Published in final edited form as:

Behav Pharmacol. 2011 September ; 22(5-6): 468–479. doi:10.1097/FBP.0b013e328349ab6c.

Drug Discrimination in Pigeons Trained to Discriminate among Morphine, U50,488, a Combination of These Drugs, and Saline

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Abstract

This study compared fixed-ratio (FR) and fixed-interval (FI) schedules to investigate the discriminative stimulus properties of mu- and/or kappa-opioid receptor agonists. Pigeons were trained to discriminate among morphine (mu agonist), U50,488 (kappa agonist), the combination, and saline under an FR 20 or FI-300 s schedule. After training, correct-key responding averaged 94.4% (FR) and 66.5% (FI) after administration of training drugs. Dose-response curves were generally quantal under the FR and graded under the FI schedules, but highly variable among subjects under the FI. Under the FR schedule, the dose of naltrexone that blocked morphine's discriminative stimuli also blocked U50,488. Combining high doses of morphine with low doses of U50,488 produced responding on the morphine key and combining high doses of U50,488 with low doses of morphine produced responding on the U50,488 key. Combining high doses of both drugs produced responding on the drug-combination key. Increasing *d,l*-pentazocine doses shifted responding from the saline key to the U50,488 key, then to the morphine key, and finally to the drug-combination key. Butorphanol and ethylketocyclazocine produced similar effects, except responding on the morphine key increased before responding on the U50,488 key. The four-choice procedure under the FR schedule has potential for determining the discriminative stimulus effects of mixed agonists.

Keywords

four-key drug discrimination; drug-combination training; morphine; U50,488; drug-combination tests; naltrexone; pentobarbital; *d,l*-pentazocine; butorphanol; ethylketocyclazocine; fixed-ratio; fixed-interval; pigeon

Introduction

Colpaert (1987) indicated that fixed-ratio schedules (FR) are attractive for maintaining responding in drug discrimination studies because FR schedules produce “a rapid and accurate method which is sensitive to the discriminative effects of low doses and allows fairly wide ranges of training dose.” He further added, “The availability of this procedure, however, should not act to discourage further methodological research” (pp.346–347). In an attempt to develop new methods for the study of drug discrimination, we have been studying

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the role of the reinforcement schedule in drug discrimination experiments. These experiments have shown that training animals to discriminate drugs under FR schedules favored the development of quantal dose-response curves, while training to discriminate drugs under fixed-interval (FI) schedules favored the development of graded dose-response curves (Massey, et al., 1992; McMillan, et al., 2001a; McMillan, et al., 2001b). This observation led to experiments to determine which schedule might be most useful to study the discriminative stimulus effects of drugs whose effects depend on multiple mechanisms. It was possible that the use of techniques that produced graded dose-response curves might be more useful in teasing out the underlying mechanisms of such drugs than would the conventional FR procedures. Opioid drugs were chosen for study because of the availability of pure agonists, pure antagonists, and partial agonists whose discriminative stimulus effects have been studied frequently using FR schedules.

Many opioid agonists produce strong discriminative stimuli in animals and humans. The mechanism by which these opioid agonists produce their discriminative stimulus effects has been shown to be their binding to and activation of specific opioid receptor subtypes (Gutstein and Akil, 2001; Holtzman, 1985), which have been labeled as mu (μ) and kappa (κ) receptors. Some opioid agonists have been shown to have a much higher affinity for μ receptors than for κ receptors, while other opioid agonists have a much higher affinity for κ receptors than for μ receptors. There are also a considerable number of opioid agonists; particularly those with mixed agonist-antagonist activity, whose agonist effects appear to be produced by binding to both μ and κ receptors (Gutstein and Akil, 2001). It has proven difficult to determine the relative degree to which the discriminative stimulus effects of these mixed agonists are dependent upon their binding to these two receptor subtypes.

Pigeons have been used widely in drug discrimination studies including experiments of the discriminative stimulus effects of opioid drugs. When trained to discriminate drugs with relatively high affinity and selectivity for μ receptors, such as morphine or fentanyl, these drugs substituted for each other. Other μ agonists, such as methadone and *l*-alpha-acetylmethadol, also substituted for the training drugs. Mixed-action opioids, such as butorphanol, buprenorphine, *l*-pentazocine and ethylketocyclazocine, substituted for μ agonists while drugs with high selectivity for κ receptors, such as U50,488 and U69,593, did not (Picker and Cook, 1997; Picker and Dykstra, 1987; Picker, et al., 1993). When U50,488 and bremazocine, drugs with potent κ agonist activity, were used as training drugs in pigeons they substituted for each other. The selective κ agonist U69,593 also substituted, but μ agonists did not (Makhay, et al., 1998; Picker and Cook, 1997; Picker and Dykstra, 1987). Testing with mixed-action opioid agonists produced more ambiguous results in pigeons trained to discriminate selective κ agonists. In two-choice discrimination studies, pigeons trained to discriminate the selective κ agonist U50,488 as the training drug responded on both the drug- and saline-appropriate keys after testing with ketocyclazocine or ethylketocyclazocine (Picker and Dykstra, 1987). Similarly, in a three-choice discrimination in pigeons trained to discriminate among U50,488, morphine and vehicle, ethylketocyclazocine produced vehicle responding at low doses, mixed U50,488- and morphine-appropriate responding at moderate doses, but substituted for morphine at high doses (Makhay, et al., 1998). In pigeons with bremazocine as the training drug versus saline in a two-choice discrimination, ethylketocyclazocine and ketocyclazocine produced only partial substitution for the training drug (Picker, 1994). In contrast, in a three-choice discrimination task among bremazocine, fentanyl and water, ethylketocyclazocine and ketocyclazocine only produced fentanyl-appropriate responding (Picker and Cook, 1997).

A few attempts have been made to study the discriminative effects of mixed agonists and partial agonists as training drugs in pigeons. In one study, pigeons were trained in separate two-choice experiments to discriminate either a low, a moderate, or a high dose of

butorphanol, a mixed κ agonist and partial μ agonist (Gutstein and Akil, 2001) from saline. At all three training doses, the butorphanol stimulus generalized to selective μ agonists including morphine, fentanyl and methadone, as well as to mixed agonists including *l*-pentazocine, buprenorphine, and nalbuphine. The selective κ agonists U50,488, U69,593 and bremazocine substituted partially for butorphanol only in the low-dose trained group and produced predominately saline-appropriate responding in the medium- and high-dose butorphanol trained groups (Picker, et al., 1996). Although these data suggest that following training of pigeons to discriminate selective μ agonists, there is generalization to other selective μ agonists but not to selective κ agonists, and after training with selective κ agonists there is generalization to other selective κ agonists, but not to selective μ agonists, it becomes more complicated when mixed agonists are considered. Fentanyl (a μ agonist) usually generalizes to mixed agonists in two-choice drug discrimination experiments in pigeons, but bremazocine (a κ agonist) generalizes to some but not all mixed agonists. Pigeons trained to discriminate butorphanol from saline often show similar responses to bremazocine. In a three-choice discrimination study using U50,488, morphine, and water (κ , μ and neither, respectively), responding occurred on both the morphine- and U50,488-appropriate keys after lower doses of ethylketocyclazocine, but higher doses of ethylketocyclazocine resulted in most of the responding occurring on the morphine key (Makhay, et al., 1998).

One of the problems with these studies is that most of the work has been done using two-choice procedures where either a μ or a κ agonist is discriminated from the drug vehicle. Even in three-choice experiments where the training conditions include a μ agonist, κ agonist and the drug vehicle, training under a mixed-agonist condition usually has not been made available. Recently, we have developed a four-choice drug-discrimination procedure that requires pigeons to respond among two different drug-appropriate keys, a drug combination-appropriate key, and a saline-appropriate key (Li, et al., 2005). Application of this procedure to the discrimination of drugs with mixed κ and μ effects might be accomplished by establishing discrimination among a μ agonist, a κ agonist, a combination of these μ and κ agonists, and the drug vehicle. Experiments from our laboratory have shown that drug discrimination under FR schedules of reinforcement usually results in dose-response curves that are quantal in nature, that is, responding shifts abruptly from the saline-appropriate key to a training drug-appropriate key within a single increment of dose. In contrast, drug discrimination under FI schedules usually generates graded dose-response curves where responding gradually shifts from the saline- to the drug-appropriate key across several dosage increments (Massey, et al., 1992; McMillan, et al., 2001a,b). Therefore, some of the present four-choice drug- discrimination experiments were conducted under both schedules of reinforcement to determine whether schedules that favor the development of graded dose-response curves might be better suited to the study of these different drug states than are schedules that favor the development of quantal dose-response curves.

Methods

Procedures used during these experiments were conducted in accordance to protocols approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences.

Subjects

For experiments using the FR schedule, six male White Carneau pigeons (Palmetto Pigeon Plant, Sumter, SC), designated numbers 445, 446, 454, 455, 457, 458, served as experimental subjects. For experiments using the FI schedule, five male White Carneau pigeons (Palmetto Pigeon Plant, Sumter, SC), designated numbers 480, 481, 482, 483, 484, served as experimental subjects. Subjects were experimentally naïve at the beginning of the

experiment. They were individually housed with free access to grit and water in a temperature- and humidity-controlled room that was maintained under a 12-hour normal phase lighting cycle. The subjects were maintained at 80–85% (FR: 412–508g; FI: 416–472g) of their free-feeding weights during experiment by post-session supplemental feeding (Purina Pigeon Chow Checkers 5405, Purina Mills, LLC, St. Louis, MO).

Apparatus

The experimental chambers were MED Associates ENV-009A Modular Test Cages (MED Associates, Inc., St. Albans, VT), measuring 32 cm wide by 30 cm long by 33 cm high, enclosed in Gerbrands Model G7211 sound- and light-attenuating enclosures. Four pigeon response keys (MED Associates, Model ENV-124AM) were mounted on the front panel, spaced 6 cm apart in a row 21 cm above the grid floor. The keys could be transilluminated with different colored lights and when operative, the left key was red, the left-center key was white, the right-center key was green, and the right key was blue. The assignment of drug- and saline-appropriate keys for the training conditions was made randomly. Pigeon chow was presented by a grain dispenser (MED Associates, ENV-205M) through a 5.5 cm by 6.5 cm opening centered under one of the middle two response keys, 2 cm above the floor, when schedule contingencies were met. A 28-V DC light mounted near the ceiling illuminated the test chamber except during food presentations when only the food opening was illuminated. A PC computer programmed with MED-PC software (MED Associates) recorded data and controlled experimental contingencies through an interface (MED Associates).

Drug discrimination under an FR 20 schedule

Training—The methods for training pigeons have been described in detail previously (e.g., Li, et al., 2005). The pigeons were trained to discriminate among 5.0 mg/kg morphine, 5.0 mg/kg U50,488, a combination of 5.0 mg/kg morphine and 5.0 mg/kg U50,488, and saline. All of the training drugs and saline were administered intramuscularly. During initial training, pigeons were trained to key peck on a single illuminated response key for food presentation (4-s access to pigeon chow) under a fixed ratio 1 schedule (FR 1). After responding was established the response requirement was incremented across sessions to the terminal schedule, FR 20. Then the second key was introduced and responding for food presentation on this key was established, and the schedule advanced to FR 20. After responding on both keys under an FR 20 was established, responding during subsequent sessions was differentially reinforced according to whether morphine or saline was administered, 10 min prior to the sessions. Under the two-choice condition, 5.0 mg/kg morphine or saline was administered before the session on a double alternation pattern, but later on a single alternation pattern across sessions. After the discrimination between morphine and saline was acquired, a third response key was illuminated and made active. Responses on the third key were reinforced only when 5.0 mg/kg U50,488 was administered before the session. Initially, responding on the third key was reinforced under an FR 1. Once responding was established, the response requirement was gradually incremented to an FR 20. After this three-choice discrimination was established, a fourth response key was illuminated and made active as before. Responses on that key were reinforced only if both morphine and U50,488 had been administered before the session. Training sessions ended after 40 min or after 20 reinforcements had been presented, whichever occurred first. Training the subjects to make this four-choice discrimination under an FR 20 schedule required about 15 months before responding was stable, accurate and showed no further improvement.

Testing—Test sessions began when performance showed no further signs of improvement for forty sessions (two months). Drug testing was conducted once a week on Friday, with training sessions continuing through Monday to Thursday. The procedure for test sessions

was similar to training sessions, except that they were made up of several test trials to facilitate cumulative dose-response testing. Each test trial began with the administration of a test dose of drug, followed 10 min later by a response period during which the response keys were illuminated and active and responding on any key was reinforced under an FR 20 schedule of food presentation. The test trial ended after the first food delivery, or 15 min had passed, whichever occurred first; then the next cumulative dose was administered, followed 10 min later by the next response period. Cumulative dosing test trials continued until a dose was reached that markedly reduced responding during the 15-min response period. During test sessions, the cumulative dose-response curves and the rates of responding were determined for the training drugs, various combinations of doses of morphine and U50,488, and other drugs.

Data analysis

The percentage of responses made on each response key was determined. If a pigeon made more than 80% of the responses on one key that key was considered the choice key and the data were plotted as the number of subjects choosing each key using bar graphs. The total number of responses on all four keys was summed and then was divided by the total time of testing and averaged across subjects to give mean overall rates of responding.

Drug discrimination under a FI-300 s schedule

Training—During initial training, pigeons were trained to peck a single illuminated response key under an FR 1 schedule reinforced by 4-s access to pigeon chow. This was repeated in separate training sessions until pigeons responded for food presentation on each of the four keys. In subsequent sessions, the response requirements for food presentation were gradually incremented across sessions to FR 20. The schedule of reinforcement was then changed to a short fixed-interval (FI) and several additional training sessions were conducted with gradually increasing FI requirements until responding under an FI-300 s schedule was established on all four response keys. Under this schedule, the first response that occurred on an illuminated key after 300 s had elapsed resulted in the presentation of food. Once FI responding for food presentation was established for all keys, all four keys were illuminated and drug discrimination training began. During drug discrimination training, pigeons were trained under a FI-300 s reinforcement schedule to discriminate among the same four drug conditions as under the FR 20 schedule (i.e., 5.0 mg/kg morphine, 5.0 mg/kg U50,488, a combination of these two doses, and saline).

Responses on the left-center response key were always reinforced after saline administration, responses on the right response key were always reinforced after the combination of morphine and U50,488 had been administered. Responses on the other two response keys (left and right-center), were reinforced after either morphine or U50,488 administration, counterbalanced across subjects. Training sessions ended after 60 min elapsed. Training continued until the performance of the subjects was judged to be stable. The duration of time it took to train the pigeons averaged 24 months.

Testing—Test sessions began when performance showed no further signs of improvement for forty sessions (two months). Drug testing was conducted once a week on Friday with training sessions continuing through Monday to Thursday. The procedures for testing under the FI schedule was essentially the same as described for cumulative dose-response testing under the FR schedule of reinforcement. During test sessions, the cumulative dose-response curves and the rates of responding were determined for the training drugs, various combinations of doses of morphine and U50,488, and other drugs.

Data analysis

The percentage of responses on each key were calculated and averaged across subjects and plotted as bar graphs. The total number of responses on all four keys was summed and then was divided by the total time of testing and averaged across subjects to give mean overall rates of responding.

Drugs

Pentobarbital sodium and U50,488 (($\pm$)-*trans*-U-50488 methanesulfonate) were purchased from Sigma-Aldrich (St. Louis, MO). Morphine sulfate was purchased from Mallinkrodt, Inc. (St. Louis, MO). Naltrexone HCl was supplied by the National Institute on Drug Abuse (Rockville, MD). Ethylketocyclazocine methanesulfonate and *d,l*-pentazocine HCl were both generously supplied by Sterling-Winthrop Research Institute (Rensselaer NY) and butorphanol tartrate was generously supplied by Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ). All drugs were dissolved in 0.9% physiological saline (University of Arkansas for Medical Sciences Pharmacy, Little Rock, AR) to an injection volume of 1.0 ml/kg. Doses are expressed as mg/kg and refer to the salt. Doses shown in figures represent the total dose administered. For all experiments, drugs were administered as intramuscular injections 10 min before behavioral sessions or trials in a volume of 1.0 ml/kg of body weight. After injection, the pigeons were placed in the darkened operant chamber until the training session or test trial began. Two sides of the breast muscles were used for injection of each drug when drug combinations were administered; alternating sides of the breast muscles were used during cumulative dosing tests.

Result

Table 1 shows the distribution of responses on each response key as a percentage of total responses and rates of responding under training conditions after discriminative stimulus control of responding was established and behavior had stabilized in subjects maintained under both schedules. As shown in the upper half of Table 1, across the four training conditions under the FR schedule, subjects distributed an average of 94.4% of their responses on the condition-appropriate key. The percentage of correct responses on the saline-appropriate key after saline was slightly greater than the percentage of responses on the morphine- and U50,488-appropriate keys after these training drugs alone, which also was slightly higher than the percentage of drug combination-appropriate responses following morphine in combination with U50,488. Following morphine administration, a higher percentage of the incorrect responses were made on the saline key rather than the other two keys. Following administration of saline, U50,488, or the drug combination, incorrect responses were more evenly distributed across the other two or three keys. Overall rates of responding during training under the FR schedule were highest following saline administration, with rates of responding progressively decreasing following U50,488, morphine, and morphine plus U50,488 (drug combination).

Training of subjects under the FI-300 s schedule was prolonged. It required two years of training to reach the levels of stability shown in the lower half of Table 1, which shows the percentage of responses on the condition-appropriate keys and rates of responding during six training sessions after responding had stabilized. Discriminative stimulus control of responding was established, as the pigeons distributed an average of 66.5% of their responses on the condition-appropriate key where responses produced the reinforcer during training sessions. There were considerable differences in performance of between subjects. Three pigeons made 74.9% to 76.5% of their responses on the condition-appropriate key that produced the reinforcer during these training sessions, while pigeons 482 and 483 made 49.9% to 57.5% of their responses on the key that produced the reinforcer. After saline,

morphine and U50,488, incorrect responses were always lowest on the drug-combination key. There were no other systematic patterns in responding on the keys that did not produce the reinforcer under any of the four drug conditions during training sessions under the FI schedule. During saline- and morphine-training sessions, rates of responding were higher than during U50,488-training sessions, which in turn were higher than during the training sessions with the drug combination.

Figure 1 shows dose-response curves for morphine (upper panel) and U50,488 (lower panel) in individual subjects under the FR schedule. For four subjects the lowest dose of morphine discriminated was 3.0 mg/kg, while for two subjects a dose of 5.6 mg/kg was required. For four subjects the lowest dose of U50,488 discriminated was 3.0 mg/kg, while for the other two subjects a dose of 5.6 mg/kg was required. Thus, pigeons discriminated the salts of these two drugs at much the same mg/kg doses. The dose-response curve for all subjects was quantal in shape in that the pigeons shifted from responding on the saline key to maximal responding on the drug key with a single increment in dose.

Figure 2 shows dose-response curves for morphine (upper panel) and U50,488 (lower panel) in individual subjects under the FI-300 s schedule. Following cumulative doses of morphine, three of the five pigeons gradually shifted dose-dependently from responding on the saline-appropriate key to responding on the morphine-appropriate key with drug-appropriate responding between 20 and 70% occurring at intermediate doses. A fourth pigeon shifted more abruptly to responding on the morphine-appropriate key. The remaining pigeon showed a gradual increase in responding on the morphine key, but responding on the morphine key never reached more than 40%. The bottom panel shows responding on the U50,488-appropriate key as the dose of that drug increased. Three of the five pigeons showed the anticipated gradual increase in responding on the U50,488 key as the dose increased, but the other two pigeons generated relatively flat dose-response curves with responding on the U50,488 key remaining less than 50% across all doses. Three subjects continued to be tested in subsequent experiments. However, in view of the poorer level of discriminative stimulus control and less consistent dose-response data in a limited number of subjects, these results will not be presented in detail and only be briefly described. In general, the results in pigeons tested under the FI-300 s schedule were similar to those obtained under the FR 20 schedule described below for the interaction studies of morphine and U50,488, for the antagonism of morphine or U50,488 with naltrexone, and for the individual drug dose-response curves for *d,l*-pentazocine, butorphanol, ethylketocyclazocine and naltrexone.

Figure 3 shows dose-response data under the FR schedule for morphine and U50,488 alone (upper panels) and dose-response data for the effects of increasing doses of naltrexone in the presence of 10.0 mg/kg of morphine or 10.0 mg/kg U50,488 (lower panels). When a dose of 5.6 mg/kg of morphine was administered, all pigeons met the criterion for responding on the morphine key. Similarly, when 5.6 mg/kg U50,488 was administered, all pigeons met the criterion for responding on the U50,488 key. When 10.0 mg/kg morphine or U50,488 was administered without naltrexone, all subjects responded on the morphine- or U50,488-appropriate key, respectively. As the dose of naltrexone increased, after the 10.0 mg/kg doses of morphine or U50,488, an increasing number of subjects responded on the saline key. Naltrexone was at least equally effective in blocking the discriminative stimulus effects of U50,488 as it was in blocking the discriminate stimulus effects of morphine. Only one pigeon failed to respond on the saline key when 10.0 mg/kg naltrexone was combined with 10.0 mg/kg morphine and all pigeons responded on the saline key when 10.0 mg/kg naltrexone was combined with 10.0 mg/kg U50,488.

Figure 4 shows interactions for different combinations of morphine and U50,488 under the FR 20 schedule. When 1.0 mg/kg morphine was combined with 1.0 mg/kg U50,488 (upper left panel), all subjects responding on the saline-appropriate key. As the dose of U50,488 increased in the presence of 1.0 mg/kg morphine, the number of subjects responding on the U50,488-appropriate key increased and all subjects responded on that key after the 5.6 and 10.0 mg/kg doses. When 3.0 mg/kg morphine was combined with 1.0 mg/kg U50,488 (upper right panel), all subjects responded on the morphine key. When the dose of U50,488 in the combination was increased to 3.0 mg/kg, two subjects responded on the morphine key, two on the U50,488 key, and two on the drug-combination key. With further increases in the dose of U50,488 combined with 3.0 mg/kg morphine, all pigeons responded on the drug combination key with the exception of one subject who responded on the U50,488-appropriate key after the highest dose of U50,488 combined with 3.0 mg/kg morphine. When 5.6 mg/kg morphine was combined with U50,488 (lower left panel), responding occurred on the morphine key after the lowest dose of U50,488. When 3.0 mg/kg U50,488 was combined with 5.6 mg/kg morphine, half the subjects responded on the morphine-appropriate key and half on the U50,488-appropriate key. When the two highest doses of U50,488 were combined with 5.6 mg/kg morphine, all subjects responded on the drug-combination key. When 10.0 mg/kg morphine was combined with increasing doses of U50,488, (lower right panel), subjects responded on the morphine-appropriate key following the 1.0 mg/kg dose of U50,488, followed by shifting to the drug-combination key as the dose of U50,488 increased, then all subjects responded on the drug-combination key at the two highest doses of U50,488 in combination with 10.0 mg/kg morphine. In summary, the combination of the lowest doses of morphine and U50,488 produced responding on the saline-appropriate key. Increases in the dose of morphine in the presence of the low dose of U50,488 produced responding on the morphine-appropriate key. Increases in the dose of U50,488 in the presence of low doses of morphine produced responding on the U50,488-appropriate key. Combinations of larger doses of morphine and U50,488 were characterized by responding on the drug-combination key.

Figure 5 shows the discrimination data for other drugs. Low doses of *d,l*-pentazocine (top left panel) produced responding on the saline key, except for one bird that made 46.2% responses on the U50,488 key and 45.2% responses on the saline key at the lowest dose of *dl*-pentazocine. At the 3.0 mg/kg dose of *d,l*-pentazocine, two subjects responded on the U50,488 key while the remaining four subjects continued to respond on the saline key. At the 5.6 mg/kg dose two subjects responded on the morphine key, three on the U50,488 key, and one on the drug combination key. After the 10.0 mg/kg dose of *d,l*-pentazocine, five of six subjects responded on the drug-combination key. The pattern after the administration of *d,l*-pentazocine can be summarized that with increasing doses of *d,l*-pentazocine, some pigeons begin to the respond on the U50,488 key, with a higher dose causing some to respond on the morphine key, and at the highest dose most subjects respond on the drug-combination key.

With ethylketocyclazocine (top right panel), subjects respond on the saline key at low doses. As the dose increased, responding by some subjects shifted first to the morphine key and at a higher dose to the U50,488-appropriate key. After the 1.0-mg/kg dose of ethylketocyclazocine (the highest dose tested), three subjects responded on the morphine-appropriate key and three on the drug-combination key. With butorphanol, low doses produced responding on the saline key. As the dose increased, subjects first shifted responding to the morphine key, while at the highest dose (1.0 mg/kg), one subject responded on the morphine key, one on the U50,488 key and four responded on the drug-combination key. Increasing doses of naltrexone and pentobarbital resulted in responding only on the saline-appropriate key for all subjects.

During training, overall rates of responding under the FR schedule were highest following saline administration, and less (in descending order) after administration of U50,488, morphine, and the drug combination (upper half of Table 1). Figure 6 shows rates of FR responding averaged across all four response keys following administration of other doses of the training drugs, test drugs, and drug combinations. Morphine and U50,488 produced dose-dependent decreases in rates of responding (upper panel). In general, combinations of morphine with U50,488 produced rate decreases greater than the decreases produced either drug given alone, although there were a few dose reversals (lower panel). *d,l*-pentazocine, butorphanol, ethylketocyclazocine and naltrexone also produced dose-dependent rate decreases (upper panel).

Discussion

These experiments showed that pigeons could be trained under an FR schedule to discriminate among four alternatives, including morphine (a selective μ agonist), U50,488 (a selective κ agonist), a combination of morphine and U50,488, and saline. It required an average of 15 months to train the pigeons tested under the FR schedule to the level of performance shown in Table 1. The FI schedule was more problematic. Under the FI schedule, even after two years of training discriminative stimulus control was considerably poorer than that under the FR schedule.

Under the FR schedule, responses were generally distributed in a quantal pattern across the four keys during training sessions and during dose-response determinations as shown by the dose-response curves for morphine and U50,488 (Figure 1). In contrast, graded dose-response curves were observed for the effects of morphine and U50,488 for the pigeons trained under the FI schedule (Figure 2). However, not all pigeons tested under the FI schedule showed increased responding on the drug-appropriate key as the doses of morphine or U50,488 increased. Although the pigeons that did respond reliably under the FI schedule in subsequent experiments produced data leading to the same general conclusions as the data from the pigeons trained under the FR schedule, the details of the FI experiments have not been included in this report because of the few number of subjects tested and the relatively poor discriminative stimulus control. Similar differences between stimulus control under FR and FI schedules have been emphasized in previous studies (Massey, et al., 1992; McMillan and Hardwick, 1996; Snodgrass and McMillan, 1991). The experiments using the FI schedule under the four-choice procedure did not produce any advantages of using this schedule compared to the FR schedule which is in agreement with the observations on the usefulness of FR schedules in drug discrimination experiments made by Colpaert (1987) a quarter of a century ago.

Under the four-choice FR 20 schedule, increasing doses of morphine produced responding that shifted from the saline key at low doses to being confined largely to the morphine-appropriate key. Similarly, increasing doses of U50,488 produced responding that shifted from the saline key to responding confined largely to the U50,488-appropriate key. This result was expected based on previous studies in pigeons (Makhay, et al., 1998; Picker and Dykstra, 1987). When 10.0 mg/kg doses of morphine or U50,488 were combined with increasing doses of naltrexone, the dose of naltrexone required to shift responding from the drug-appropriate key to the saline-appropriate key was the same for morphine and U50,488. These findings contrast with previous results in other biological systems where it has generally been reported that opioid antagonists block the effects of μ agonists at lower doses than they block the effects of κ agonists (Locke, et al., 1989; Makhay, et al., 1998; Millan, et al., 1989). Such disparate results may reflect different procedural features of these studies or other, as yet unspecified factors.

Drug combinations were studied systematically under the FR schedule and when low doses of morphine were combined with low doses of U50,488, responding was largely confined to the saline-appropriate key. Increasing the dose of U50,488 in the presence of low doses of morphine produced responding on the U50,488-appropriate key and increasing the dose of morphine in the presence of low doses of U50,488 produced responding on the morphine-appropriate key. Combinations of higher doses of these two drugs produced responding largely on the drug-combination key. The picture described above strongly suggested that the procedure would be a productive assay for studying the discriminative stimulus effects of mixed-action opioids that act at both μ and κ receptors (or other drugs whose discriminative stimulus effects are determined by multiple mechanisms). It would be anticipated that at low doses of a mixed agonist, responding would occur predominately on the saline key. As the dose of the mixed agonist increased, responding might first occur on one of the two drug-associated keys or on the drug-combination key, which could lead to inferences as to which mechanism or mechanisms contributed to the discriminative stimulus effects of different doses of the drugs acting at more than one receptor site.

Pentazocine generally has been considered to be a partial agonist at μ receptors and an agonist at κ receptors (Gutstein and Akil, 2001). In the present experiments under the FR schedule, as the dose of *d,l*-pentazocine increased, responding shifted from the saline-appropriate key to the U50,488-appropriate key and then to the morphine-appropriate key and finally to the drug-combination key. This is not completely consistent with previous observations that low doses of *d,l*-pentazocine activate μ receptors and higher doses result in κ -receptor activation. Nevertheless, both sets of experiments support the contention that both μ and κ receptors are involved in the discriminative stimulus properties of *d,l*-pentazocine. Response distributions in individual subjects following *d,l*-pentazocine were more graded in shape than observed with other test drugs under the FR schedule; that is, there was a wider distribution of responding across keys and some subjects distributed less than 90% of their responses on a preferred key after one or more doses.

Ethylketocyclazocine has been considered to produce effects similar to *d,l*-pentazocine, perhaps with stronger κ activity (Gutstein and Akil, 2001). Under the FR schedule, as the dose of ethylketocyclazocine increased, responding first occurred on the morphine-appropriate key, while at higher doses some subjects responded on the U50,488-appropriate key before responses on the drug-combination key predominated. These results suggest greater μ -receptor activity than κ -activity at low doses of ethylketocyclazocine. In previous studies with pigeons using a three-choice procedure with U50,488, morphine, and saline as the training-drug stimuli (Makhay, et al., 1998), ethylketocyclazocine produced responding on U50,488-appropriate key at moderate doses and on the morphine-appropriate key at higher doses. Although the data are conflicting as to the activation of which receptor population is responsible for the discriminative stimulus effects of lower doses of ethylketocyclazocine, again all of these experiments strongly support a role for both μ and κ receptors in the discriminative stimulus effects of ethylketocyclazocine. At higher doses of ethylketocyclazocine in the present study, pigeons responded predominately on the drug-combination key indicating the influence of both μ and κ effects, which could not be seen in the three-choice procedure where there is no option to report on combined effects. These findings contrast with the idea that some drugs that act on more than one opioid receptor have discriminative stimulus effects that are κ -like in rat and monkey and μ -like in the pigeon (Picker, 1994).

Butorphanol has been considered to produce μ and κ effects similar to those of pentazocine and ethylketocyclazocine (Gutstein and Akil, 2001). The present experiments suggest that at low doses of butorphanol μ effects predominate, while κ effects become apparent at higher doses. Pentobarbital was studied as a control to show that drugs that do not produce any

effects mediated by μ or κ receptors would elicit only responding on the saline-appropriate key, and this is what was observed. Naltrexone was studied to show that it did not produce stimulus effects of its own in this procedure thereby removing the possibility that naltrexone was not blocking the effects of morphine and U50,488 by producing stimulus effects that overshadowed those of these two drugs. As expected, naltrexone alone produced responding largely confined to the saline-appropriate key.

A pattern occasionally seen on the effects of these mixed-acting drugs was for subjects to respond on the saline-appropriate key at low doses and at higher doses, switching to dividing responses across the single-drug keys. For example, after the 5.6 mg/kg dose of *d,l*-pentazocine, one subject distributed 33% of responses on the morphine-appropriate key and 67% of responses on the U50,488-appropriate key. The question arises as to why the subject did not respond on the drug-combination key rather than splitting responses between the two single-drug keys. Presumably, both the μ - and κ -discriminative stimuli would be present, so one might predict responding on the drug-combination key. One possibility is that as responding shifts from the saline-appropriate key to a drug key, for example the U50,488-appropriate key, the low dose is producing interoceptive effects discriminated as weak κ effects. As the dose increases and responding begins to predominate on the morphine-appropriate key, μ effects are beginning to overshadow the κ effects. However, at even higher doses, both μ and κ effects are present to produce responding on the drug-combination key.

It seems likely that there are other possible explanations for these observations, and it should be remembered that the results in the present experiments could have been influenced by the choice of the training doses of morphine and U50,488 used, especially in the drug combination condition. In defense of this choice, the doses for training of the drug combination was chosen because 5.0 mg/kg doses of morphine and U50,488 were equally and maximally discriminable (Figure 1). Combining a strong discriminable dose of one drug with a less discriminable dose of a second drug might bias all subsequent experiments toward responding to the more discriminable alternative. Therefore, it seemed appropriate to choose training doses for the drug combination that provided equally strong discriminative stimuli rather than choosing doses with weak discriminative stimuli or that were unequal in strength for these initial experiments.

In summary, under this four-choice procedure the FR schedule generated quantal dose-response curves and the FI schedule generated graded dose-response curves, as expected, but the marginal discrimination accuracy under the FI schedule and the failure of some birds to generate reliable dose-response curves made the FR schedule the schedule of choice for future experiments. The utility of the four choice procedure in providing an additional choice option for the subjects as was shown in the current study suggests that it may be useful for future studies on the discriminative stimulus effects of drug mixtures and the discriminative stimulus effects of drugs whose effects depend on more than one mechanism, despite the labor involved in establishing the complex drug discrimination.

Acknowledgments

This research was supported by a grant from the National Institute for Drug Abuse, DA2251-25.

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Figure 1.

Dose-response curves for morphine and U50,488 in 6 individual subjects under the FR 20 schedule. Abscissae: drug dose in mg/kg. Ordinates: percentage of responses on the morphine-appropriate key following morphine (top panel) or percentage of responses on the U50,488-appropriate key following U50,488 (bottom panel). Each point is a single observation in one subject.

Figure 2.

Dose-response curves for morphine and U50,488 in 5 individual subjects under the FI-300 s schedule. Abscissae: drug dose in mg/kg. Ordinates: percentage of responses on the morphine-appropriate key following morphine (top panel) or percentage of responses on the U50,488-appropriate key following U50,488 (bottom panel). Each point is a single observation in one subject.

Figure 3.

Effects of different doses of morphine alone (top left panel) and U50,488 alone (top right panel) and antagonism of 10.0 mg/kg morphine (bottom left panel) or 10.0 mg/kg U50,488 (bottom right panel) by increasing doses of naltrexone under the FR 20 schedule. Abscissae: drug dose in mg/kg. Ordinates: number of subjects responding on each response key. A key to the bar graphs is shown at the bottom of the figure.

Figure 4. Discriminative stimulus effects of different combinations of morphine and U50,488 under the FR 20 schedule. Abscissae: drug dose in mg/kg. Ordinates: number of subjects responding on each response key. A key to the bar graphs is shown at the bottom of the figure.

Figure 5.

Discriminative stimulus effects of other drugs under the FR 20 schedule. Abscissae: drug dose in mg/kg. Ordinates: number of subjects responding on each response key. See the bottom right panel for the key to the bar graphs.

Figure 6.

Dose-response curves for the effects of single drugs (top panel) and combinations of morphine and U50,488 (bottom panel) on overall rates of responding across the four keys under the FR schedule. Abscissae: drug doses in mg/kg (top panel) and mg/kg doses of U50,488 (bottom panel). Ordinates: rate of responding in responses/s. The data are means from six subjects.

Table 1

Distribution of responding on each of four response keys (percentage of total responses) during training sessions (data prior to delivery of first reinforcer) after administration of each training drug and overall rate of responding across all keys in responses/s (last column) for pigeons trained under the FR 20 and FI 300 schedules. Data are means (SE) for 6 individual subjects under the FR schedule and 5 individual subjects under the FI schedule.

Training Drug	Percentage of responses on each key under FR 20 schedule				Overall response rate (responses/s)
	Saline Key	Morphine Key	U50,488 Key	Combination Key	
Saline	98.1 (0.6)	1.0 (0.4)	0.5 (0.3)	0.4 (0.1)	1.91 (0.05)
Morphine	4.2 (1.3)	94.2 (1.2)	0.9 (0.7)	0.4 (0.8)	0.78 (0.04)
U50,488	2.6 (0.9)	0.8 (0.3)	94.6 (1.1)	2.0 (0.8)	1.18 (0.07)
Combination	0.8 (0.3)	5.6 (1.5)	3.1 (1.4)	90.6 (1.8)	0.48 (0.06)

Training Drug	Percentage of responses on each key under FI-300 s schedule				Overall response rate (responses/s)
	Saline Key	Morphine Key	U50,488 Key	Combination Key	
Saline	71.9 (2.6)	15.3 (1.7)	8.4 (1.0)	4.4 (0.8)	0.51 (0.02)
Morphine	15.3 (1.4)	66.4 (2.8)	12.2 (1.9)	8.0 (1.1)	0.47 (0.03)
U50,488	16.9 (2.2)	12.2 (1.3)	65.7 (2.6)	5.4 (0.9)	0.40 (0.03)
Combination	11.5 (1.5)	15.5 (1.4)	11.1 (1.0)	61.9 (2.6)	0.24 (0.02)