at random, was preceded by a dual injection of saline. The order of administration of the drug combinations within each test period was determined in an essentially random way. After completing the acute morphine-naloxone antagonism experiment, one rat became ill and died. The three remaining animals participated in a second experiment involving chronic (daily) administration of morphine. For 25 consecutive sessions, each rat received an i.p. injection of morphine at a dose of 30 mg/kg, given 5 min before each session. Since responding remained severely suppressed throughout the period of 25 sessions (see Results, Fig. 5A), the

dose of morphine was reduced and for the next 35 days, each session was preceded by an injection of morphine at a dose of 10 mg/kg, given 5 min before each session.

Chronic daily administration of morphine (10 mg/kg i.p.) was continued throughout a series of consecutive sessions, some of which were preceded by an injection of saline or by naloxone at doses of 0.01, 0.1 or 1 mg/kg i.p. Two determinations of the effects of each morphine-naloxone dose combination were obtained on separate occasions; naloxone test sessions were preceded by not less than two sessions in which either morphine alone (10 mg/kg) or morphine and a second (control) injection of saline were given 5 min before the session.

Combined effects of dl-cyclazocine and naloxone Three male rats, used previously to determine the cyclazocine dose-effect curve, received on two separate occasions, naloxone at a dose of 1 mg/kg in combination with either vehicle or cyclazocine at doses of 0.1, 1, 5.6 and 10 mg/kg. At least two sessions, one preceded by dual injection of vehicle as a control procedure intervened between cyclazocine-naloxone drug combination tests.

Combined effects of d-amphetamine and naloxone Two male rats (used previously to determine the amphetamine dose-effect curve) received, on separate occasions, each

of the following amphetamine- or saline-naloxone dose combinations: 0.1 mg/kg amphetamine and 1, 3, or 10 mg/kg naloxone; 1 mg/kg amphetamine and 1, 3, or 10 mg/kg naloxone; 3 mg/kg amphetamine and 1, 3, or 10 mg/kg naloxone; and saline and 1, 3, or 10 mg/kg naloxone. Not less than two sessions, one preceded by two injections of saline as a control procedure intervened between amphetamine-naloxone drug combination tests; each combination of the two drugs was tested on three separate occasions per animal in a randomized sequence.

Behavioral measures For each individual session, and for each subject, reinforcement frequencies and average response rates per second were computed from data collected on the counters and elapsed time meter. Interresponse times (IRTs) were also obtained. All IRTs less than 50 sec were recorded in 10 5-sec class intervals or bins, and all IRTs greater than 50 sec registered in the 11th class interval. The frequency distribution of IRTs expressed in percentage form derived from single sessions or individual subjects were computed for the drug treatments and for some control sessions. The average group response rates and reinforcement frequencies for the various drug treatments were compared with the mean rates of their respective control sessions. The standard errors of the means were also calculated.

CHAPTER III

Results

Control performances

Throughout the whole study, at least two non-drug control days, one of no injection and one of saline or vehicle pretreatment intervened between individual drug sessions. For each experimental manipulation, the mean response rates and averaged number of reinforcements per session obtained by animals performing under these two control conditions were calculated and tabulated in Tables 1 and 2.

Insert Tables 1 and 2 about here

For all of the experimental conditions, control measures did not significantly differ whether animals were tested without preliminary injection or with saline or vehicle pretreatment (see Tables 1 and 2). For each of the separate experiments, different groups of animals were used. In each group and during each phase of the different experiments, the control rates of responding were consistently maintained at low values (range of mean values for all animals: .041 to .047 responses/sec), and the averaged number of reinforcements obtained ranged between 86% to 98% of available reinforcers. It

Table 1

Mean Response Rates per second ± SEM for both No Injection and Saline or Vehicle control sessions under the various Experimental Conditions

Experimental conditions[a]	No injection	Saline or Vehicle pretreatment
Experiment 1		
Acute morphine	.044 ± .001 (68)[b]	.042 ± .001 (64)
Acute naloxone	.044 ± .001 (32)	.043 ± .001 (52)
Acute morphine-naloxone	.045 ± .001 (76)	.044 ± .001 (72)
Experiment 2		
Acute dl-cyclazocine	.045 ± .001 (93)	.044 ± .001 (93)
dl-Cyclazocine-naloxone	.044 ± .001 (52)	.043 ± .001 (21)
Experiment 3		
Acute d-amphetamine	.047 ± .001 (36)	.047 ± .001 (36)
d-Amphetamine-naloxone	.044 ± .001 (102)	.044 ± .001 (54)

[a] Experimental conditions are presented as three separate experiments; each experiment used different animals.

[b] Numbers in parentheses refer to the number of observations on which each mean was based.

Table 2

Averaged Number of Reinforcements per session ± SEM for both No Injection and Saline or Vehicle control sessions under the various Experimental Conditions

Experimental conditions[a]	No injection	Saline or Vehicle pretreatment
Experiment 1		
Acute morphine	96 ± 1 (68)[b]	94 ± 2 (64)
Acute naloxone	93 ± 2 (32)	94 ± 2 (52)
Acute morphine-naloxone	98 ± 1 (76)	97 ± 1 (72)
Experiment 2		
Acute dl-cyclazocine	92 ± 1 (93)	95 ± 1 (93)
dl-Cyclazocine-naloxone	86 ± 3 (52)	87 ± 4 (21)
Experiment 3		
Acute d-amphetamine	89 ± 6 (36)	91 ± 2 (36)
d-Amphetamine-naloxone	90 ± 1 (102)	92 ± 2 (54)

[a] Experimental conditions are presented as three separate experiments; each experiment used different animals.

[b] Numbers in parentheses refer to the number of observations on which each mean was based.

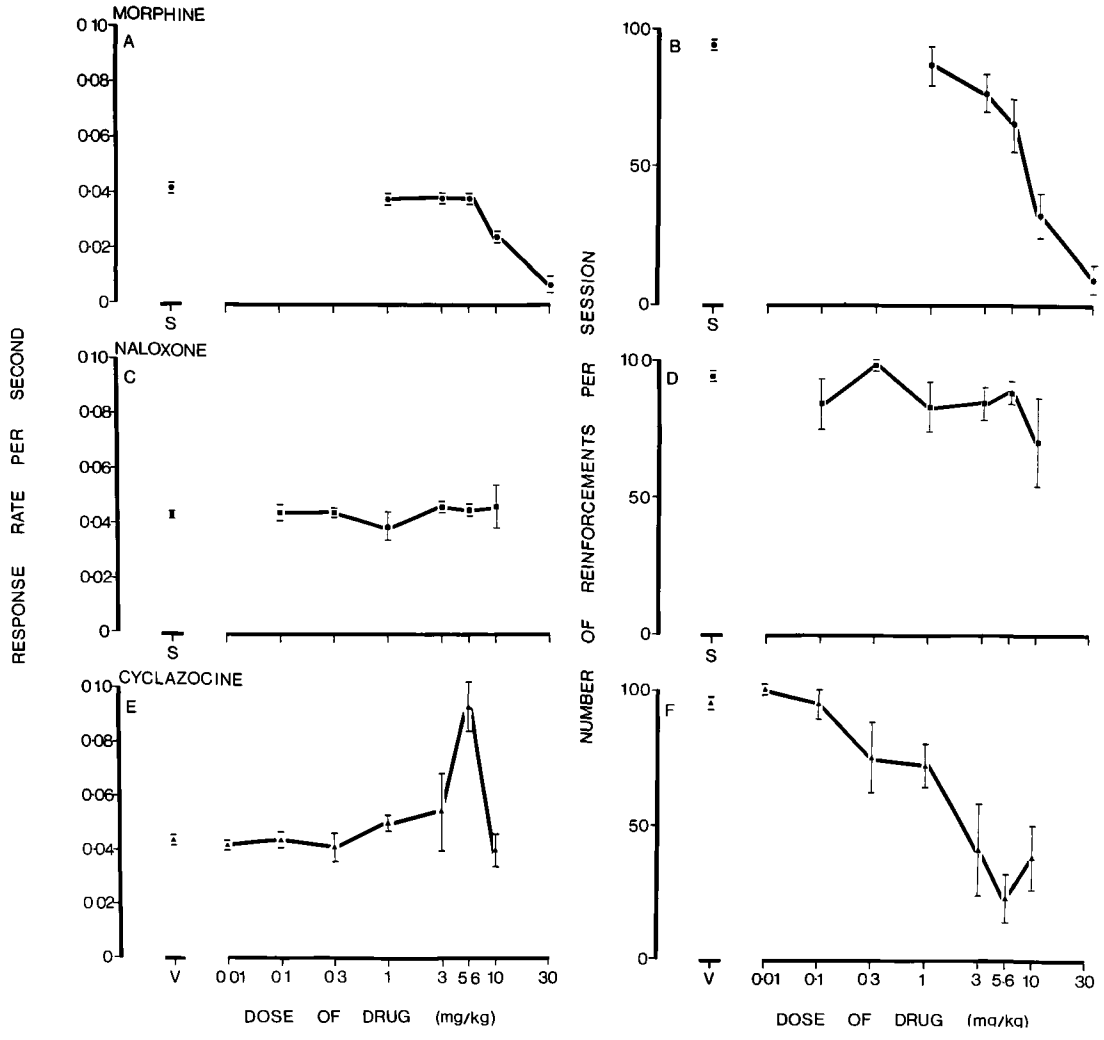
is evident, therefore, that the animals developed and maintained stable, efficient baseline behaviour throughout the study.

Samples of interresponse times (IRTs) distribution from control (saline) sessions are shown in the histograms A of Figures 2, 6 and 8. The animals characteristically display distributions of IRTs that peak at time intervals conducive to reinforcement, i.e., IRTs > 20 sec but < 30 sec. The shaded bars in the distributions show that percentage of food-reinforced responses that occurred between 20 sec and 30 sec after a preceding response.

Seperate effects of morphine, naloxone, dl-cyclazocine and d-amphetamine

Dose-response curves were determined for morphine, naloxone, cyclazocine and amphetamine. The effects of these drugs on rate of responding and number of reinforcements per session are plotted in Figures 1 and 3. In Figure 1, the drug means are based upon twelve observations for morphine (three determinations per dosage for each of four animals); eight observations for naloxone (two administrations of a given dose to each of four rats), and finally nine observations for cyclazocine (three determinations per dosage per animal). Tables 1 and 2 give the number of observations represented by each of the

Figure 1. Graphs showing the separate effects of morphine, naloxone and dl-cyclazocine on response rate per second (left hand graphs: A, C and E) and number of reinforcements per session (right hand graphs: B, D and F) under the DRL 20 sec LH 10 sec schedule of food presentation. Data points denote the mean ±SEM. For the morphine and naloxone dose-effect curves, S denotes saline pretreatment and for the cyclazocine dose-effect curves, V refers to vehicle alone as the respective control procedures.


MORPHINE
A
B
NALOXONE
C
D
CYCLAZOCINE
E
F
RESPONSE RATE PER SECOND
NUMBER OF REINFORCEMENTS PER SESSION
0·10
0·08
0·06
0·04
0·02
0
100
50
S
V
0·01
0·1
0·3
1
3
5·6
10
30
DOSE OF DRUG (mg/kg)

control (saline) means.

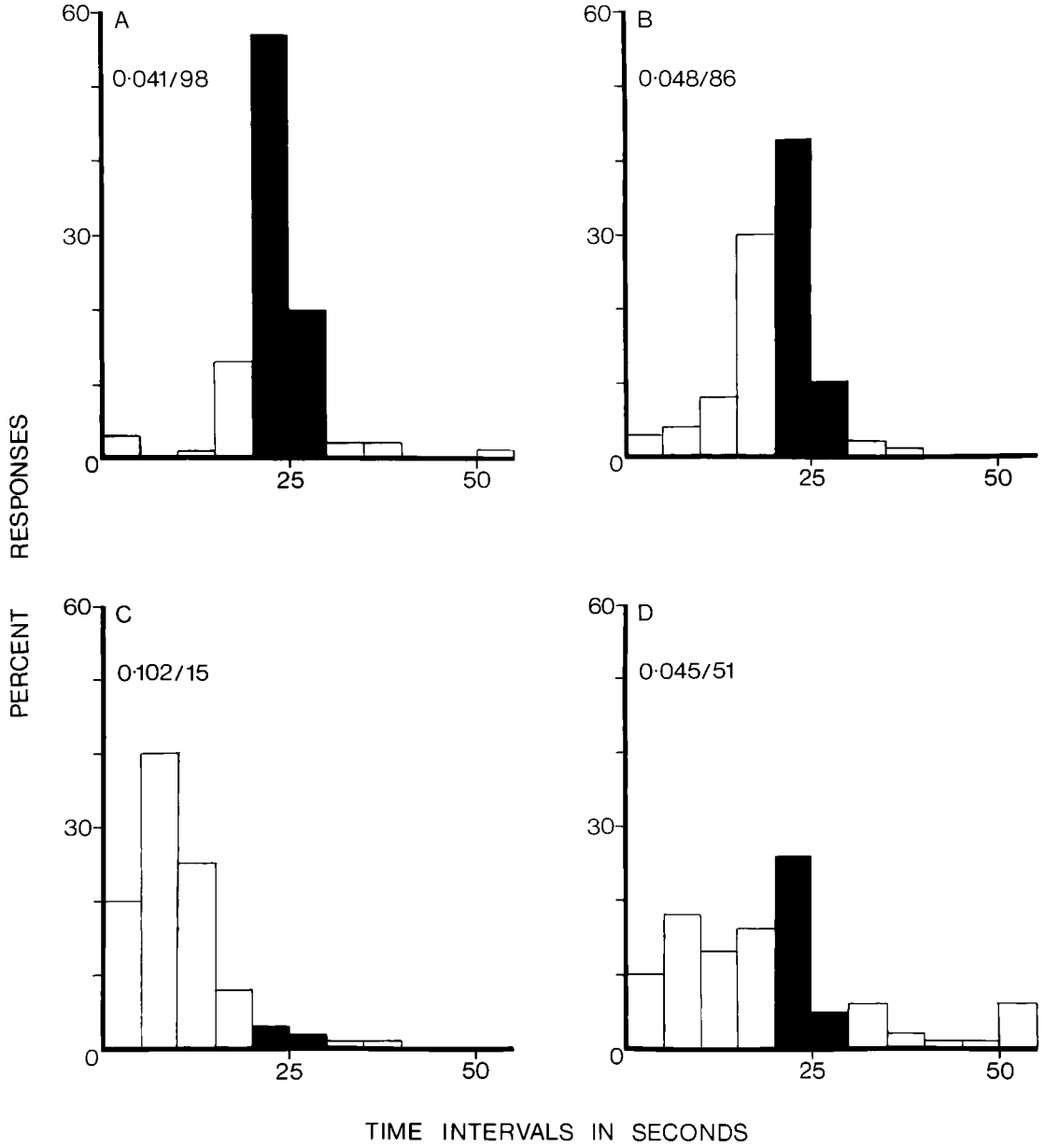
Insert Figure 1 about here

Morphine did not produce any significant effect on responding at doses of 1 - 5.6 mg/kg; at 10 mg/kg, a 57% decrease in responding was observed in comparison with control performance and responding was even more severely depressed by 30 mg/kg of morphine (Figure 1A). Though overall rates of responding were not affected by morphine at small doses (1 - 5.6 mg/kg), a dose-dependent decrease in the number of reinforcements per session is clearly evident in Figure 1B.

Naloxone did not alter responding at doses ranging from 0.1 to 10 mg/kg (Figure 1C); at 10 mg/kg, however, overall response rates were more variable and there was an average decrease of 24% in the number of reinforcements per session (Figure 1D).

Cyclazocine at a dose of 5.6 mg/kg induced a substantial increase (116%) in the overall rate of responding in comparison with control (vehicle) performance; the rate-increasing effect of the drug was partly evident at a dose of 3 mg/kg though the effect was variable (see Figure 1E). When a dose of 10 mg/kg was given, overall response rates were disrupted and significantly

Figure 2. (A-D) shows averaged frequency distributions of IRTs, expressed in percentage form, derived from three single sessions (one per animal). The two numbers shown with each distribution refer respectively, to response rate per second and number of reinforcements per session, averaged for the three sessions on which each distribution is based. A refers to control (vehicle) performance; B-D shows the acute effects of cyclazocine at doses of 1.0, 5.6 and 10 mg/kg, respectively. Columns in black denote reinforced responses; the last column of each distribution refers to any IRT > 50 sec.


A
0·041/98
B
0·048/86
C
0·102/15
D
0·045/51
60
30
0
25
50
PERCENT RESPONSES
TIME INTERVALS IN SECONDS

lower than the rate-increasing effect observed at a dose of 5.6 mg/kg. Cyclazocine produced dose-dependent decreases in the number of reinforcements; the effect is first evident at a dose of 0.3 mg/kg and at doses of 3, 5.6 and 10 mg/kg, the average number of reinforcements per session was reduced by 60% or more in comparison with control data.

Insert Figure 2 about here

Figure 2 consists of averaged frequency distributions of IRTs expressed in percentage form and based upon three sessions (one per animal) in each case. Distribution A refers to vehicle control performance; distribution C-E describes the acute effects of cyclazocine at doses of 1, 5.6 and 10 mg/kg, respectively. The substantial increase in rate of responding following the administration of 5.6 mg/kg of cyclazocine (see Figure 1E) is accompanied by a modification in the slope of the IRT distribution (see Figure 2). In comparison with control performance (A) the relative frequency of short IRTs following cyclazocine treatment (5.6 mg/kg) is greatly increased, to the extent that reinforced IRTs are almost totally suppressed (C). At a smaller dose (1.0 mg/kg), the increase in the incidence of IRTs $\angle$ 20 sec is also evident, but to a lesser

degree (C). Eventhough the mean response rate following an injection dose of 10 mg/kg cyclazocine (.040 responses/sec; see Figure 1E) was comparable to the overall response rate recorded under vehicle pretreatment conditions (.044 responses/sec; see Figure 1E), cyclazocine nevertheless disrupted DRL performance, as evidenced by the increase in the relative frequency of both short and long IRTs and the marked reduction in the percentage of reinforced IRTs (see Figure 2D).

Insert Figure 3 about here

Amphetamine produced a dose-dependent, biphasic effect on responding (see Figure 3B). At doses of 0.3, 1 and 3 mg/kg, rates of responding increased generally as the dose increased; the effect was slight at 0.3 mg/kg (a 29% increase). At doses of 1 and 3 mg/kg overall rate increases of, on average, 178% to 238%, respectively, were observed. As the dose of amphetamine increased, however, rates of responding became extremely variable and during some individual sessions, amphetamine at a dose of 3 mg/kg suppressed responding. At a dose of 5.6 mg/kg, responding was severely suppressed in two animals; the third rat, however, continued to respond at rates (0.17, 0.10 and 0.13 responses/sec) that greatly

Figure 3. Graphs showing the effects of d-amphetamine on the number of reinforcements per session (A) and response rate per second (B). S refers to saline (control) pretreatment. Control means are based upon 36 observations (12 determinations for each of 3 animals); the remaining data points represent 6 observations (2 administrations of a given dose to each of 3 rats) in each case. Vertical lines denote the standard error of the respective means.

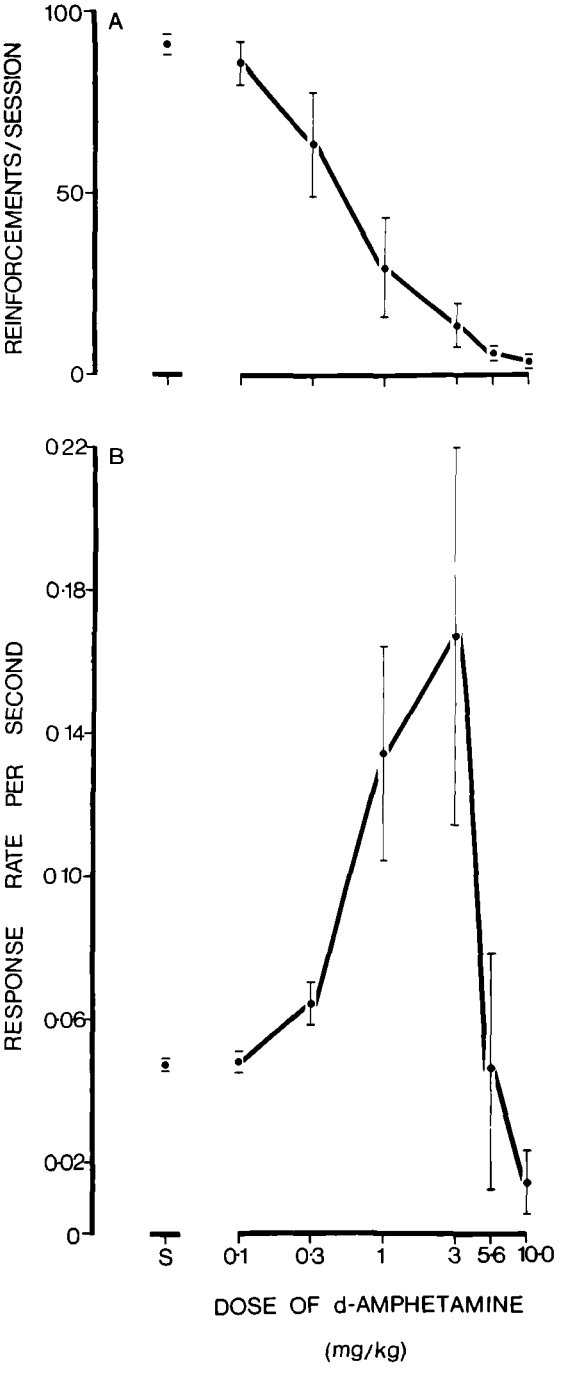
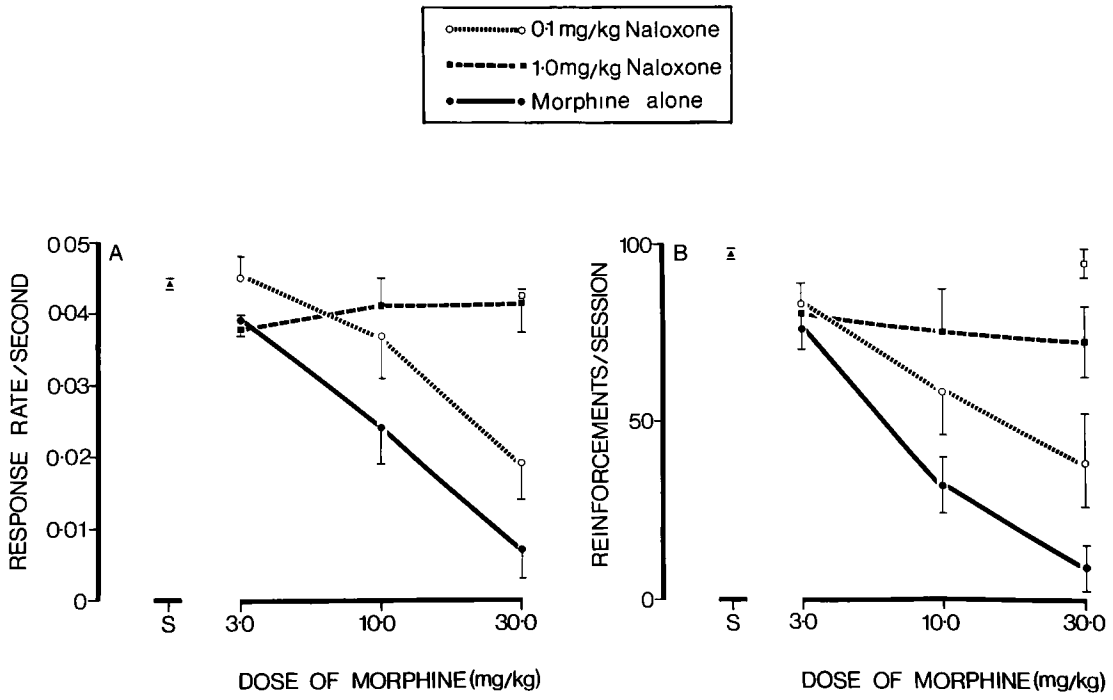

A
100
50
0
REINFORCEMENTS/SESSION
B
0·22
0·18
0·14
0·10
0·06
0·02
0
RESPONSE RATE PER SECOND
S
0·1
0·3
1
3
5·6
10·0
DOSE OF d-AMPHETAMINE
(mg/kg)

Figure 4. Graphs showing the effects on response rate per second (A) and the number of reinforcements per session (B) of naloxone (0.1 and 1 mg/kg) given in combination with morphine at doses of 3, 10 and 30 mg/kg. The effects of morphine alone at these three doses are also plotted to illustrate the extent to which naloxone antagonized the rate-suppressive effects of morphine. Data points denote the mean ± SEM and S refers to saline (control) pretreatment. Note that naloxone at a dose of 1 mg/kg (■ - - - ■), given in combination with morphine (30 mg/kg), restored response rates to control values though the number of reinforcements per session remained below the control level. Naloxone at a dose of 3 mg/kg, tested in conjunction with 30 mg/kg morphine (□), fully reinstated DRL performance to control (saline) baseline.


0·1 mg/kg Naloxone
1·0mg/kg Naloxone
Morphine alone
RESPONSE RATE / SECOND
0·05
0·04
0·03
0·02
0·01
0
A
S
3·0
10·0
30·0
DOSE OF MORPHINE (mg/kg)
REINFORCEMENTS / SESSION
100
50
0
B
S
3·0
10·0
30·0
DOSE OF MORPHINE (mg/kg)

exceeded the control (saline) baseline, i.e., 0.047 responses/sec. Amphetamine at a dose of 10 mg/kg produced an almost complete cessation of responding in two rats and partially suppressed responding in the third animal in comparison with control data.

Response rate increases or decreases were produced by amphetamine at different doses, often in conjunction with appreciable variability; a consistent dose-dependent decrease in the number of reinforcements per session was observed, however, as shown in Figure 3A. The effect was slight at a dose of 0.1 mg/kg and then increased progressively as the dose of amphetamine increased.

Combined effects of morphine and naloxone in non-tolerant and morphine-tolerant rats

The data shown in Figure 4 relates to the effects of combinations of doses of naloxone and morphine administered in non-tolerant animals. Each drug combination point is based upon 8 observations (2 determinations in each of 4 animals). Control means were based on 72 observations (18 per animal).

Insert Figure 4 about here

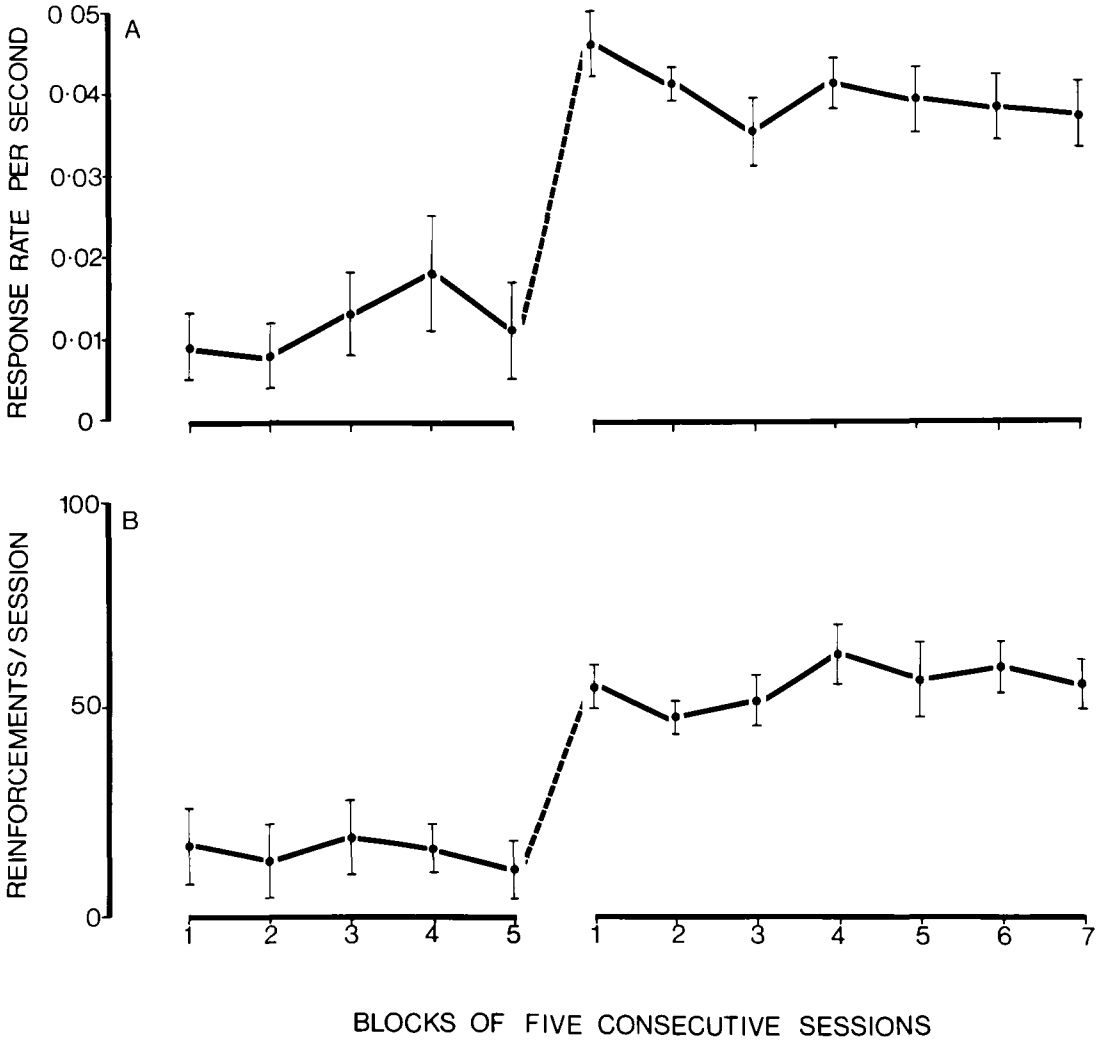
Figure 4A shows that naloxone at a dose of 0.1 mg/kg only partially antagonized the effects of morphine at a dose of 10 mg/kg; when given in combination with a larger dose of morphine (30 mg/kg), naloxone at a dose

of 0.1 mg/kg did not significantly attenuate the severe rate-depressive effect of morphine. At a dose of 1 mg/kg, naloxone fully reversed the rate-decreasing effects of morphine observed at doses of 10 and 30 mg/kg. Even though naloxone (1 mg/kg) antagonized the rate-decreasing effects of large doses of morphine to the extent that overall rates of responding returned to control values, the number of reinforcements per session was reduced, on average, by 24.7%, when morphine (30 mg/kg) and naloxone (1 mg/kg) were given together in comparison with control (saline) performance (see Figure 4B). It is worth noting, however, that when naloxone at a dose of 3 mg/kg was given in conjunction with morphine (30 mg/kg), the suppressive effect of morphine, assessed either in terms of overall response rates or the number of reinforcements per session was abolished and performance returned to the control (saline) baseline.

Insert Figure 5 about here

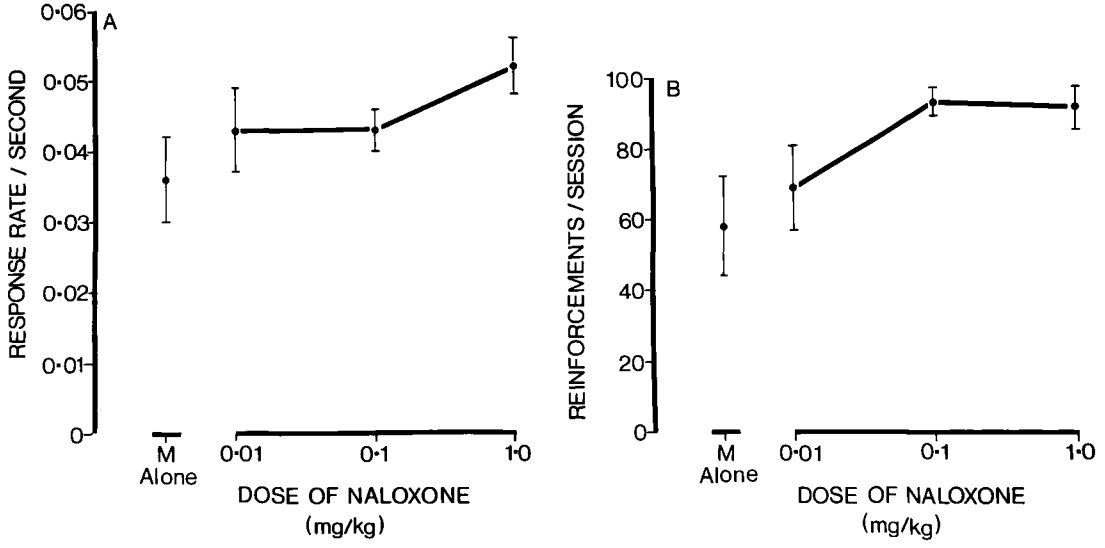
Data summarized in Figure 5 indicates that morphine (30 mg/kg) suppressed responding and decreased the number of reinforcements per session throughout a series of 25 consecutive sessions, each session being preceded by an i.p. injection of morphine at this dose. The daily pre-

Figure 5. Graphs showing the effects of daily morphine pretreatment on DRL performance assessed by response rate per second (A) and the number of reinforcements per session (B). For the first 5 blocks of consecutive sessions (N = 25), each session was preceded by morphine at a dose of 30 mg/kg i.p. For the next 7 blocks of consecutive sessions (N = 35), each session was preceded by morphine at a dose of 10 mg/kg i.p. Data points refer to the mean, based upon 15 observations (5 per animal), ± SEM.


A
0 05
0·04
0·03
0·02
0·01
0
RESPONSE RATE PER SECOND
B
100
50
0
REINFORCEMENTS/SESSION
1
2
3
4
5
1
2
3
4
5
6
7
BLOCKS OF FIVE CONSECUTIVE SESSIONS

session dose of morphine was then reduced from 30 to 10 mg/kg for the next 35 consecutive sessions and it is clearly apparent that reducing the dose led to an increase in responding from an overall rate of 0.01 responses/sec during the fifth block of sessions at 30 mg/kg of morphine to an overall rate of 0.046 responses/sec during the first block of sessions at a dose of 10 mg/kg of morphine. In the original morphine dose-effect curve (see Figure 1A), responding was reduced to 0.024 responses/sec following morphine pretreatment at a dose of 10 mg/kg. It is evident, therefore, that tolerance to the rate-decreasing effect of morphine developed during chronic daily administration of the drug since 10 mg/kg of morphine (0.046 responses/sec) no longer suppressed responding in comparison with prior saline pretreatment data (0.042 responses/sec; see Figure 1A). Even though response rates following chronic daily administration of morphine (10 mg/kg) were comparable to overall response rates recorded previously under control (saline pretreatment) conditions, the number of reinforcements per session was lower (an overall reduction of 40.5%) following repeated daily injections of morphine in comparison with prior control data (see Figures 5B and 1B). Although responding was no longer suppressed by morphine at a dose of 10 mg/kg, following daily exposure to the drug, therefore, the number

Figure 6. Graphs showing the effects of morphine alone (M) and doses of naloxone given in conjunction with morphine pretreatment at a dose of 10 mg/kg i.p. on response rate per second (A) and the number of reinforcements per session (B) after chronic daily morphine administration. Data points refer to the mean ± SEM. Each drug combination mean is based upon 6 observations (2 determinations in each of 3 animals). Data points for morphine alone are based on 96 observations in each instance.


A
RESPONSE RATE / SECOND
0·06
0·05
0·04
0·03
0·02
0·01
0
M
Alone
0·01
0·1
1·0
DOSE OF NALOXONE
(mg/kg)
B
REINFORCEMENTS / SESSION
100
80
60
40
20
0
M
Alone
0·01
0·1
1·0
DOSE OF NALOXONE
(mg/kg)

of reinforcements per session did not return to pre-drug (control) values.

Insert Figure 6 about here

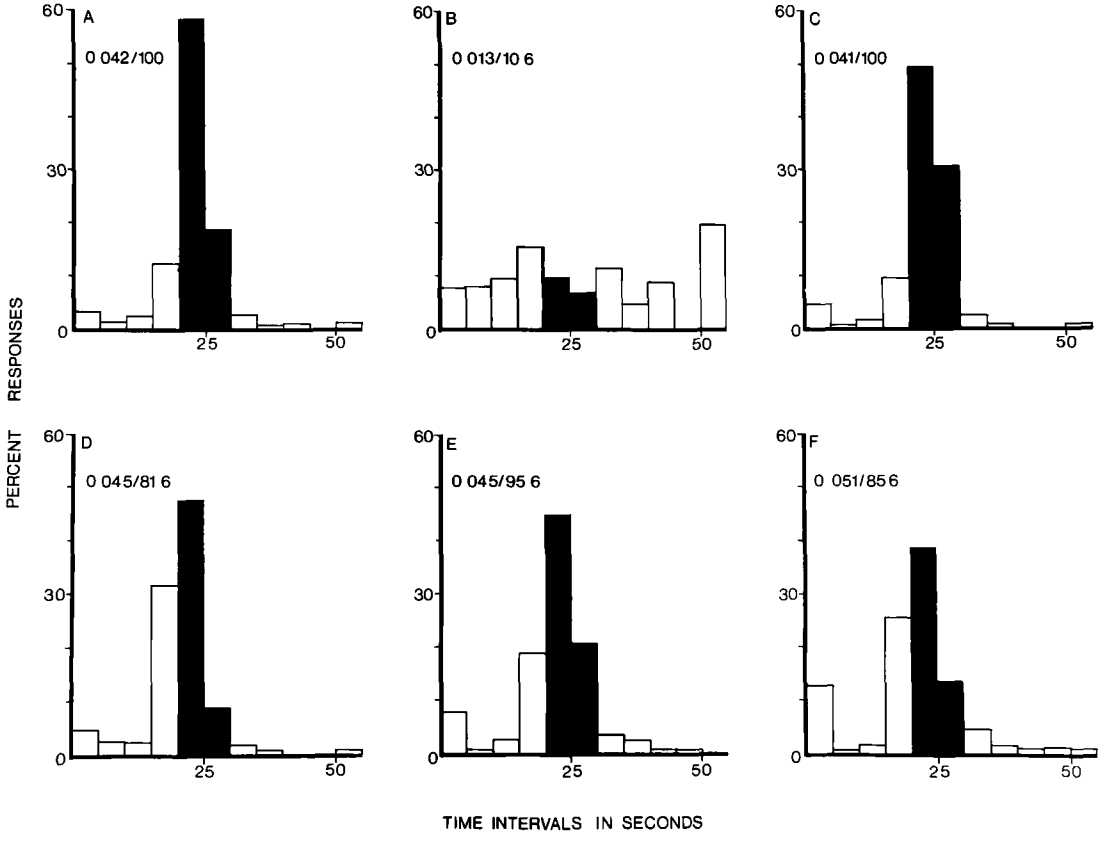
Figure 6A shows that naloxone at doses of 0.01 and 0.1 mg/kg, when given in conjunction with morphine (10 mg/kg), did not significantly affect overall rates of responding in morphine-tolerant rats. When naloxone was injected at a dose of 1 mg/kg in addition to morphine (10 mg/kg), an increase in responding of, on average, 52.9%, was observed. Figure 6B shows, however, that naloxone at doses of 0.1 and 1 mg/kg restored the number of reinforcements per session to pre-drug (control) values in these morphine-tolerant animals. It is evident that although overall rates of responding returned to pre-drug (control) values for animals receiving morphine (10 mg/kg) on a daily basis, the number of reinforcements per session remained depressed. In morphine-tolerant animals, naloxone (0.1 mg/kg), in conjunction with morphine, did not exert any detectable effect upon overall rates of responding though, at this dose, naloxone restored to control (pre-drug) values the number of reinforcements per session. At a dose of 1 mg/kg, naloxone exerted a marked rate-increasing effect in morphine-

pretreated, tolerant animals without diminishing the overall number of reinforcements per session by more than 3.3% in comparison with control (pre-drug) data.

Insert Figure 7 about here

Figure 7 consists of averaged frequency distributions of IRTs expressed in percentage form and based upon three sessions (one per animal) in each instance. Distribution A refers to pre-drug (saline) performance; the acute effects of morphine at a dose of 10 mg/kg are plotted in B from which it is evident that morphine tended to increase the relative frequency of both short and long IRTs while producing an overall suppression of responding (see Figure 1A). Naloxone (1 mg/kg) antagonized the acute effects of morphine (10 mg/kg) as shown in C. Distribution D represents the 30th session of chronic morphine pretreatment (10 mg/kg), demonstrating the development of tolerance to the rate-decreasing effects of the drug when compared to B. There is, however, a greater incidence of IRTs $>$ 15 sec but $<$ 20 sec in comparison with pre-drug (control) performance (A); this effect was attenuated by naloxone at a dose of 0.1 mg/kg (E), accompanied by an increase in the relative frequency of reinforced IRTs. Naloxone (1 mg/kg) increased responding in conjunction

Figure 7. (A-F) shows averaged frequency distributions of IRTs, expressed in percentage form, derived from three single sessions (one per animal). The two numbers shown with each distribution refer respectively to response rate per second and the number of reinforcements per session, averaged for the three sessions on which each distribution is based. A refers to control (saline) performance; B shows the acute effects of morphine at a dose of 10 mg/kg. C shows the effects of 10 mg/kg morphine given in conjunction with naloxone (1 mg/kg) Distribution D shows the results obtained on the 30th session of chronic morphine pretreatment at a dose of 10 mg/kg and should be compared to distribution B which shows the acute effects of morphine at this dose. E and F show the effects of naloxone at doses of 0.1 mg/kg (E) and 1 mg/kg (F) given in conjunction with morphine (10 mg/kg) in morphine-tolerant animals. Columns in black denote reinforced responses; the last column of each distribution refers to any IRT $\geq$ 50 sec.


A
0 042/100
B
0 013/10 6
C
0 041/100
D
0 045/81 6
E
0 045/95 6
F
0 051/85 6
60
30
0
25
50
PERCENT RESPONSES
TIME INTERVALS IN SECONDS

with morphine (10 mg/kg) in morphine-tolerant animals (see Figure 6A); this effect is seen indirectly in distribution F when IRTs < 20 sec are compared in terms of their relative frequency with the incidence of IRTs < 20 sec in distribution E.

Combined effects of dl-cyclazocine and naloxone

Insert Figures 8 and 9 about here

Figures 8 and 9 plot the effects on performance of pretreating rats with naloxone (1 mg/kg) in addition to several individual doses of dl-cyclazocine. To assure proper experimental control, naloxone (1 mg/kg) was also tested in comtination with vehicle, on two separate occasions per animal. The overall response rate and averaged reinforcement frequency were .045 responses/sec and 91 reinforcements/session, respectively. Since these values did not significantly differ from the data obtained under vehicle pretreatment conditions (.043 responses/sec and 87 reinforcements/session; see Figures 8 and 9), only the latter means were plotted.

The significant rate-increasing effect of cyclazocine alone (5.6 mg/kg) was unaffected by naloxone pretreatment. Nor did naloxone (1 mg/kg) alter responding observed after cyclazocine pretreatment at doses of 0.1 and 1 mg/kg. In

Figure 8. Effects of dl-cyclazocine (CYC) alone at doses of 0.1, 1.0, 5.6 and 10.0 mg/kg and dl-cyclazocine given in combination with 1.0 mg/kg of naloxone on response rate per second. V denotes vehicle (control) pretreatment. In the dl-cyclazocine-naloxone combination tests, each drug combination point refers to the mean of two observations for each of three animals (N = 6); vertical lines denote SEM.

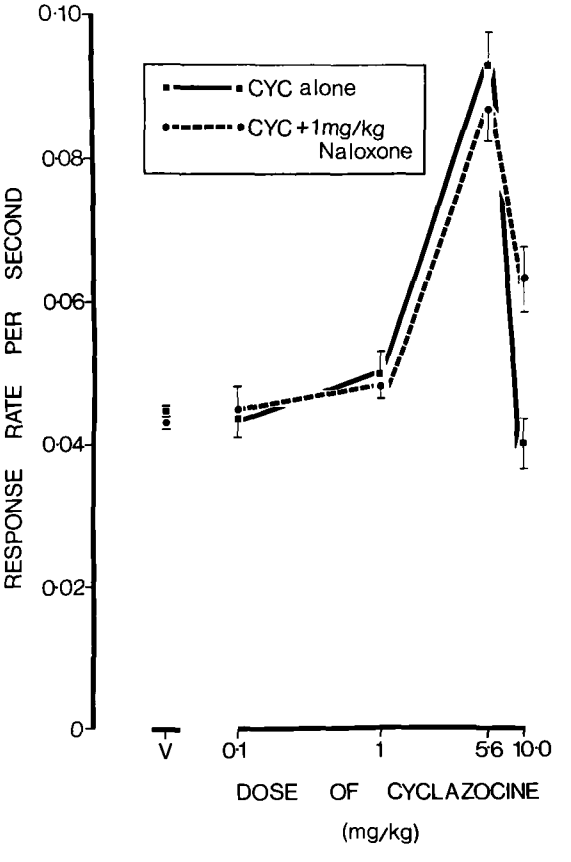
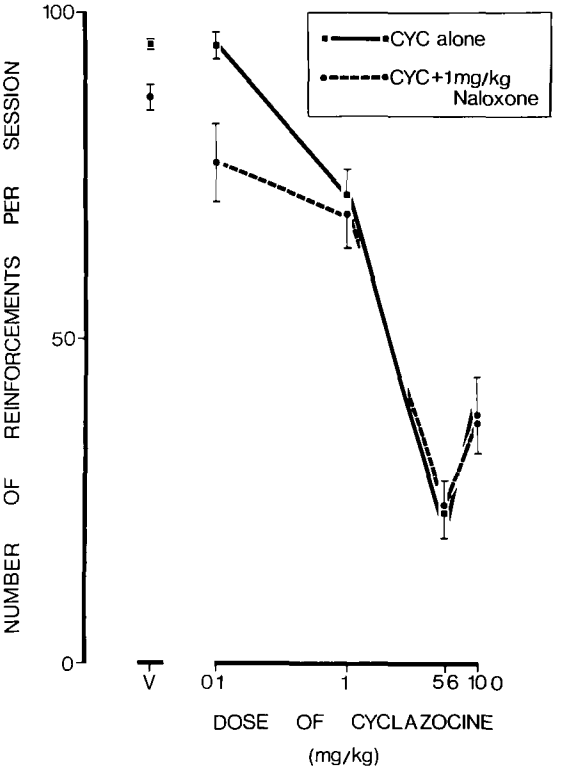

0·10
0·08
0·06
0·04
0·02
0
RESPONSE RATE PER SECOND
CYC alone
CYC +1mg/kg
Naloxone
V
0·1
1
5·6
10·0
DOSE OF CYCLAZOCINE
(mg/kg)

Figure 9. Graph showing the effects of dl-cyclazocine (CYC) alone at doses of 0.1, 1.0, 5.6, and 10.0 mg/kg and dl-cyclazocine given in combination with 1.0 mg/kg of naloxone on the number of reinforcements per session. V denotes vehicle (control) pretreatment. In the dl-cyclazocine-naloxone combination tests, each drug combination point refers to the mean of two observations for each of three animals ($N = 6$); vertical lines denote SEM.


100
50
0
NUMBER OF REINFORCEMENTS PER SESSION
CYC alone
CYC+1mg/kg Naloxone
V
01
1
56
100
DOSE OF CYCLAZOCINE
(mg/kg)

comparison with the effects of cyclazocine alone at a dose of 10 mg/kg, naloxone pretreatment (1 mg/kg) increased responding though the effect was associated with considerable variability. Since cyclazocine alone (10 mg/kg) disrupted responding during several previous sessions (see Figure 1C), naloxone antagonized the rate-decreasing effect of cyclazocine but did not alter the rate-increasing effect observed at a smaller dose (5.6 mg/kg).

The dose-dependent decreases in the reinforcement frequencies following acute administration of cyclazocine at doses of 1.0, 5.6 and 10.0 mg/kg, was unaffected by naloxone pretreatment (see Figure 9). Naloxone (1 mg/kg) in conjunction with a smaller dose of cyclazocine (0.1 mg/kg) which did not exert any detectable effect upon DRL performance when given alone, slightly reduced, by 18%, the overall number of reinforcements per session.

Combined effects of d-amphetamine and naloxone

Naloxone at doses of 1.0, 3.0 and 10.0 mg/kg was tested in combination with either saline or d-amphetamine at dose levels of 0.1, 1.0, and 3.0 mg/kg. Table 3 presents the mean response rates and averaged number of reinforcements per session obtained under the saline-naloxone control conditions as well as the saline pretreatment control procedure.

Table 3

Mean Response Rates per second and Averaged Number of Reinforcements per session ± SEM under various control conditions

Control conditions	Response Rate	Reinforcement frequency
Saline + 1.0 mg/kg naloxone	.047 ± .002	97 ± 4
Saline + 3.0 mg/kg naloxone	.044 ± .002	95 ± 4
Saline + 10 mg/kg naloxone	.045 ± .004	96 ± 3
Saline + Saline	.044 ± .001	92 ± 2

Note. Each mean is based upon 6 observations (2 per animal) with the exception of Saline + Saline control condition which represents 54 observations.

Insert Table 3 about here

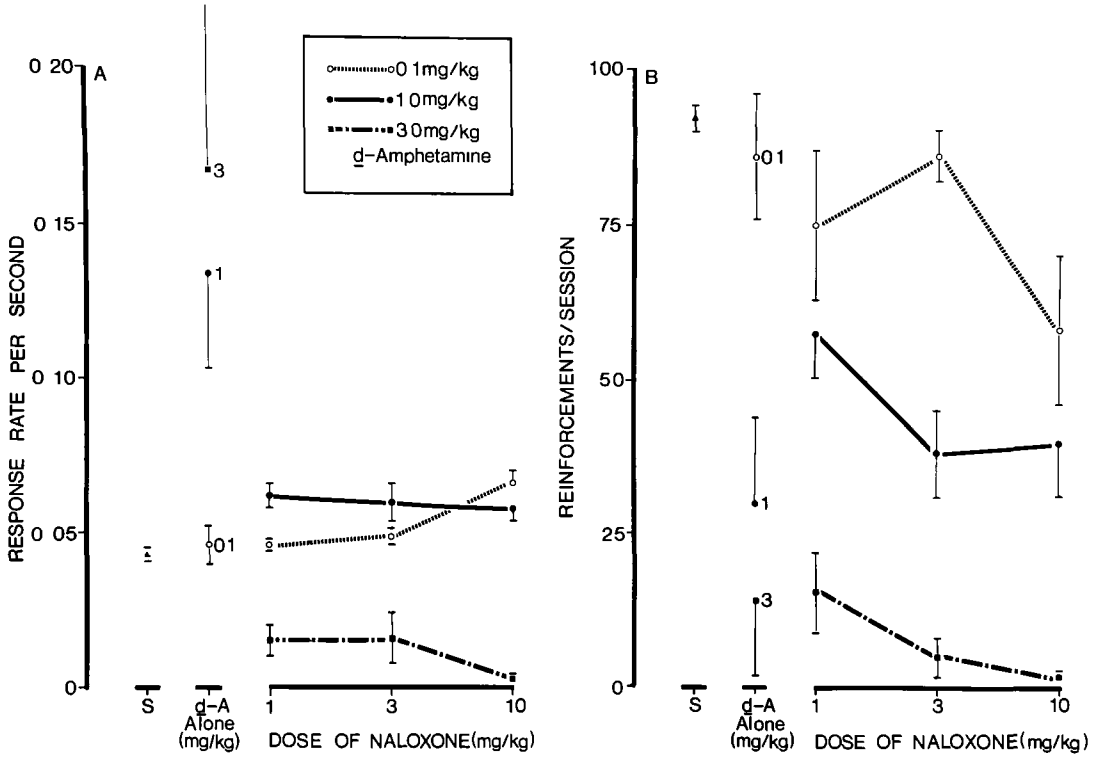
DRL behaviour, whether assessed in terms of response rate per second or number of reinforcements per session, was unaffected by the concomitant administration of saline and the various doses of naloxone (1.0, 3.0, and 10.0 mg/kg); no significant difference appeared between performance recorded under either saline (dual injection) pretreatment or naloxone-saline control conditions (see Table 3).

Insert Figure 10 about here

Figure 10 summarizes the effects on DRL performance of combined doses of d-amphetamine and naloxone. At doses of 1 and 3 mg/kg, naloxone did not alter the negligible effects of d-amphetamine alone when given at a dose of 0.1 mg/kg. Naloxone at a dose of 10 mg/kg increased responding by 37.8% (Figure 10A) when combined with a small dose of d-amphetamine (0.1 mg/kg), an effect which was accompanied by a reduction (33.5%) in the number of reinforcements per session (Figure 10B).

It was found previously (see Figure 3B) that, when given alone, d-amphetamine at a dose of 1 mg/kg exerted a substantial rate-increasing effect. Naloxone at doses of 1, 3 and 10 mg/kg uniformly antagonized the rate-increasing

Figure 10. Effects of d-amphetamine alone at doses of 0.1, 1 and 3 mg/kg and d-amphetamine given in combination with naloxone at doses of 1, 3 or 10 mg/kg on response rate per second (A) and on the number of reinforcements per session (B). S denotes saline (control) pretreatment. In the d-amphetamine-naloxone combination tests, each data point refers to the mean of three observations for each of two animals (N=6); vertical lines denote SEM.


A
B
0 1mg/kg
1 0mg/kg
3 0mg/kg
d-Amphetamine
RESPONSE RATE PER SECOND
REINFORCEMENTS/SESSION
0 20
0 15
0 10
0 05
0
100
75
50
25
0
S
d-A Alone (mg/kg)
1
3
10
DOSE OF NALOXONE(mg/kg)

effect of amphetamine. The number of reinforcements per session increased by 91.6% when amphetamine (1 mg/kg) was given in conjunction with the smallest dose of naloxone (1 mg/kg) but this aspect of performance was unaffected at larger doses of naloxone (i.e., 3 and 10 mg/kg).

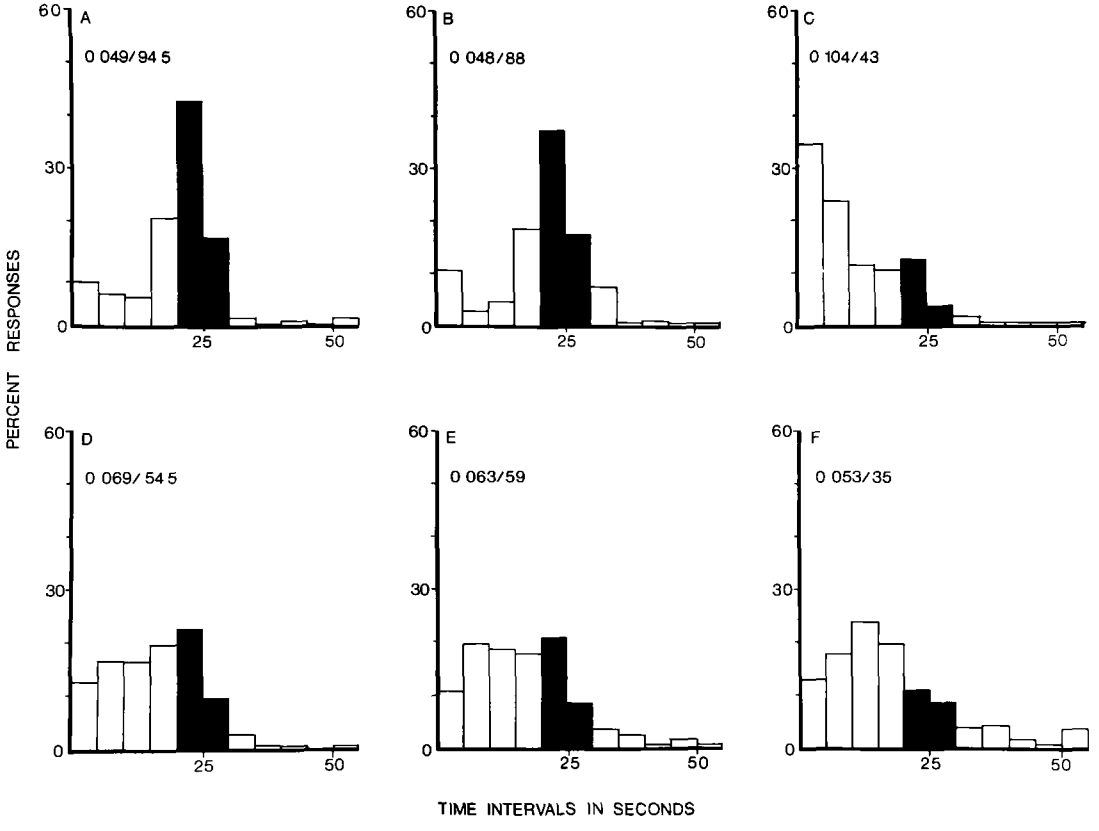
The effects of d-amphetamine at a dose of 3 mg/kg (see Figure 3B) were previously found to be extremely variable since, on average, the drug increased responding by 238% though, on some occasions, responding was suppressed. Naloxone at each of three doses antagonized the rate-increasing effects of amphetamine (3 mg/kg), causing a severe suppression of responding (see Figure 10A).

These data show that naloxone antagonizes the rate-increasing effects of d-amphetamine on DRL performance; however, naloxone (10 mg/kg) increased responding that otherwise was unaffected by a small dose of d-amphetamine (0.1 mg/kg) given alone.

Insert Figure 11 about here

Averaged frequency distributions of IRTs, expressed in percentage form and based on 4 sessions (two per animal) are shown in Figure 11. Distribution A refers to pre-drug (saline) performance and, by comparison, it

Figure 11. (A-F) shows averaged frequency distributions of IRTs, expressed in percentage form, derived from four single sessions (two per animal). The two numbers shown with each distribution refer to response rate per second and number of reinforcements per session, averaged for the four sessions on which each distribution is based. A refers to control (saline) pretreatment; distributions B and C show the effects of d-amphetamine at doses of 0.1 and 1 mg/kg, respectively. Distribution D shows the effects of d-amphetamine (0.1 mg/kg) given in combination with naloxone at a dose of 10 mg/kg. Distributions E and F show the effects of d-amphetamine (1 mg/kg) given in combination with naloxone at doses of 1 mg/kg (E) and 10 mg/kg (F). Columns in black denote reinforced responses; the last column of each distribution refers to any IRT >50 sec.


A
0 049/94 5
B
0 048/88
C
0 104/43
D
0 069/54 5
E
0 063/59
F
0 053/35
60
30
0
25
50
PERCENT RESPONSES
TIME INTERVALS IN SECONDS

is evident that d-amphetamine exerted little effect at a dose of 0.1 mg/kg (B). At a dose of 1 mg/kg (C), amphetamine caused a substantial increase in the relative frequency of IRTs < 5 sec, consistent with the rate-increasing effect of the drug, and a significant reduction in the relative frequency of reinforced IRTs. Distribution D refers to the combined effects of amphetamine (0.1 mg/kg) and naloxone (10 mg/kg). This combination of the two drugs caused an increase in overall responding relative to the effects of amphetamine alone (see Figure 10A) and, in comparison with distribution B, an increase in the relative frequencies of IRTs < 20 sec is evident. In distributions E and F, naloxone at doses of 1 and 10 mg/kg attenuated the substantial increase in the relative frequency of IRTs < 5 sec caused by amphetamine (1 mg/kg) alone (C); although naloxone antagonized the rate-increasing effect of amphetamine, each IRT distribution shows a greater relative incidence of IRTs < 20 sec in comparison with control (saline, A) performance and an associated reduction in the relative frequencies of reinforced IRTs.

CHAPTER IV

Discussion and Conclusions

The various drug-behaviour manipulations reported in the present dissertation were aimed at extending some of Holtzman's experiments on the effects of morphine and morphine-antagonist drugs upon locomotor activity and electric-shock avoidance behaviour in rats to a situation in which responding was maintained by food-presentation. Consequently, the present results will be discussed chiefly in terms of Holtzman's previous work.

Operant behaviour maintained by a schedule of inter-response time reinforcement of food presentation in the rat was differentially affected by morphine, naloxone, dl-cyclazocine and d-amphetamine. The effects obtained with the acute administration of morphine in the present experiments were similar to the effects this drug has been reported recently to exert on low rates of responding maintained by a comparable DRL schedule of food reinforcement in the rat (Ford and Balster, 1976). The present results differ, however, with the previous observations of Ford and Balster in that morphine did not have a biphasic effect upon DRL behaviour. Rate-increasing effects of morphine at doses of 1 - 5.6 mg/kg were not observed in the present experiment. However, in their

study, as in the present work, morphine at large doses (10 - 30 mg/kg) decreased responding and produced an increment in the relative frequency of short and long IRTs. Also in keeping with Ford and Balster's (1976) findings, a dose-dependent decrease in the number of reinforcements per session was observed, becoming apparent at doses of 3.0 and 5.6 mg/kg. Although mean response rates were unaffected by these smaller doses (see Figure 1), it is evident that morphine nevertheless, disrupted DRL performance when assessed in terms of reinforcement frequency.

In another experiment (Holtzman and Jewett, 1972b), the effects of morphine were evaluated upon electric-shock avoidance behaviour in rats. It was found that morphine (0.5 - 8 mg/kg) exerted graded increases in the rate of avoidance responding over a 16-fold dose range exceeding 170% of the control rate at some doses. Present observations relating to the lack of behavioural stimulation following acute morphine treatment taken in conjunction with Ford and Balster's (1976) finding that slight, but statistically insignificant rate increments occurred at low doses of morphine suggest that DRL performance in comparison to shock avoidance behaviour is relatively insensitive to any stimulant component of morphine's action. Further experiments are needed to

determine whether these differences in the behavioural effects of morphine depend primarily upon the ongoing rates of responding or whether the nature of the event maintaining behaviour (e.g., food versus electric-shock presentation) can also exert a specific effect.

In marked contrast to morphine, naloxone, when administered alone, had no systematic effect on DRL performance whether assessed in terms of rate of responding or the number of reinforcements per session. Holtzman (1974b, 1976a) similarly found little effect of naloxone on shock avoidance behaviour in the rat and the squirrel monkey.

Unlike naloxone, the mixed agonist-antagonist analgesic, dl-cyclazocine substantially modified DRL performance. Appropriate doses of cyclazocine (3 and 5.6 mg/kg) were found to increase responding; an effect being most prominent at the larger dose. At a dose of 10 mg/kg, the mean response rates fell below control values. Cyclazocine-induced disruptions in the pattern of DRL responding were also noted: low doses of the drug (1 - 5.6 mg/kg) produced increases in the frequency of short IRTs whereas a larger dose (10 mg/kg) resulted in an increment in the incidence of both short ($<$ 20 sec) and long ($>$ 30 sec) IRTs. This disruption in the pattern of responding, taken in conjunction with the effects of

cyclazocine on overall response rates, resulted in a dose-dependent decrease in the number of reinforcements per session which was observed over a broad range of doses.

These results are generally consistent with previous work. McMillan and Harris (1972), for instance, compared the effects of the optical isomers of cyclazocine, and pentazocine on responding in pigeons maintained by a multiple FI FR schedule of food reinforcement. At small doses, both narcotic antagonists and their respective isomers increased the response rate under the FI component and at larger doses, decreased responding under both components of the multiple schedule.

In other experiments (Holtzman, 1974b, 1976a; Holtzman and Jewett, 1973), the behavioural effects of dl-cyclazocine have been evaluated on electric-shock avoidance behaviour in rats and squirrel monkeys. In both cases, cyclazocine increased avoidance responding in a graded manner over broad ranges of doses; responding was disrupted at larger doses, falling at or below control levels. Unlike other prior experiments, cyclazocine was recently reported to produce only dose-related decreases in responding maintained by electrical self-stimulation of the lateral hypothalamus in rats (Holtzman, 1976b). Whether these differences in the effects of cyclazocine result from divergence in the ongoing rates of responding or the

nature of the maintaining event needs further investigation.

In comparing the effects of cyclazocine in several experiments, it is evident that the behavioural actions of the drug are qualitatively similar in several animal species, whether responding is maintained by a DRL schedule of food reinforcement, a multiple FI FR schedule of food presentation, or by a schedule of electric-shock avoidance. Quantitative rather than qualitative differences in the effects of cyclazocine are apparent when the present results and Holtzman's observations using electric-shock avoidance responding in rats are compared. In his studies (Holtzman, 1974b; Holtzman and Jewett, 1973), dl-cyclazocine produced graded increases in the rate of avoidance responding over a 16-fold dose range that exceeded at some doses 260% of control during the first hour of the four-hour session, whereas the present rate increases in DRL responding reached a maximum of 210% of control and were observed over a narrower range of doses, i.e., about a 6-fold dose range. Since behavioural stimulation following cyclazocine pretreatment is graded over a broader range of doses under a schedule of electric-shock avoidance, it is possible that continuous avoidance behaviour in comparison with food-reinforced responding may be a more

useful operant baseline in evaluating the stimulant effects of narcotic-antagonist analgesic drugs on behaviour.

In keeping with Dews (1958a) rate-dependency hypothesis, d-amphetamine, a psychomotor stimulant drug was found to increase at relatively small doses (0.3 - 3 mg/kg) the low response rates obtained under a DRL schedule, and to shift, as a function of increasing dosage, the relative distributions of IRTs toward the shorter intervals (e.g., Figure 11). Marked suppression of lever-press responding occurred at larger doses (5.6 - 10 mg/kg). d-Amphetamine also produced dose-dependent decreases in the number of reinforcements per session. These effects are consistent with other published reports on the effects of amphetamines on DRL performance (Kelleher et al., 1961; Segal, 1962; Sidman, 1955, 1956). A similar biphasic effect of d-amphetamine on the mean rate of avoidance responding was noted by Holtzman and Jewett (1973) and Holtzman (1974c).

In the drug-interaction experiments, naloxone, which when given alone did not affect DRL responding, antagonized the acute rate-decreasing effects of morphine (10 and 30 mg/kg). Though naloxone at a dose of 1 mg/kg fully reversed the marked rate-decreasing effect of morphine at 30 mg/kg, the number of reinforcements remained below control values. When the dose of naloxone, acting as an antagonist was

increased to 3 mg/kg, the number of reinforcements per session as well as overall rates of responding returned to control baseline. Furthermore, morphine-induced alterations in the patterning of DRL performance, observed under the higher doses of morphine were also reversed in the presence of naloxone (e.g., Figure 7). Naloxone has consistently been found to be a strong antagonist of acute morphine effects upon operant behaviours maintained by either electric-shock or food delivery in rats (Holtzman, 1976b), squirrel monkeys (Byrd, 1976; Goldberg et al., 1976; Holtzman, 1976a) and pigeons (McMillan, 1974; McMillan et al., 1970). Naloxone effects in morphine pretreated, tolerant animals were also investigated in the present work. These results will be discussed after the behavioural effects of naloxone in combination with either acute dl-cyclazocine or acute d-amphetamine pretreatments have been considered.

Naloxone (1 mg/kg) generally failed to antagonize the rate-increasing effects of cyclazocine. The rate-disruptive effects produced by the largest dose of cyclazocine tested (10 mg/kg) were however, slightly attenuated in the presence of naloxone. This is in contrast to Holtzman and Jewett (1973) and Holtzman's (1974b) reports that all of cyclazocine actions on continuous avoidance behaviour of rats were antagonized by naloxone. In their studies,

selected doses of naloxone (8 and 16 mg/kg) were found to reduce both the stimulant and disruptive effects of cyclazocine on avoidance behaviour. On the other hand, identical doses of naloxone failed to alter the effects of cyclazocine on locomotor activity and brain catecholamines levels in the rat. Similar findings were also reported when other mixed agonist-antagonist drugs, such as pentazocine and levallorphan were tested in the presence of naloxone (Holtzman, 1974b). From these observations, Holtzman (1974b) concluded that the differential antagonism of the effects of the mixed-acting narcotic antagonists by naloxone suggest that the agonist component of action of some mixed narcotic antagonists is mediated by several mechanisms, at least one of which is not blocked by naloxone.

Comparison of the present results with Holtzman's (1974b) findings on naloxone antagonism of cyclazocine effects upon avoidance behaviour in rodents is difficult, since only a single dose of naloxone (i.e., 1 mg/kg) was used in the present experiment. Although such a dose level of naloxone readily antagonizes cyclazocine actions on operant behaviour of squirrel monkeys (Holtzman, 1976a), it may not be sufficiently potent to produce similar effects in the rat. Further experiments using larger doses of naloxone in combination with graded doses of cyclazocine

need to be conducted before more conclusive statements can be formulated concerning the antagonism of cyclazocine effects upon DRL performance in rats by naloxone.

Naloxone, in addition to being an antagonist of morphine-induced behavioural depression and of cyclazocine-induced disruption in DRL responding, also modified the behavioural effects of d-amphetamine upon DRL performance. At doses of 1 - 10 mg/kg, naloxone uniformly reduced the substantial rate-increasing effects of d-amphetamine seen at doses of 1 and 3 mg/kg (see Figure 10). d-Amphetamine-induced changes in the patterning of DRL behaviour, i.e., the substantial increase in the relative frequency of IRTs < 5 sec were also attenuated in the presence of naloxone (e.g., Figure 11). According to recent reports (Holtzman, 1974c, 1976b; Holtzman and Jewett, 1973), naloxone also significantly modified the effects of d-amphetamine on continuous avoidance behaviour, on locomotor activity and on responding maintained by intra-cranial self-stimulation of the lateral hypothalamus in rats. The present experiments confirm and extend these observations to a situation in which responding was maintained by food-presentation.

Present results differ, however, in one respect from these previously published observations. When a behaviourally inactive dose of d-amphetamine (0.1 mg/kg) was tested

in combination with 10 mg/kg of naloxone, increases in DRL responding were observed, accompanied by a corresponding reduction in the number of reinforcements per session (see Figure 10). Other researchers have not reported overall response-rate increases following the concomitant administration of naloxone and d-amphetamine. It must also be noted that in the former studies, naloxone was not tested in combination with doses of amphetamine that were behaviourally inactive when administered alone. It is suggested that the nature of the interaction between naloxone and d-amphetamine may differ at the lower end of the psychomotor stimulant dose-effect curve. However, further d-amphetamine-naloxone interaction studies extending the range of doses of both drugs are necessary to confirm and possibly clarify the nature of their interaction.

To recapitulate, present observations, taken in conjunction with those of Holtzman (1974c; 1976b), and Holtzman and Jewett (1973), show that a narcotic antagonist, naloxone can alter the behavioural effects of a non-opioid psychoactive drug in rats. In an attempt to clarify the nature of d-amphetamine-naloxone interaction, Holtzman (1974) suggests that naloxone may exert a non-specific rate-decreasing effect which becomes apparent only when activity has been elevated above

baseline rates. Naloxone is ineffective, however, in reducing the stimulation of locomotor activity produced by narcotic antagonists such as pentazocine and cyclazocine (Holtzman and Jewett, 1973). It follows that the precise mechanism of action underlying the interaction between d-amphetamine and naloxone has yet to be defined.

Tolerance to the effects of morphine on schedule-controlled behaviour has previously been described in pigeons (Heifetz and McMillan, 1971) and in rats (Rhodus et al., 1974). In both experiments, morphine was given by systemic injection only once per day preceding each experimental session (either 10 or 15 min beforehand). Heifetz and McMillan (1971) found evidence for the development of tolerance to the rate-decreasing effects of morphine and also methadone in separate experiments. Using a multiple FR FI schedule of food presentation, it was found that tolerance to the depressant effects of morphine (5.6 mg/kg) or methadone (5.6 mg/kg) was less complete under the fixed-ratio (FR) component of the schedule than under the fixed-interval (FI) component. Rhodus et al., (1974) studied the effects of repeated administration of equivalent doses of morphine (7.5 mg/kg) and heroin hydrochloride (3.0 mg/kg) on responding maintained under a multiple FI 60 sec FI 60 sec schedule of either food pellet or saccharin solution

presentation. After an initial phase in which responding was markedly lowered and even suppressed in some animals, substantial increases in responding were evident but unlike Heifetz and McMillan's (1971) study, pre-drug levels of responding were not reached for most animals. Withdrawal of the drugs resulted in an immediate return to control (pre-drug) baseline rates.

In the present experiments, a single dose of morphine was given 5 min preceding daily test sessions. Throughout a series of 25 consecutive sessions, morphine at a dose of 30 mg/kg consistently depressed responding, an effect which was accompanied by a substantial decrease in the number of reinforcements per session. Evidence for the development of tolerance to the rate-decreasing effects of morphine was obtained when the dose was reduced from 30 to 10 mg/kg for a further sequence of 35 consecutive sessions; responding recovered from an overall rate of 0.01 responses/second during the last five sessions preceded by morphine at a dose of 30 mg/kg to 0.046 responses/second during the first five sessions in which the dose had been reduced to 10 mg/kg. Inasmuch as morphine at a dose of 10 mg/kg reduced the overall rate of responding to 0.024 responses/second when the acute effects of the drug were determined previously (see Figure 1A), it is evident that tolerance to the rate-decreas-

ing effects of morphine at this dose developed during chronic daily administration of the drug, though the effect was apparently masked by the larger dose of morphine (30 mg/kg) during the initial series of 25 pretreatments.

Even though tolerance to the rate-decreasing effects of morphine (10 mg/kg) became apparent, the number of reinforcements per session was lower (an overall reduction of 40.5%, on average) following repeated daily injections of morphine in comparison with prior control performance. Present results support previous studies (Heifetz and McMillan, 1971; Rhodus et al., 1974) showing that tolerance to some of the behavioural effects of morphine can occur after repeated daily administration of a single dose of morphine.

Tolerance to the effects of morphine on schedule-controlled behaviour has been demonstrated in other experiments using rats (Babbini et al., 1976; Ford and Balster, 1976; Gellert and Sparber, 1977) and also rhesus monkeys (Holtzman and Villarreal, 1973). Each of these experiments involved either two or three daily injections of morphine or, in a recent experiment (Gellert and Sparber, 1977), subcutaneous implantation of a pellet containing 75 mg/kg of morphine base. Comparisons between the results obtained in these experiments and the

present work are made difficult owing to differences in behavioural testing procedures as well as differences in drug dosages and treatment regimens.

A procedure involving a rapidly-increasing dose regimen of twice-daily morphine administration was adopted by Ford and Balster (1976); these workers noted that tolerance development was not permitted to occur prior to further increases in dose and suggested that their procedure masked most of the evidence for tolerance. Ford and Balster (1976) did not observe any pronounced suppression of responding maintained by a DRL schedule of food presentation in rats as the chronic regimen of morphine treatments progressed. A 35% decrease in the number of reinforcements per hour during session 21 was observed, however, six hours after a dose of 300 mg/kg of morphine sulphate had been given (600 mg/kg over the preceding 24 h), suggesting that tolerance had developed under this dose regimen. It is noteworthy that a comparable average reduction (40.5%) in the number of reinforcements per session was observed consistently over a series of 35 consecutive sessions in the present experiments, each session being preceded by an injection of morphine at a dose of 10 mg/kg given 5 min beforehand. Whereas overall response rates did not differ from the control (pre-drug) baseline,

DRL performance in morphine-tolerant rats remained disrupted, as evidenced by the observed reduction in the number of reinforcements per session.

In comparison with Ford and Balster's (1976) recent work, as well as previous tolerance development studies, the results of the present morphine tolerance experiment exemplify the need for systemic analysis of response rates characteristically engendered by various schedules of reinforcement in conjunction with different morphine dose regimens and time-course relationships. It is evident from presently-available information that, in experiments designed to assess the development of tolerance to the behavioural effects of morphine, the schedule employed to maintain responding itself exerts significant effects (Heifetz and McMillan, 1971). For example, Babbini et al., (1976) observed an initial decrease followed by highly-significant increases in responding maintained by a fixed-interval (FI 2 min) schedule of food presentation in rats during a period of 44 days in which, after two days, the animals continued to receive morphine hydrochloride which was injected twice daily (20 mg/kg per injection). Responding maintained under the DRL schedule of food-presentation in the present experiments did not increase above control rates during a protracted series of daily morphine pretreat-

ments. These differences between experiments may be attributed to different morphine dose regimens but also to the quite different rates and patterns of responding engendered by fixed-interval, fixed-ratio and DRL schedules of food-presentation.

It was suggested at the end of Chapter I, that inasmuch as the ongoing behaviour of an organism is a primary determinant of drug action (Kelleher and Morse, 1968), naloxone antagonism of morphine effects may differ whether animals are in a state of non-tolerance or in a state of morphine tolerance. As previously discussed, acute morphine administration (10 and 30 mg/kg) produced severe depression of DRL performance, whether assessed in terms of rate of responding or the number of reinforcements per session. However, during the chronic morphine treatment, response rates are no longer decreased by the administration of 10 mg/kg of morphine; the number of reinforcements per session remained slightly lower than control (pre-drug) levels. Despite the differences in ongoing behaviours of non-tolerant and morphine-tolerant animals, it was found that the actions of naloxone in combination with either acute or chronic morphine pretreatment were substantially the same. Appropriate doses of naloxone partially or fully antagonized whatever effects morphine was exerting upon DRL performance. Thus, in non-tolerant

animals, naloxone (0.1 mg/kg) antagonized in a dose-dependent manner morphine's (10 mg/kg) suppressive effect upon DRL responding and reinforcement frequency. In morphine-pretreated tolerant rats, naloxone (.01 and .1 mg/kg) did not induce significant response-rate changes, though at doses of 0.1 and 1 mg/kg, it restored the number of reinforcements per session to control (pre-drug) levels.

In contrast to the acute morphine-naloxone experiment, some increases in the overall response rate were observed when a dose of 1 mg/kg was given in conjunction with morphine (10 mg/kg) in morphine-tolerant animals. Similar increases in fixed-interval responding were observed in pigeons when morphine was withdrawn following chronic daily treatment at a dose of 5.6 mg/kg (Heifetz and McMillan, 1971). DRL response rate increases and decreases were also reported by Ford and Balster (1976) following withdrawal of morphine in morphine-dependent rodents. Moreover, Holtzman and Villareal (1973) noted an increase in low rates of punished responding during morphine withdrawal in rhesus monkeys, who were receiving a daily maintainance dose of 8.0 mg/kg of morphine. They suggested that low response rates may, in general, be increased by morphine withdrawal. In the present experiment, naloxone-precipitated increases in the low rates of DRL responding in morphine-pretreated, tolerant

animals confirm and extend this finding. Present results also support the widespread view that behavioural baselines are extremely sensitive in detecting morphine tolerance-dependence development (Holtzman and Villareal, 1973; Gellert and Sparber, 1977).

In conclusion, no major qualitative differences in the individual and combined effects of the psychoactive drugs tested, with the exception of morphine when given alone, were apparent, whether responding was maintained by food presentation, as presently studied, or by electric-shock delivery as previously reported by Holtzman (1974a, 1974b, 1976a). It is thus suggested that the behavioural properties of naloxone and dl-cyclazocine, as well as d-amphetamine are largely independent of the type of reinforcing stimulus and the schedule contingencies used in maintaining the operant behaviour in rats. While the apparent differential effects of morphine in Holtzman's work and the present study may represent a dependence of this drug's effect on the nature of the maintaining event, as previously proposed by McKearney (1974), it is most likely that the different ongoing rates and patterns of responding engendered by the different schedules used contribute to the discrepancy observed in the effects of morphine. However, as already stated, further experiments are needed to determine whether these differences in the

behavioural effects of morphine depend largely upon the ongoing rates of responding or whether the nature of the maintaining event can also exert a specific effect.

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