JOURNAL OF THE EXPERIMENTAL ANALYSIS OF BEHAVIOR 2009, **92,** 387–412 NUMBER 3 (NOVEMBER)

EFFECTS OF DRUGS AND DRUG COMBINATIONS IN PIGEONS TRAINED TO DISCRIMINATE AMONG PENTOBARBITAL, DIZOCILPINE, A COMBINATION OF THESE DRUGS, AND SALINE

D. E. McMillan, William D. Wessinger, and Mi Li

UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Drugs with multiple actions can have complex discriminative-stimulus properties. An approach to studying such drugs is to train subjects to discriminate among drug combinations and individual drugs in the combination so that all of the complex discriminative stimuli are present during training. In the current experiments, a four-choice procedure was used to train pigeons to discriminate among dizocilpine (noncompetitive NMDA receptor blocker), pentobarbital ($GABA_A$ receptor agonist), a fixed-dose combination of these two drugs, and saline. Following extended training, low doses of pentobarbital or dizocilpine administered alone produced saline-appropriate responding. Higher doses of pentobarbital produced responding on the pentobarbital-appropriate key and higher doses of dizocilpine produced responding on the dizocilpine key. Administering the lowest doses of pentobarbital and dizocilpine together resulted in responding on the saline-appropriate key. Increasing the dose of pentobarbital in the presence of low doses of dizocilpine produced responding primarily on the pentobarbital-appropriate key; increasing the dose of dizocilpine in the presence of the lowest dose of pentobarbital produced responding primarily on the dizocilpine-appropriate key. Combining the higher doses of pentobarbital and dizocilpine resulted in responding primarily on the drug-combination key. Low doses of phencyclidine or ethanol produced responding on the saline-appropriate key, but intermediate doses resulted in individual subjects responding predominately on either the pentobarbital key, the dizocilpine key, or the drug-combination key depending on the subject. After the highest dose of phencyclidine or ethanol, most subjects responded predominantly on the drug-combination key. Low doses of other drugs tested produced responding on the saline-appropriate key. With the highest diazepam doses responding was largely confined to the pentobarbital-appropriate key. The highest doses of dextromethorphan or dextrorphan resulted in responding on the dizocilpine key more frequently than on other keys. Across a range of doses, morphine produced responding predominantly on the saline key. The results using the four-key procedure emphasized the role of both $GABA_A$ and NMDA receptors in the complex discriminative stimulus properties of phencyclidine and of ethanol.

Key words: four-key drug discrimination, drug-combination training, pentobarbital, dizocilpine, drug-combination tests, phencyclidine, ethanol, diazepam, morphine, dextromethorphan, dextrorphan, pigeons

Both exteroceptive and interoceptive discriminative stimuli can vary in more than one dimension. For example, visual discriminative stimuli can vary in color, brightness, shape, size, and position, as well as other attributes. In establishing discriminative control by visual stimuli, it can be difficult to determine to which of these stimuli the subject is attending. Similarly, the development of stimulus control by interoceptive stimuli such as those produced by drugs can depend on multiple drug actions. In the present study, we used a new method for the study of the discrimination of drugs with complex actions using both individual drugs and drug mixtures in a four-choice discrimination procedure in pigeons.

The focus of these experiments was directed toward the use of this new procedure to study the effects of drugs purported to act at $GABA_A$ receptors and NMDA receptors, receptor populations which have been implicated in the effects of benzodiazepines, barbiturates, ethanol, and other drugs. For example, benzodiazepines and barbiturates are usually considered to act at $GABA_A$ receptors although at somewhat different sites on the receptor (Charney, Mihic, & Harris, 2001). Dizocilpine is generally considered to act as an noncompetitive blocker of NMDA receptors (Dingledine, Borges, Bowie, & Traynelis, 1999), while ethanol effects have been pro-

This research was supported by NIDA grant DA-2251-25 from the National Institute for Drug Abuse.

Address correspondence to William D. Wessinger, Department of Pharmacology and Toxicology, University of Arkansas for Medical Science, 4301 W. Markham Street, PO Box 611, Little Rock, Arkansas 72205-7199 (e-mail: wdwessinger@uams.edu).

Mi Li is now at the National Center for Toxicological Research, 3900 NCTR Road, Jefferson, Arkansas 72079-9502.

doi: 10.1901/jeab.2009.92-387

posed to be due to effects at both $GABA_A$ receptors and NMDA receptors (Fleming, Mihic, & Harris, 2001). Teasing out the relative contributions of NMDA and $GABA_A$ receptors to the discriminative stimulus effects of some of these drugs has been a difficult task.

Using the usual two-choice discrimination procedure (a training drug versus saline) for studying drug discrimination, when the training drug is replaced with a different drug, responding can occur predominately on the drug manipulandum (substitution), predominately on the saline manipulandum (no substitution), or distributed across both manipulanda (partial substitution). Even with these limited response options, the results can be quite complex. For example, with pentobarbital (a $GABA_A$ prototype) and the drug vehicles as the training drugs, other barbiturates and benzodiazepines substitute for the training drug (Herling, Valentino, & Winger, 1980), but ethanol, phencyclidine, dizocilpine and dextrorphan only partially substitute for pentobarbital (Herling et al., 1980; Kline & Young, 1986; Snodgrass & McMillan, 1991; Willetts & Balster, 1989). Using dizocilpine (a noncompetitive NMDA antagonist) and the drug vehicles as the training drugs, phencyclidine and dextrorphan substitute for dizocilpine but pentobarbital does not substitute (Butelman, France, & Woods, 1991), and ethanol only partially substitutes for dizocilpine (Butelman, Baron, & Woods, 1993). Thus, using two-choice drug discrimination procedures, ethanol partially substitutes for both pentobarbital and dizocilpine, but while dizocilpine partially substitutes for pentobarbital, pentobarbital does not substitute for dizocilpine. Dextrorphan, which substitutes for dizocilpine, substitutes only partially for pentobarbital.

Another approach to studying the discriminative stimulus effects of depressant drugs with complex mechanisms of action such as ethanol is by training subjects to discriminate drug mixtures. Stolerman and Olufsen (2001) found that 3 g/kg (intragastric) ethanol produced a high level of drug-appropriate responding (76%) in rats trained under a two-choice procedure to discriminate a mixture of 5 mg/kg chlordiazepoxide (a $GABA_A$ receptor modulator) and 0.08 mg/kg dizocilpine (NMDA receptor antagonist) from the no-drug condition. In subjects trained to discriminate a mixture of 8 mg/kg pentobarbital and 0.08 mg/kg dizocilpine from no drug, very little drug-appropriate responding was observed after 3.0 g/kg ethanol. However, after retraining the discrimination using a mixture of 12 mg/kg pentobarbital and 0.04 mg/kg dizocilpine, drug-appropriate responding after the same dose of ethanol returned to 75%. Thus, the ratio of the mixture components appeared to be important.

An approach to increasing the sensitivity of the drug discrimination procedure has been to train the subjects under three-choice procedures. For example, when using dizocilpine, ethanol and water as the training drugs (Gatto, Bowen, & Grant, 1995), phencyclidine, which substitutes for both ethanol and dizocilpine in two-choice studies, only substitutes for dizocilpine under the three-choice procedure (Bowen & Grant, 1998). In rats trained to discriminate among pentobarbital, ethanol, and water, partial substitution for pentobarbital occurs after dizocilpine, diazepam, and phencyclidine (Bowen, Gatto & Grant, 1997). The partial substitution of diazepam for pentobarbital in three-choice discriminations is consistent with two-choice discrimination data from rats and pigeons, but the partial substitution of diazepam for pentobarbital in three-choice discriminations contrasts with the more robust substitution of diazepam for pentobarbital in two-choice discriminations. Clearly, providing an additional choice to the drug-discrimination procedure produces different substitution patterns than are observed under two-choice procedures. Drug discrimination studies which include drug mixtures during training as well as the individual drugs in the mixtures may well reveal subtle differences among the discriminative-stimulus effects of drugs that appear to be similar under simpler procedures.

Recently, we have shown that pigeons can come under stimulus control of a four-choice procedure in which subjects are trained to discriminate among three active drugs and saline (Li & McMillan, 2001). Not only can pigeons perform such discriminations, but they are also able to discriminate among two drugs, a combination of the two drugs, and the drug vehicle using four-choice drug discrimination procedures (McMillan & Li, 2002). Training subjects to learn a discrimination

among two drugs that produce discriminative stimuli by different mechanisms, a combination of these two drugs, and the absence of all three drug states (vehicle) might permit a separation of the relative contribution of different mechanisms to the discriminative stimulus effects of a drug whose effects were a blend of the effects of the two mechanisms. For example, if drug C produces responding on the key associated with training drug A, but not the key associated with training drug B or the drug-combination key, this would suggest that drug A mechanisms mediate the discriminative stimuli produced by drug C. In contrast, responding on the drug-combination key would suggest that elements of both drug A and drug B mechanisms were contributing to the discriminative stimulus effects of drug C. Toward this end pigeons were trained to discriminate among pentobarbital (a drug acting at $GABA_A$ receptors), dizocilpine (an uncompetitive NMDA receptor blocker), a combination of pentobarbital and dizocilpine, and saline. Subsequently, the effects of other drugs were studied with a particular emphasis on ethanol, which is purported to act at both $GABA_A$ receptors and NMDA receptors.

METHOD

Subjects

Six adult male White Carneau pigeons (Palmetto Pigeon Plant, Sumter, SC) served as subjects in these experiments. All were experimentally naïve at the beginning of these experiments. Pigeons were housed individually with free access to water and grit in a temperature- and humidity-controlled room that was maintained under a 12-h normal-phase lighting cycle. During the study, the pigeons were maintained at 80–85% (410–530 g) of their free-feeding weights by food earned during the experimental sessions and postsession supplemental feeding (Purina Pigeon Chow Checkers 5405, Purina Mills, LLC, St. Louis, MO). Procedures used during these experiments were in accord with the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences.

Apparatus

The experimental chamber was a MED Associates ENV-009A Modular Test Cage (MED Associates, Inc., St. Albans, VT) enclosed in a Gerbrands Model G7211 sound- and light-attenuating enclosure. A 28-V DC light mounted near the ceiling illuminated each test chamber during behavioral sessions, except during food presentations. The chamber was equipped with four pigeon response keys that could be transilluminated with different colored lights (MED Associates, Model ENV-124AM). These were mounted on the front panel in a row 21 cm above the grid floor, each spaced 6 cm apart. 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. Beneath the left–center key, 2 cm above the grid floor was an opening through which pigeon chow could be presented when schedule contingencies were met. During food presentations the houselight was extinguished and the food opening was illuminated. A desktop computer, programmed using MED-PC software (MED Associates), controlled the experimental contingencies and recorded the data through an interface (MED Associates).

Procedure

The methods for training subjects have been discussed in detail (Li, Wessinger, & McMillan, 2005). Initially, pecks on a single illuminated response key were reinforced by food presentation (4-s access to pigeon chow) under a fixed-ratio 1 schedule (FR1). Once responding was established the schedule of reinforcement was gradually incremented across sessions to FR 20. Then a second key was illuminated and responding on it was reinforced by food presentation in a similar manner. After responding on both keys under an FR 20 schedule was established, responding during subsequent sessions was differentially reinforced depending upon whether an i.m. injection of saline or 5 mg/kg pentobarbital was administered 10 min before the session. Thus, if saline had been administered before the session, only responding on one of the keys was reinforced under the FR 20 schedule. If 5 mg/kg pentobarbital had been administered 10 min before the session, only responding on the other key was reinforced under the FR 20 schedule. After the two-key drug discrimination had been established, a third key was introduced by illuminating it also. Responding on the third key was first estab-

lished as before, and in subsequent sessions responses on the third key were reinforced under an FR 1 schedule of food presentation only if 0.13 mg/kg dizocilpine had been administered 10 min before the session. Once responding was established on the third key, the response requirement was gradually increased to FR 20. After the three-key discrimination had been established, the fourth key was introduced and responding was established as before. In this case, responses on the fourth key were reinforced only if both 5 mg/kg pentobarbital and 0.13 mg/kg dizocilpine had been administered 10 min before the session. The assignment of drug-appropriate keys was varied randomly across subjects. Training sessions ended after 20 reinforcer presentations or 40 min, whichever occurred first. Training sessions were conducted 5 or 6 days a week with at least one session under each stimulus condition occurring each week. Training the subjects to make this four-choice discrimination required about 13 months before responding was stable, accurate and showed no further improvement.

During subsequent test sessions, cumulative doses of pentobarbital or dizocilpine were administered to establish dose–response relationships. Test sessions were similar to training sessions, except that they were made up of several test trials and responding on any of the four keys was reinforced. Each test trial began with the administration of a test dose of drug. Following a presession period (10 min; except, 15 min for ethanol) to allow time for drug absorption, a response period ensued during which responding on any key was reinforced under an FR 20 schedule of 4-s access to food. The trial ended immediately after the food presentation, or after 15 min, whichever occurred first; then the next cumulative dose was administered and the next test trial began. For example, to determine the pentobarbital dose–response curve a 1.0 mg/kg dose of pentobarbital was administered i.m. 10 min before the first test trial. After 20 responses had been made on any key the food reinforcer was presented and the trial ended; or if the subject failed to complete an FR 20 schedule within 15 min the trial was ended. The subject then received a 2.0 mg/kg dose of pentobarbital for a cumulative dose of 3.0 mg/kg and a second response period ensued 10 min later. Cumulative dosing trials continued until a dose was reached that eliminated responding for the 15-min response period. Similar dosing procedures were used to determine dose–response curves for all drugs. Drug substitution tests were generally conducted once a week, with training sessions continuing on 4 or 5 other days of the week. One cumulative dose–response curve was conducted in each of the 6 pigeons for every drug or drug-combination condition.

After determining the dose–response curves for pentobarbital and dizocilpine alone, various combinations of doses of pentobarbital and dizocilpine were studied. To study dose combinations, a fixed dose of pentobarbital was administered along with the lowest dose of dizocilpine and the first test trial was conducted. In subsequent test trials, the cumulative dose of dizocilpine was incremented in the same manner as when dizocilpine was tested alone. Single ascending doses of pentobarbital were tested (1.0, 3.0, 5.6 and 10.0 mg/kg) in combination with cumulative doses of dizocilpine. Pentobarbital doses ranged from low doses that produced exclusively saline-appropriate responding when tested alone up to doses that exceeded the pentobarbital-training dose. Upon completion of these dose–response curves, a series of experiments using cumulative dosing was performed with drugs other than pentobarbital and dizocilpine. Cumulative dosing with these drugs was conducted as previously described for pentobarbital and dizocilpine alone.

Data Analysis

The percentage of responses on each key was calculated by dividing the number of responses on each key by the total number of responses on all four keys and converting to a percentage. Response keys are identified by the training drugs that were associated with reinforced responses on the respective keys. The total number of responses on all keys was divided by the session time to calculate the overall rate of responding during the session (responses/s). Response-rate data are reported in the Appendix as mean rates of responding in tabular form.

Drugs

The drugs used were purchased from commercial sources or obtained from the

Table 1

Percentage of responses on each key after each training drug (first four rows of data) and the mean rate of responding across all keys in responses/sec (last row of data) for subjects trained under the FR 20 schedule.

Training Drug	Saline Key	Pentobarbital Key	Dizocilpine Key	Combination Key
Saline	96.6 (1.0)	2.5 (0.8)	1.2 (0.6)	0.1 (0.1)
Pentobarbital	4.5 (1.1)	92.9 (1.4)	1.0 (0.6)	0.5 (0.4)
Dizocilpine	10.9 (2.1)	0.8 (0.4)	87.5 (2.2)	0.8 (0.6)
Combination	4.0 (1.1)	5.7 (1.5)	3.4 (1.0)	87.1 (1.6)
Responses/ Second	1.35	1.37	0.74	0.26

Note. Values in parentheses are standard errors.

National Institute on Drug Abuse. The drugs were pentobarbital sodium, dizocilpine hydrogen maleate, phencyclidine hydrochloride, morphine sulfate, dextrorphan tartrate, dextromethorphan hydrobromide, diazepam as the commercial preparation for injection, and 10% (w/v) ethanol. Doses are expressed in these forms and were administered as i.m. injections 10 min before the training sessions or test sessions in a volume of 1.0 ml/kg of body weight, except ethanol which was administered orally through a rubber tube 15 min before the beginning of the session. After drug administration, the pigeons were placed in the darkened test chamber until the session began. When cumulative dosing was used, subsequent i.m. injections were made into alternating sides of the breast muscle. When drug combinations were studied, pentobarbital was given into one side of the breast muscle, followed by injection of the first of a series of cumulative doses of dizocilpine into the opposite breast muscle. Subsequent cumulative doses alternated injection sites.

RESULTS

Table 1 shows the percentage of responses on each key and rates of responding after responding stabilized under training conditions. Across all four training conditions the pigeons made an average of 91% of their responses on the appropriate key. The percentage of correct responses on the saline key after saline administration was slightly greater than the percentage of pentobarbital-appropriate responses after a training dose of pentobarbital, which also was slightly higher than the nearly equal percentage of drug-appropriate responses on the dizocilpine and the drug-combination keys after these drugs. After training doses of pentobarbital and dizocilpine, most of the incorrect responses occurred on the saline key. During training sessions following administration of saline or the combination of pentobarbital and dizocilpine, incorrect responses were more equally distributed across two or three keys. Overall rates of responding on the saline and pentobarbital keys were almost two times higher than the rate of responding on the dizocilpine key, which in turn was about three times higher than the rate of responding on the drug-combination key.

Figure 1 shows the dose–response curves for pentobarbital in individual subjects. Responses were distributed to the saline-appropriate key at the lowest dose of pentobarbital. At intermediate doses of pentobarbital (3 or 5.6 mg/kg) the subjects switched from responding on the saline key to responding on the pentobarbital key; the dose at which they switched keys varied among subjects. Almost no responses were made on the dizocilpine-appropriate key or the drug-combination key after any dose of pentobarbital.

Figure 2 shows the dose-response curves for dizocilpine in individual subjects. At low doses of dizocilpine, responding occurred primarily on the saline key. As the dose of dizocilpine increased, the subjects switched to responding on the dizocilpine-appropriate key and made relatively few responses on the pentobarbital key or the drug-combination key. P460 responded primarily on the dizocilpine-appropriate key after 0.056 mg/kg, while the remaining 5 subjects discriminated dizocilpine after 0.1 mg/kg.

Figures 3–6 show the effects of combinations of different doses of pentobarbital with cumulative doses of dizocilpine. Figure 3 shows that when 1.0 mg/kg pentobarbital was

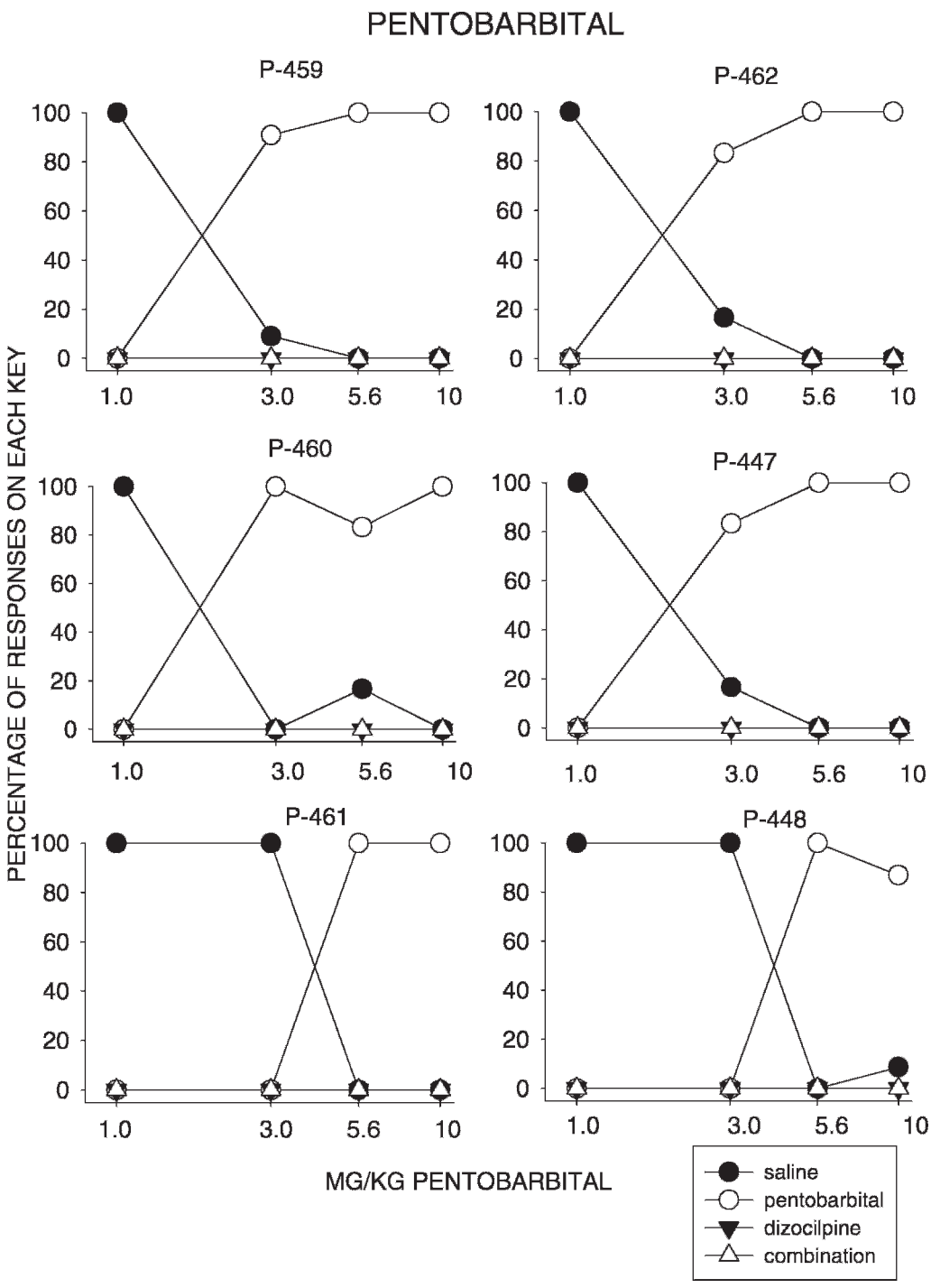

Fig. 1. Discrimination of pentobarbital in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

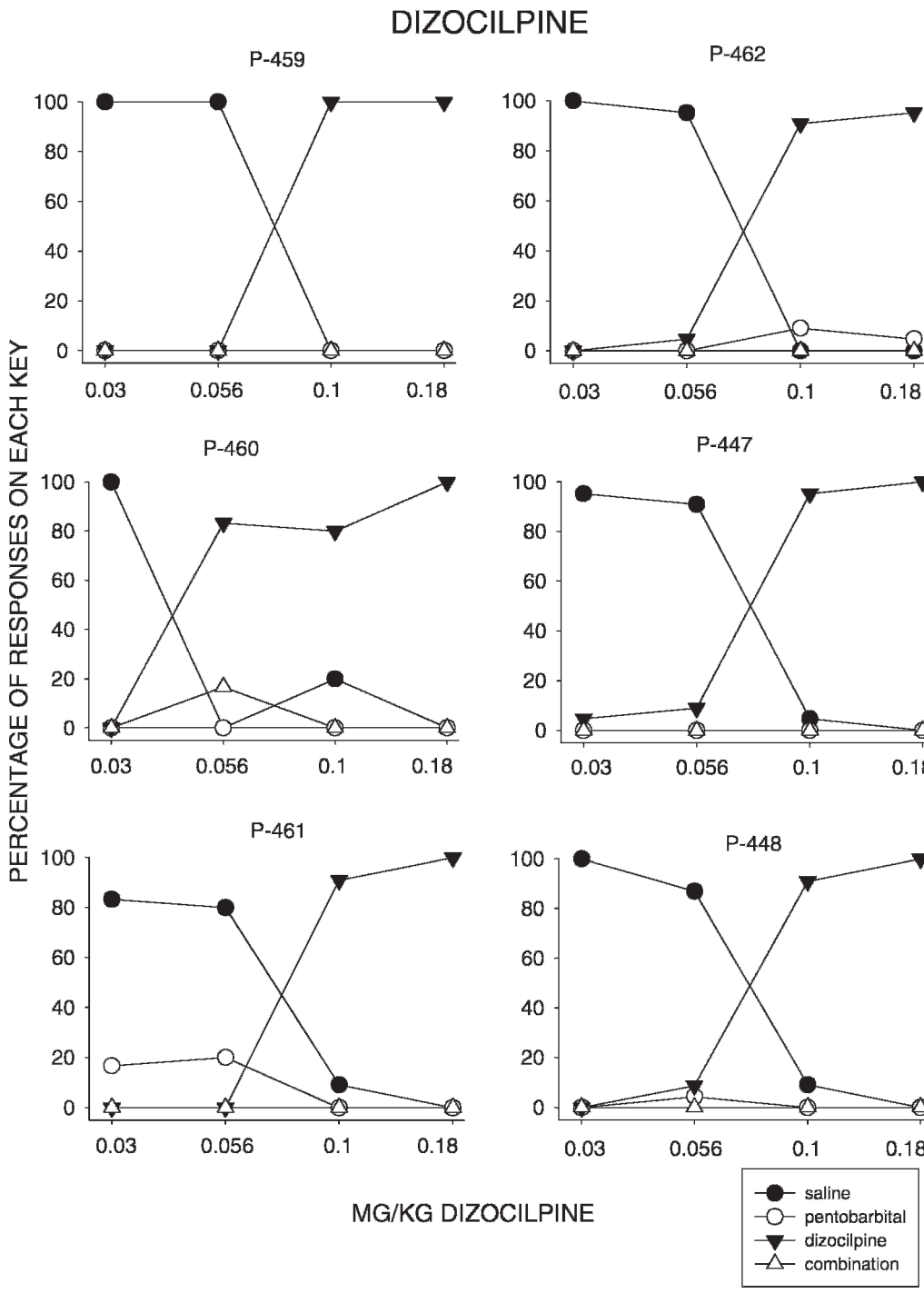

Fig. 2. Discrimination of dizocilpine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

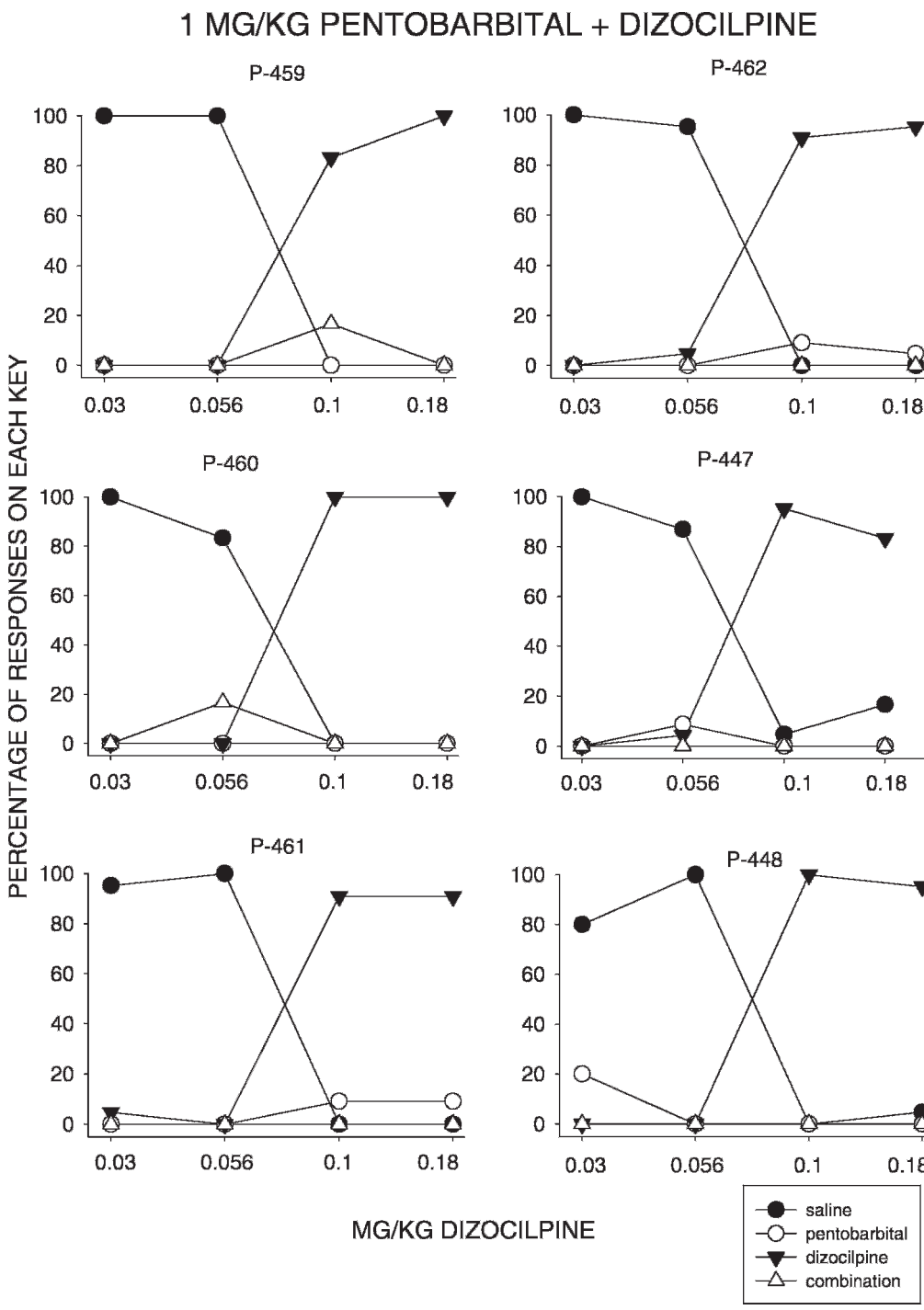

Fig. 3. Discrimination of combinations of 1 mg/kg pentobarbital with increasing doses of dizocilpine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

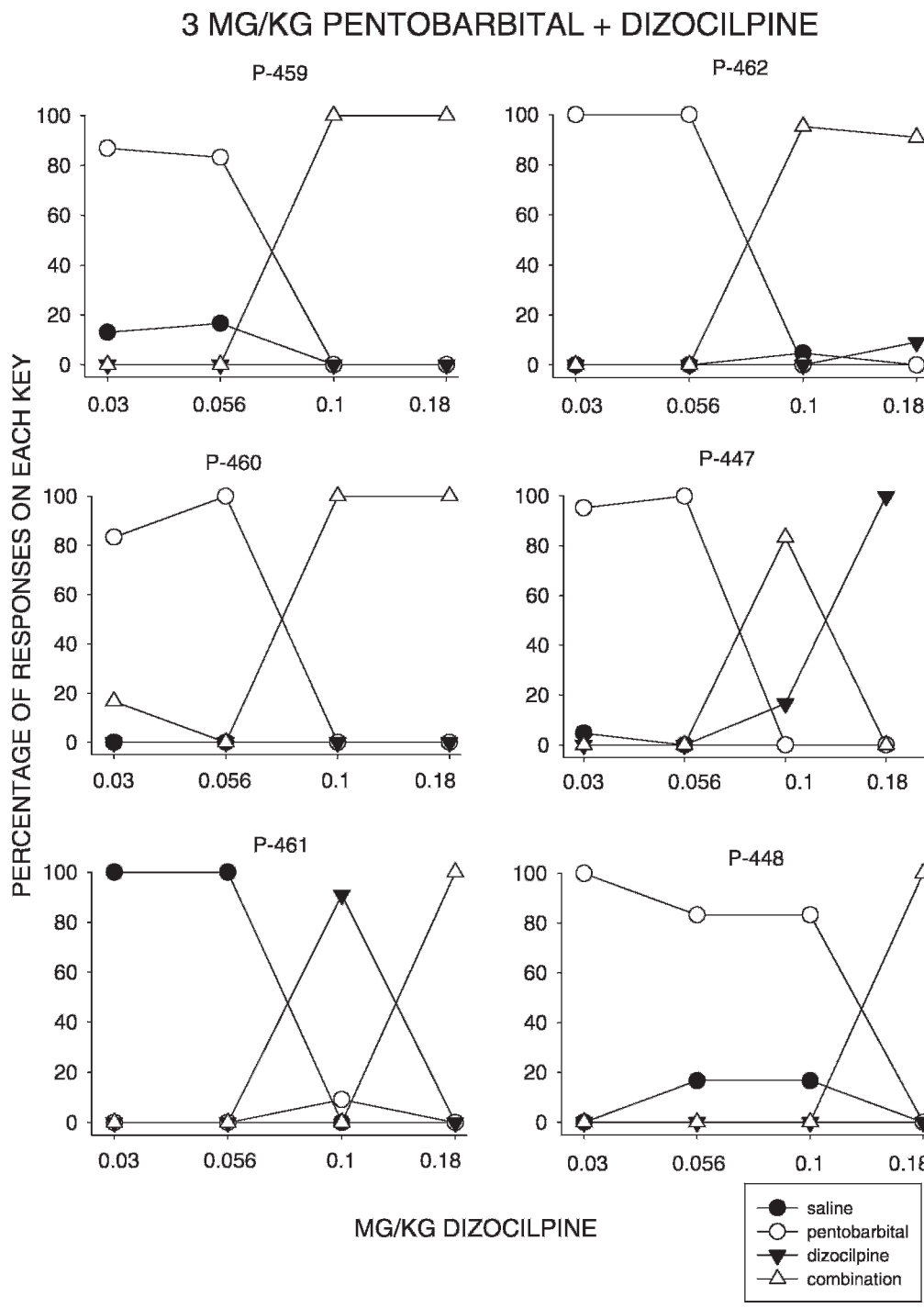

Fig. 4. Discrimination of combinations of 3 mg/kg pentobarbital with increasing doses of dizocilpine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

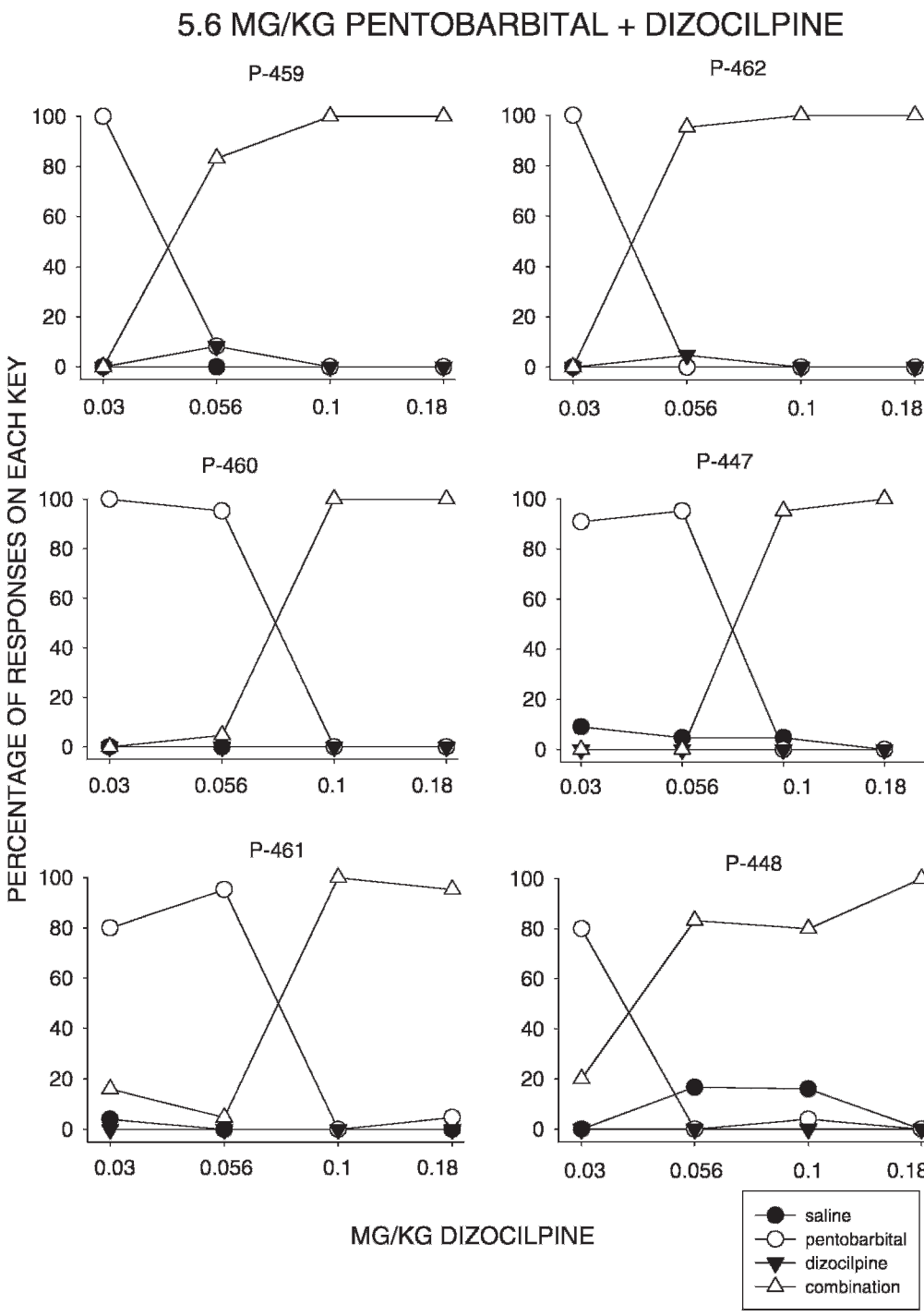

Fig. 5. Discrimination of combinations of 5.6 mg/kg pentobarbital with increasing doses of dizocilpine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

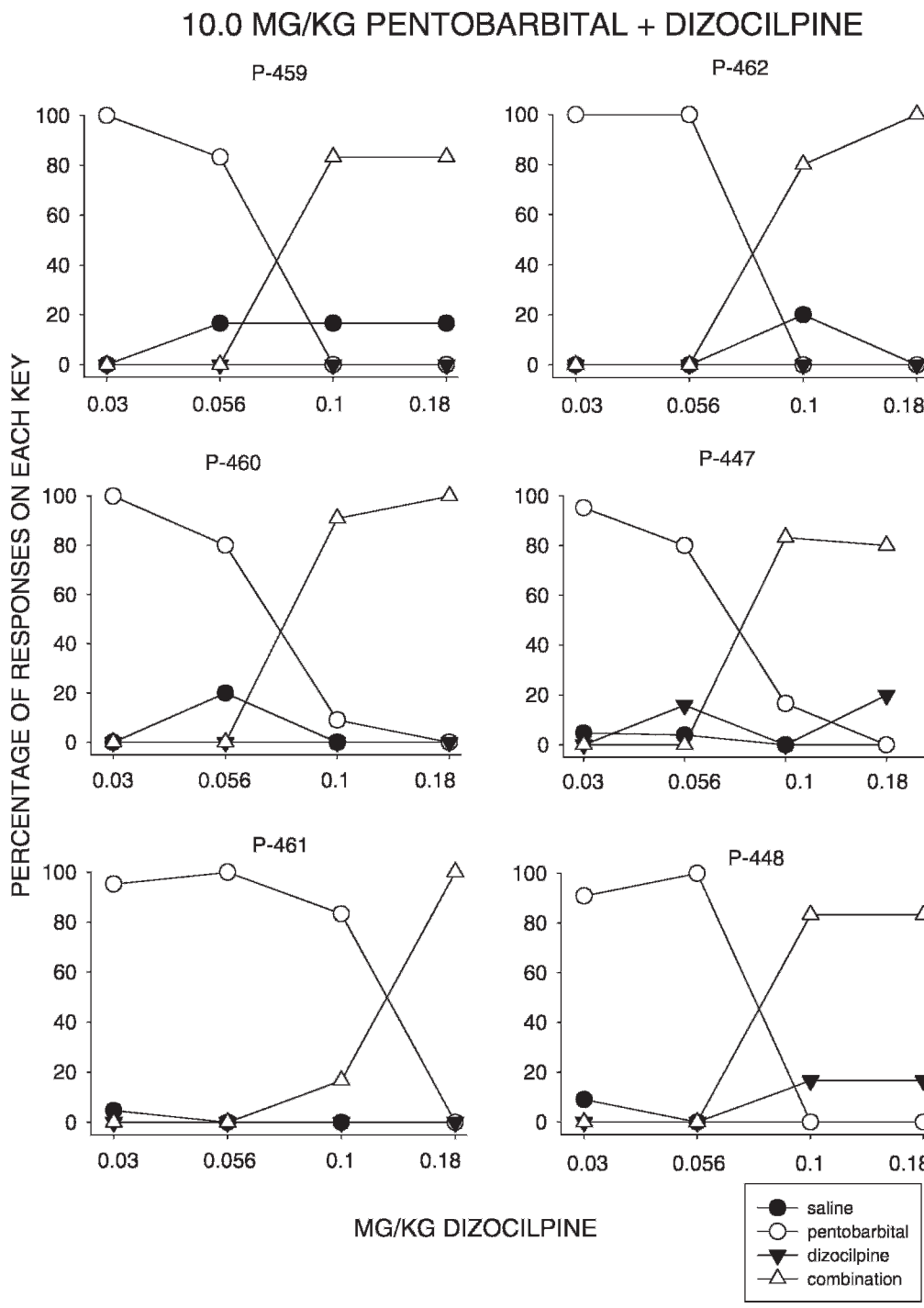

Fig. 6. Discrimination of combinations of 10 mg/kg pentobarbital with increasing doses of dizocilpine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

combined with 0.03 and 0.056 mg/kg dizocilpine all pigeons responded on the saline key. When 1.0 mg/kg pentobarbital was combined with higher cumulative doses of 0.1 and 0.18 mg/kg dizocilpine, all pigeons responded on the dizocilpine-appropriate key. Figure 4 shows that when 3.0 mg/kg pentobarbital was combined with doses of 0.03 and 0.056 mg/kg dizocilpine, 5 of the 6 subjects responded on the pentobarbital-appropriate key with the remaining subject responding on the saline key. At higher doses of dizocilpine, most subjects switched from the pentobarbital key to the drug-combination key, although pigeon P461 responded predominantly on the dizocilpine key after the 0.1 mg/kg dose of dizocilpine before switching to the drug-combination key after 0.18 mg/kg. When the highest cumulative dose of dizocilpine (0.18 mg/kg) was combined with 3.0 mg/kg pentobarbital, all subjects responded on the drug-combination key except P447 who responded on the dizocilpine key after this dose combination. Figure 5 shows that when 5.6 mg/kg pentobarbital was combined with 0.03 mg/kg dizocilpine, all subjects responded on the pentobarbital-appropriate key. As the dose of dizocilpine in the drug combination was increased, the pigeons switched from the pentobarbital key to the drug-combination key after either the 0.056 or 0.1 mg/kg dose of dizocilpine, depending on the subject. Figure 6 shows that when 10 mg/kg pentobarbital was combined with the 0.03 and 0.056 mg/kg doses of dizocilpine, all subjects responded predominately on the pentobarbital-appropriate key. At a cumulative dose of 0.1 mg/kg dizocilpine combined with 10 mg/kg pentobarbital 5 of 6 subjects responded on the drug-combination key and at the highest cumulative dose of dizocilpine combined with 10 mg/kg pentobarbital, all subjects responded on the drug-combination key.

Figure 7 summarizes these complex drug combination data in quantal form by showing the number of pigeons choosing each key at each dose combination. The combination of the two lower doses of pentobarbital and dizocilpine produced responding on the saline-appropriate key. Higher doses of pentobarbital (3–10 mg/kg) in combination with lower doses of dizocilpine (0.03–0.056 mg/kg) caused most subjects to respond predominately on the pentobarbital key, and higher doses of dizocilpine (0.1–0.18 mg/kg) in combination with the 1 mg/kg dose of pentobarbital caused most subjects to respond on the dizocilpine key. Combinations of the two highest doses of dizocilpine (0.1–0.18 mg/kg) with the three highest doses of pentobarbital (3.0–10.0 mg/kg) produced responding largely confined to the drug-combination key for most subjects.

Figure 8 shows the dose–response curves for phencyclidine for individual pigeons. After 0.3 and 0.56 mg/kg phencyclidine, all pigeons responded predominantly on the saline-appropriate key. At 1.0 mg/kg of phencyclidine, 2 pigeons responded on the pentobarbital key, 2 others responded on the dizocilpine key, and 2 others responded on the drug-combination key. After a cumulative dose of 1.8 mg/kg phencyclidine, 5 of 6 pigeons responded on the drug-combination key, while the remaining subject (P447) continued to respond on the dizocilpine-appropriate key. Although all the subjects except for P447 ended up responding on the drug-combination key after the highest dose of phencyclidine, the pattern of response-key choices at intermediate doses varied across subjects.

Figure 9 shows the individual dose–response curves for diazepam. All subjects responded on the saline-appropriate key after 0.1 mg/kg diazepam. As the cumulative dose of diazepam was increased, progressively more subjects responded on the pentobarbital-appropriate key with all 6 subjects responding predominately on this key after cumulative doses of 1.0 and 3.0 mg/kg.

Figure 10 shows the effects of ethanol on the response patterns of individual subjects. All pigeons responded on the saline key after the 0.25 g/kg dose, and 3 subjects continued to respond on the saline key after the 0.50 g/kg doses of ethanol. As the cumulative dose increased, 4 pigeons switched from the saline- to the pentobarbital-appropriate key and the other 2 switched from the saline- to the dizocilpine-appropriate key. All pigeons, except P460, switched from the pentobarbital or dizocilpine key to the drug-combination key after the highest dose of ethanol (1 g/kg). P460 continued to respond on the pentobarbital-appropriate key at doses of 0.5 to 1.0 g/kg of ethanol.

Figure 11 shows the dose–response curves for dextrorphan for individual subjects. All subjects responded on the saline-appropriate

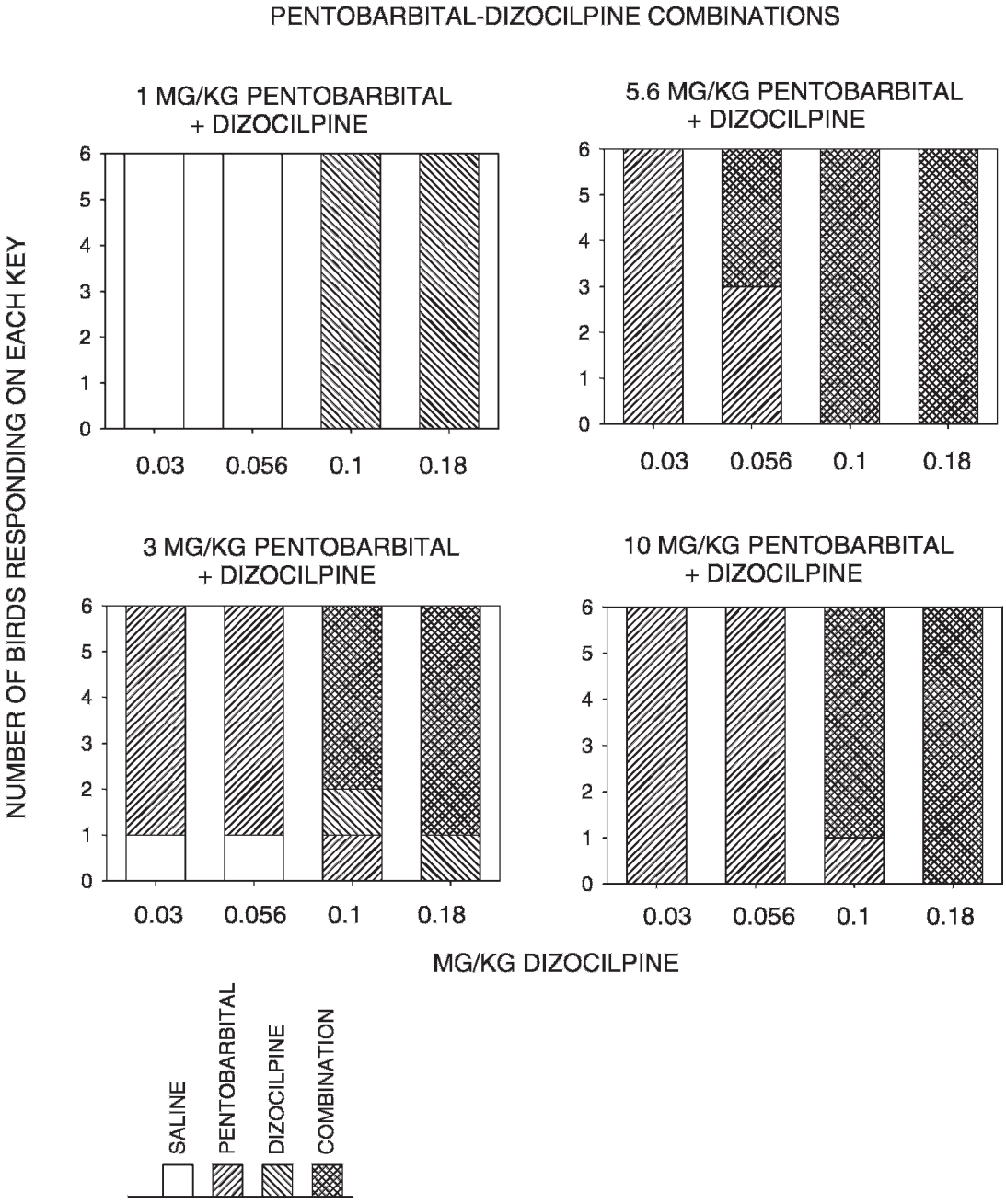

Fig. 7. Summary graph showing group data for the effects of 1.0, 3.0, 5.6 and 10.0 mg/kg of pentobarbital (individual panels) administered in combination with increasing cumulative doses of dizocilpine in pigeons trained to discriminate among pentobarbital, dizocilpine, a fixed combination of these drugs and saline. Each stacked bar shows the number of subjects (n = 6) that responded predominately on each of the four response keys (see legend at bottom of figure for key designations) following cumulative doses of dizocilpine.

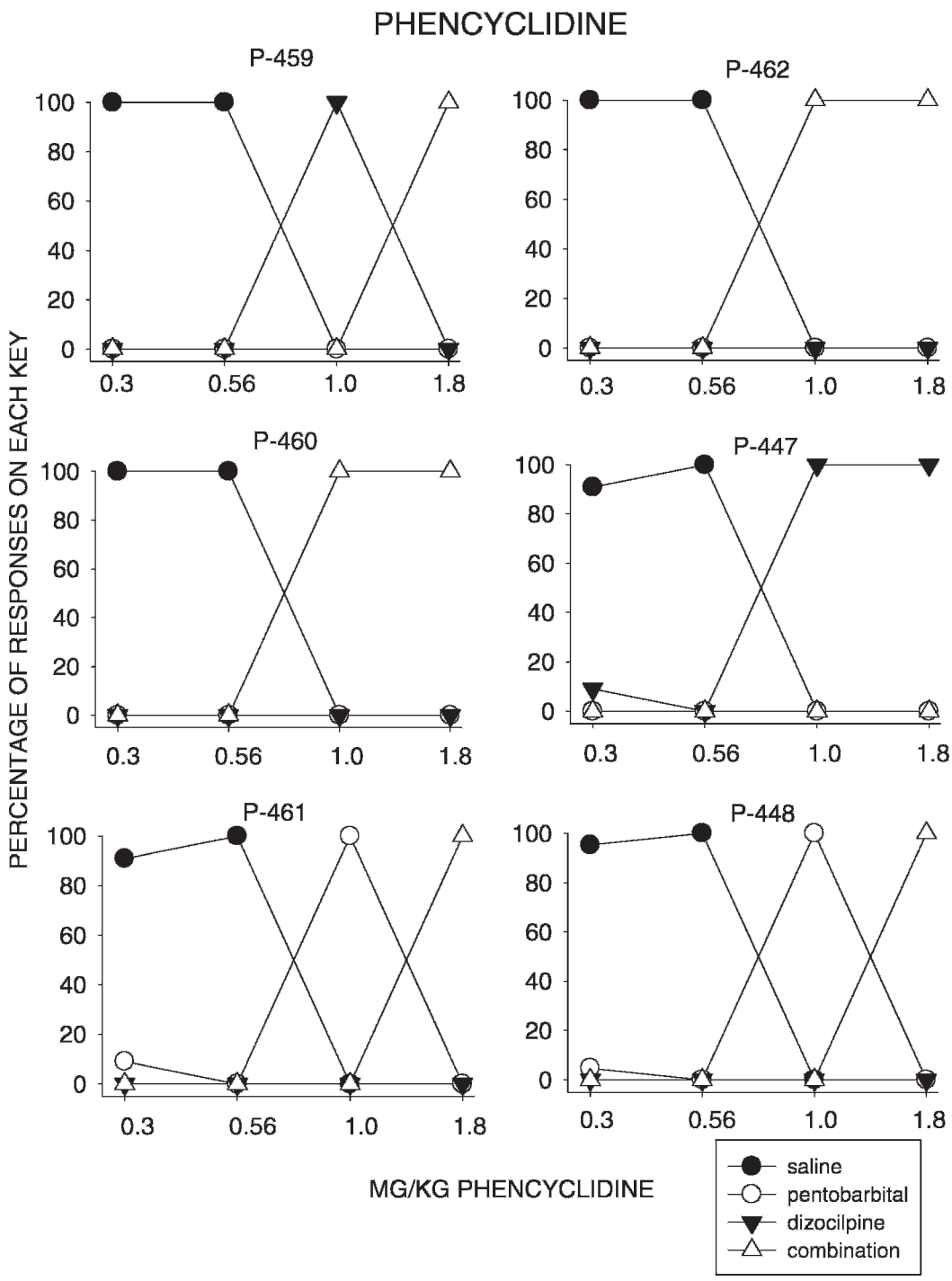

Fig. 8. Discrimination of phencyclidine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

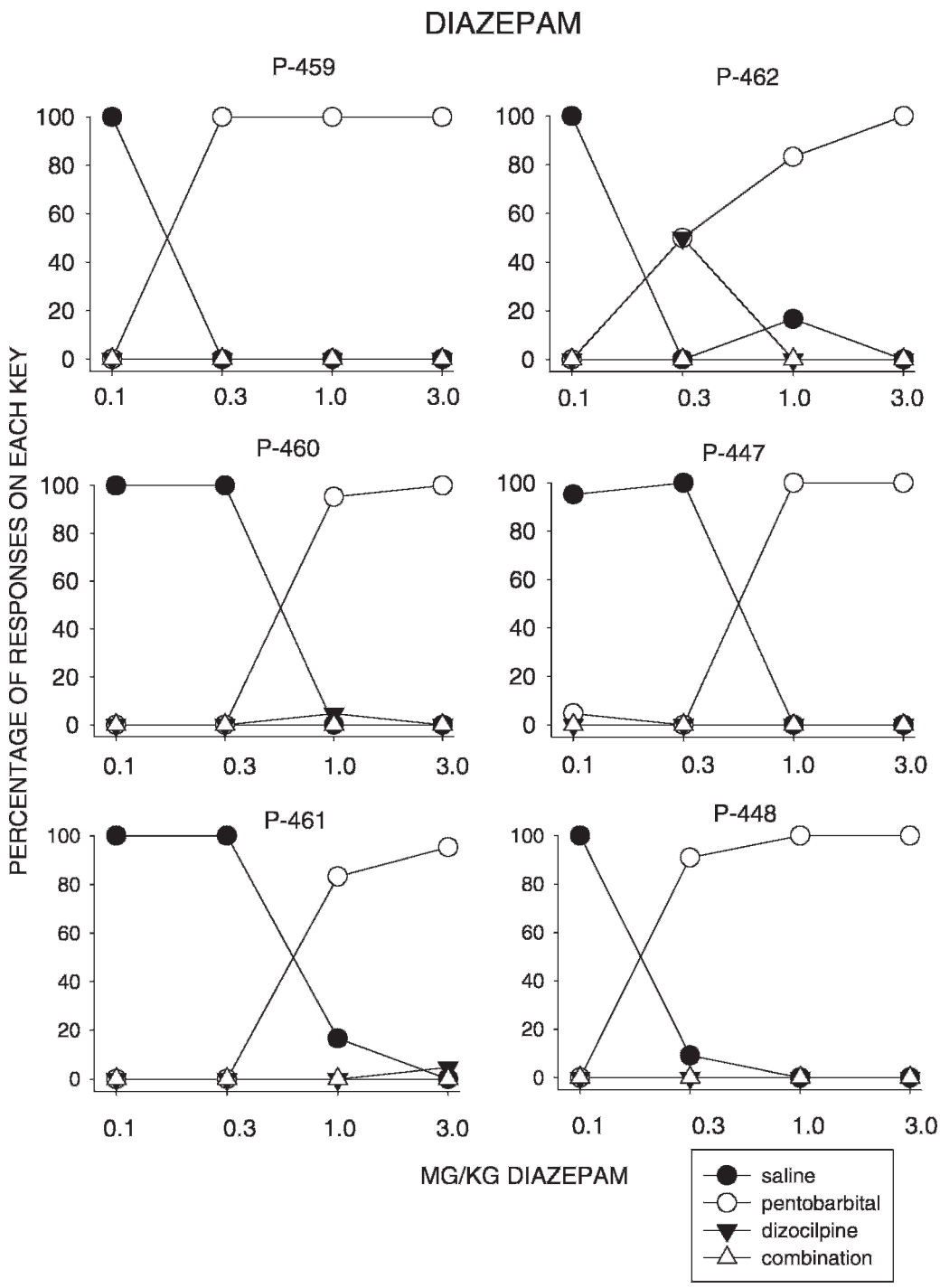

Fig. 9. Discrimination of diazepam in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

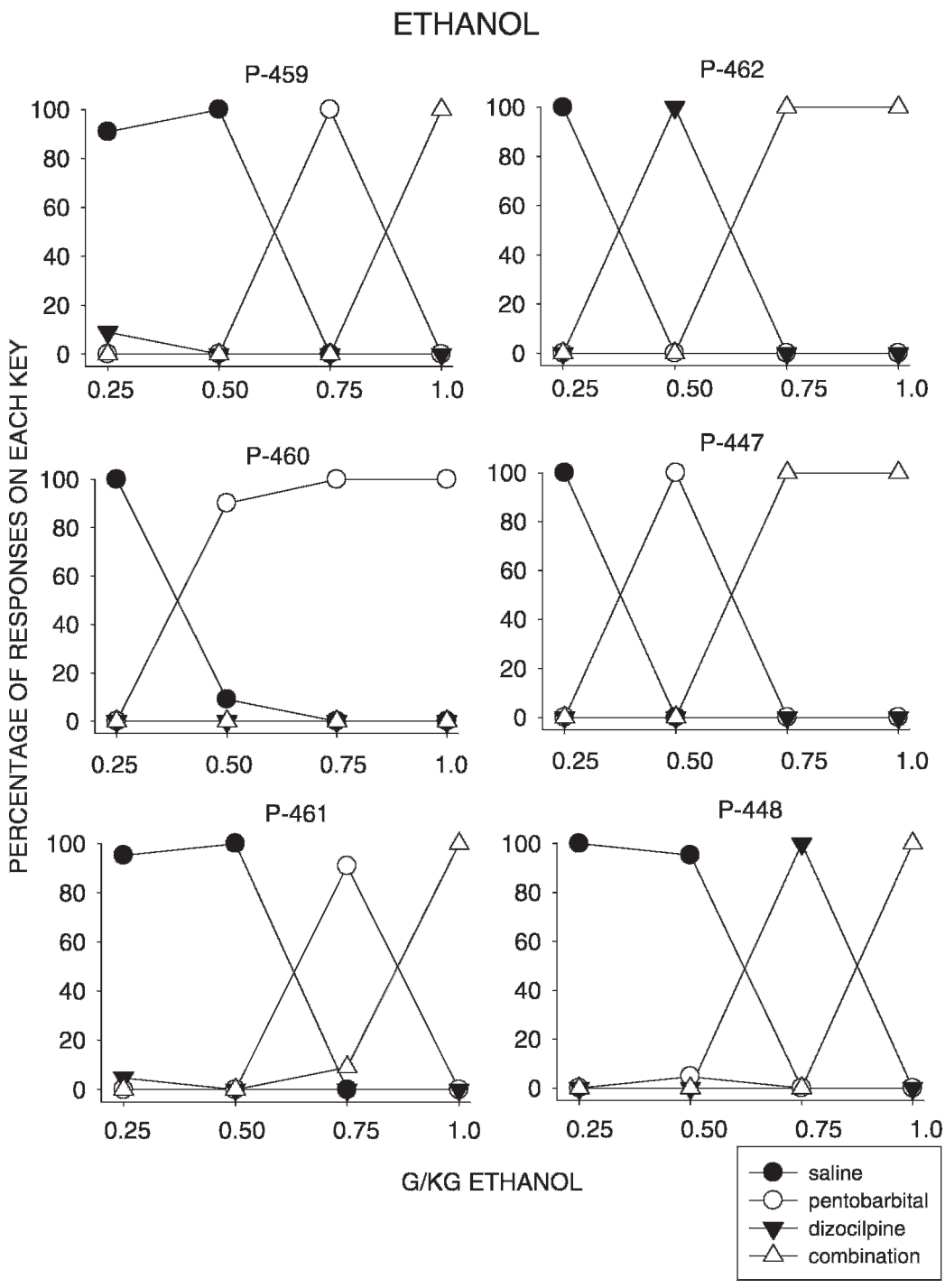

Fig. 10. Discrimination of ethanol in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

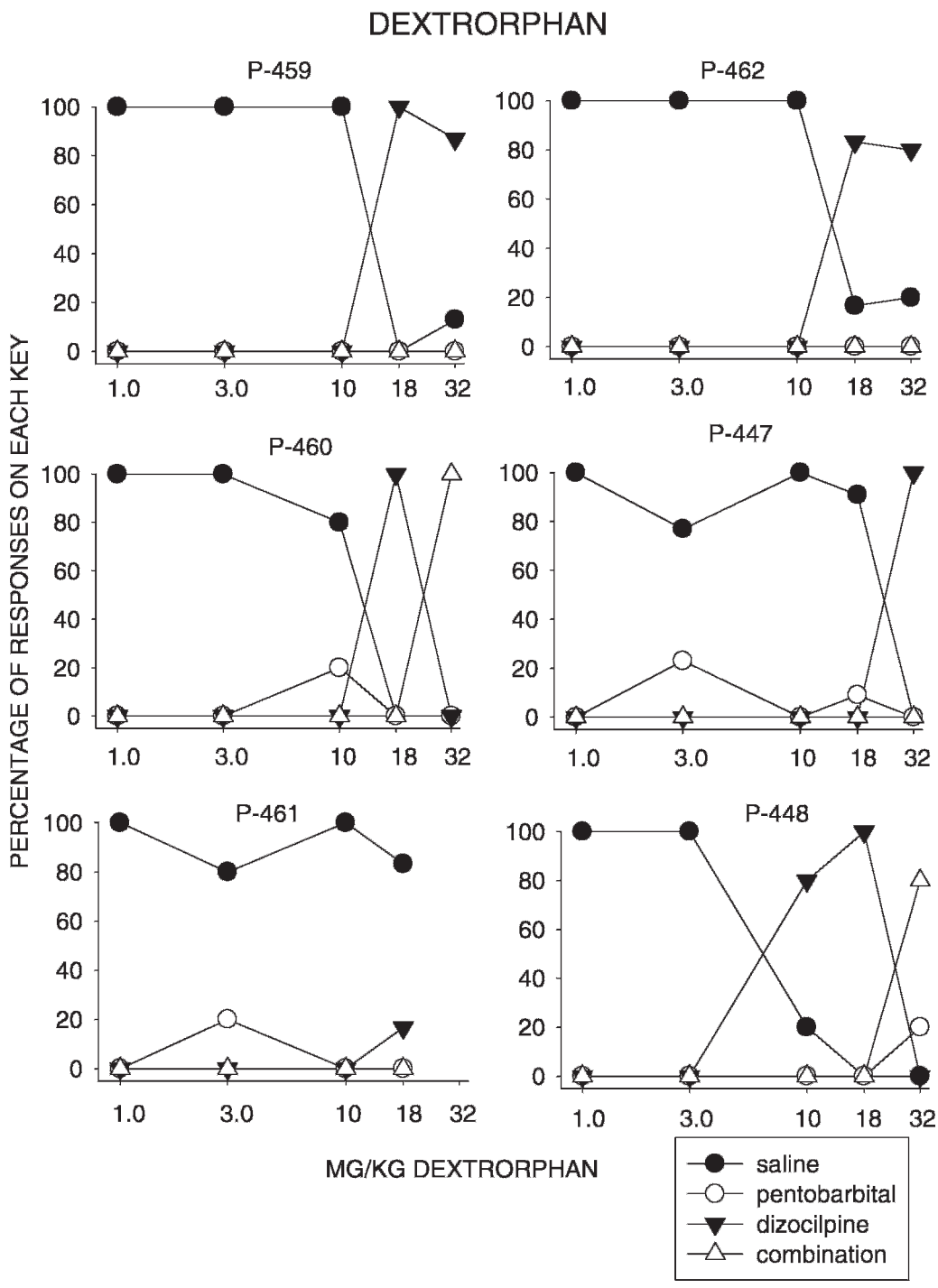

Fig. 11. Discrimination of dextrorphan in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

key at the two lowest doses of dextrorphan; 5 of 6 were still responding predominately on the saline key after cumulative doses of 10 mg/kg, and 2 were still responding on the saline key at 10 mg/kg. As the cumulative dose of dextrorphan increased to 10 mg/kg, P488 switched to the dizocilpine key, while 4 more pigeons switched to the dizocilpine-appropriate key at the 18 mg/kg dose. At the highest cumulative dose of 32 mg/kg dextrorphan, 3 pigeons continued to respond on the dizocilpine key, while 2 pigeons switched to the drug-combination key. Pigeon P461 did not respond on any of the keys at this dose. Thus following doses of dextrorphan, none of the subjects responded predominately on the pentobarbital-appropriate key, although 2 subjects did respond on the drug-combination key after the highest dose of dextrorphan.

Figure 12 shows the effects of dextromethorphan on the response patterns of individual subjects. Pigeons P459 and P448 responded predominately on the saline-appropriate key at all doses of dextromethorphan. The other 4 subjects responded on the saline key at low doses of dextromethorphan before switching to the dizocilpine-appropriate key after either 5.6 or 10 mg/kg dextromethorphan. None of the pigeons responded appreciably on the pentobarbital-appropriate key or the drug-combination key.

Morphine was used as a negative control; thus, it was expected that responding would be confined largely to the saline-appropriate key. As expected, 5 subjects responded on the saline key after all four doses of morphine (Figure 13). P461 responded on the saline key after doses of 1.0 and 5.6 mg/kg, and then equally divided responses on the saline- and dizocilpine-appropriate key after a cumulative dose of 10 mg/kg morphine.

Figure 14 summarizes the data from the individual test drugs in a single graph by showing the number of pigeons responding on each key after each dose of each drug. The 0.3 and 0.56 mg/kg doses of phencyclidine resulted in all subjects responding on the saline-appropriate key. Two subjects responded on each of the three drug-associated keys after the 1.0 mg/kg dose of phencyclidine, while after the 1.8 dose of phencyclidine, all except 1 subject responded on the drug-combination key. Similarly, ethanol produced complex effects with all subjects responding on the saline-appropriate key after the 0.25 g/kg dose of ethanol. After an intermediate dose of 0.75 g/kg ethanol, subject choice was distributed among all three drug-appropriate response keys. At the 1.0 g/kg dose, 5 of the 6 subjects responded on the drug-combination key. All pigeons responded on the saline-appropriate key after the 0.1 mg/kg dose of diazepam. As the dose of diazepam increased to 0.3 mg/kg there was a shift toward responding on the pentobarbital-appropriate key, and all subjects responded on the pentobarbital-appropriate key after the two highest doses of diazepam (1.0–3.0 mg/kg). After dextrorphan, most subjects shifted from the saline-appropriate key at doses of 3.0 and 10.0 mg/kg to the dizocilpine-appropriate key at 18.0 and 32.0 mg/kg doses, but at the 32.0 mg/kg dose 2 subjects responded on the drug-combination key. Dextromethorphan produced a dose-dependent shift from responding on the saline-appropriate key at doses of 1.0 and 3.0 mg/kg to responding on the dizocilpine-appropriate key after 5.6 and 10.0 mg/kg doses, although 2 pigeons responded only on the saline key across all doses. Morphine caused predominately saline-appropriate responding by all subjects after all doses.

The effects of drug combinations on overall rates of responding are shown in the Appendix (Table A1) and the effects of the various test drugs tested alone are shown in Table A2. In general, increasing doses of drugs or drug combinations produced decreases in overall rates of responding.

DISCUSSION

Although training was difficult and its time prolonged, pigeons were able to discriminate among a noncompetitive NMDA receptor blocker (dizocilpine at 0.13 mg/kg), a drug acting at the $GABA_A$ receptor complex (pentobarbital at 5.0 mg/kg), the combination of these two drugs at these doses, and saline. After responding stabilized under training conditions, a high degree of stimulus control was observed. This was evidenced by the high percentage of drug-appropriate responding that occurred following the administration of saline, or the training dose of the training drugs, or the combination. Mean percent responding on the correct (or drug-appropri-

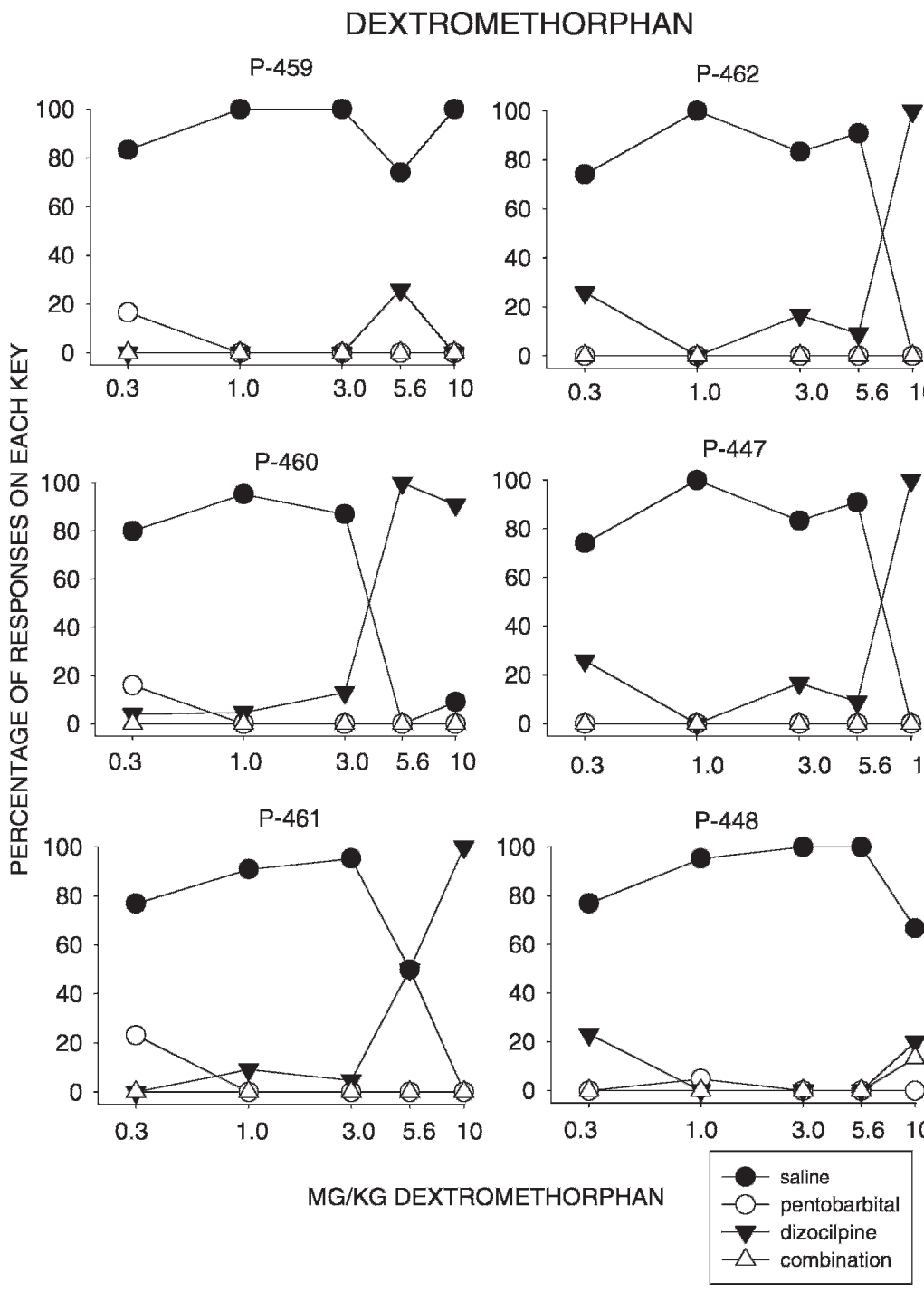

Fig. 12. Discrimination of dextromethorphan in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

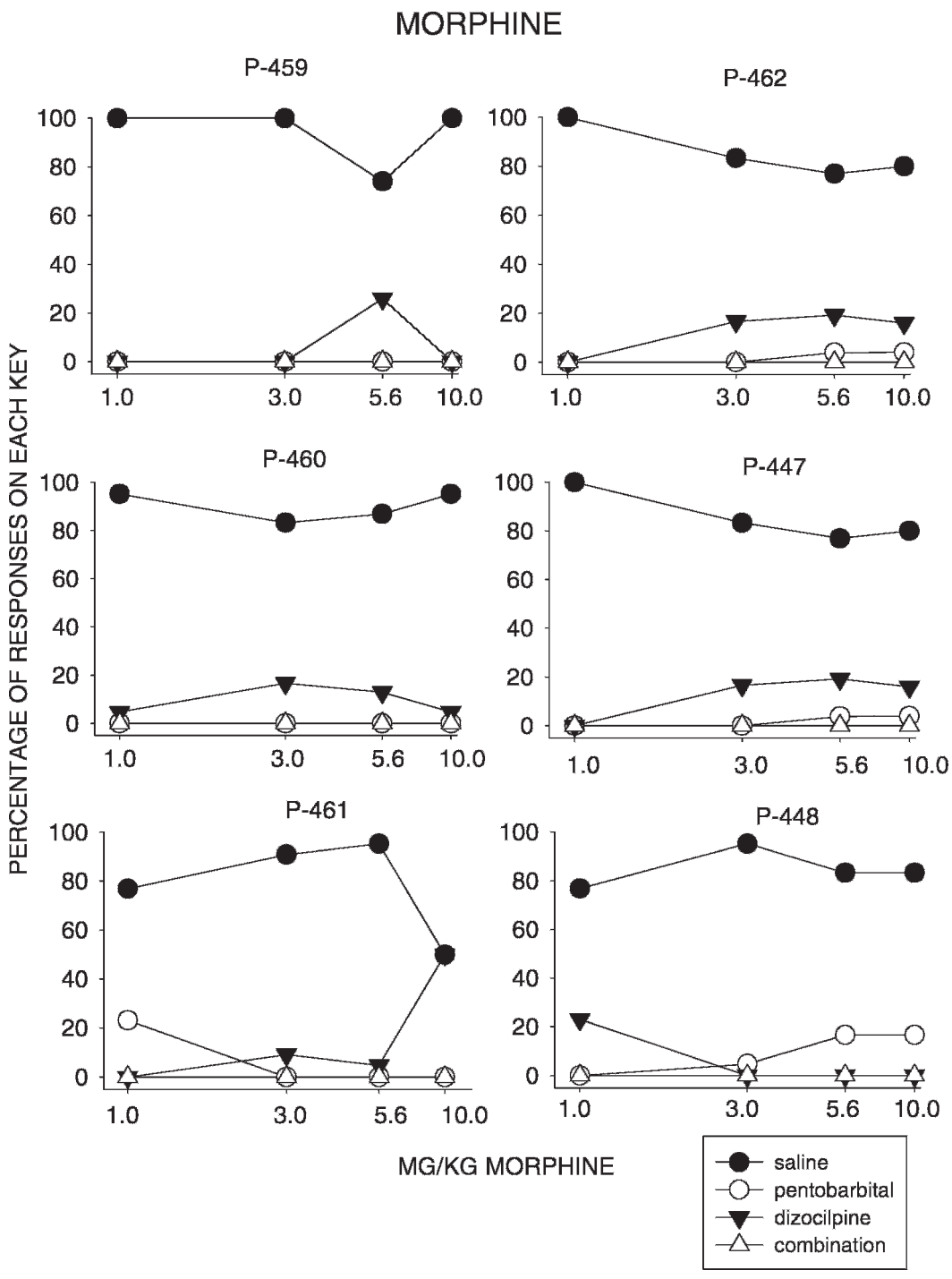

Fig. 13. Discrimination of morphine in individual pigeons trained to discriminate among pentobarbital, dizocilpine, a combination of these drugs and saline. Filled circles represent responding on the saline key, unfilled circles represent responding on the pentobarbital key, filled triangles represent responding on the dizocilpine key, and unfilled triangles represent responding on the drug-combination key. Each point is a single observation in each of 6 pigeons.

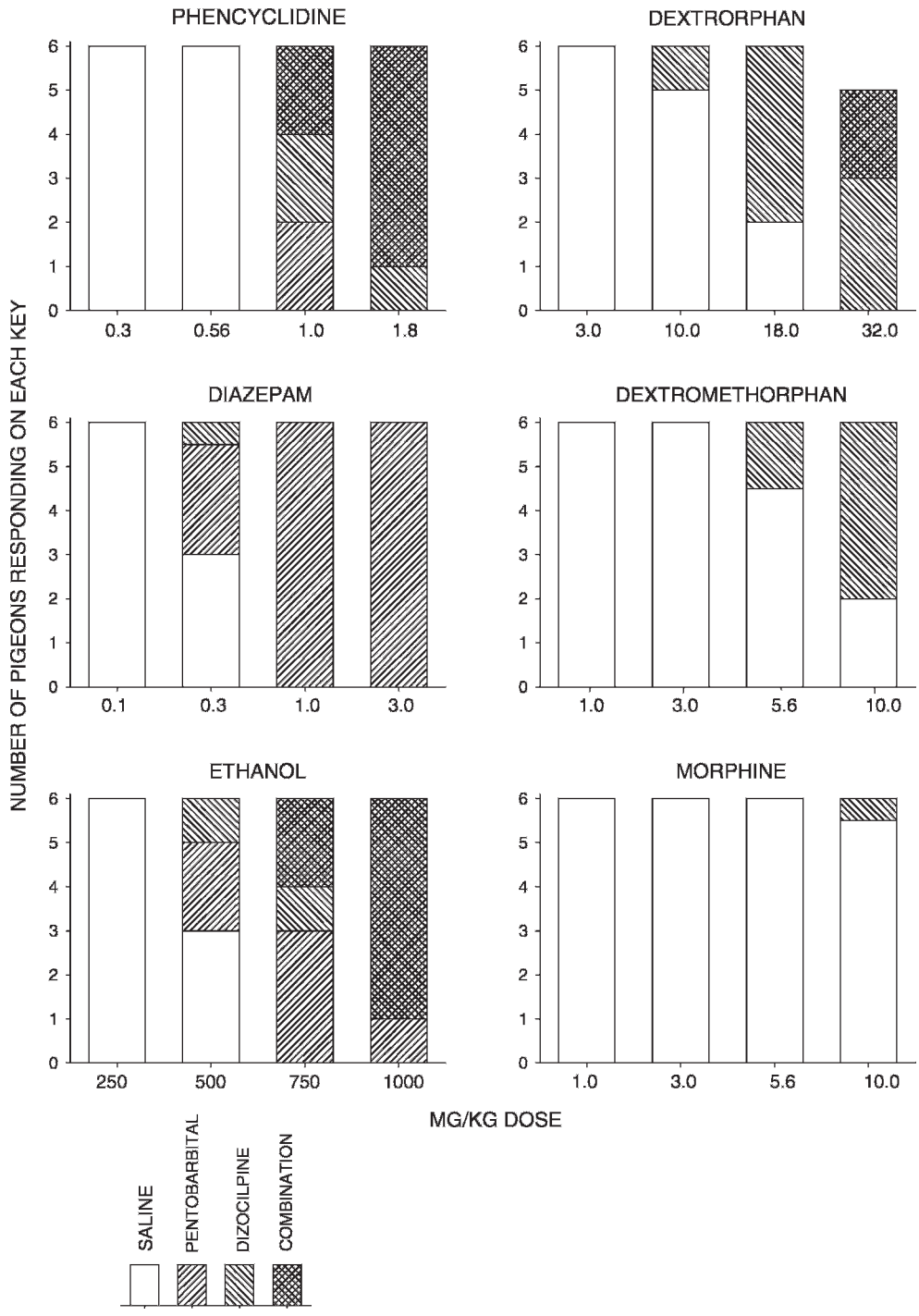

Fig. 14. Summary graph showing group data for the effects of different drugs (individual panels) administered as cumulative doses in pigeons trained to discriminate among pentobarbital, dizocilpine, a fixed combination of these drugs and saline. Each stacked bar shows the number of subjects (n = 6) that responded predominately on each of the four response keys (see legend at bottom of figure for key designations) following cumulative doses of the test drugs. In three cases (0.3 mg/kg diazepam, 5.6 mg/kg dextromethorphan, 10 mg/kg morphine) one of the subjects did not respond predominately on a single key, but rather distributed responses equally among two keys; these cases are reported in half units. One subject failed to respond on any key following 32 mg/kg dextrorphan.

ate) key ranged from 87.1 to 96.6% of responses under training conditions. When cumulative dose–response curves for the training drugs alone were determined, the lowest doses at which individual subjects discriminated pentobarbital were 3.0 to 5.6 mg/kg pentobarbital. The threshold doses for discriminating dizocilpine were 0.056 to 0.1 mg/kg dizocilpine. Considering group data, pentobarbital was discriminated by all subjects at a cumulative dose 5.6 mg/kg, the closest dose tested to the training dose (5.0 mg/kg). Dizocilpine was discriminated by all subjects at a cumulative dose of 0.1 mg/kg, also the closest dose tested to the training dose (0.13 mg/kg).

When testing different doses of pentobarbital in combination with cumulative doses of dizocilpine, the key upon which the subjects responded depended on the dose ratio of the two drugs in an orderly manner. When the lowest dose of pentobarbital was combined with cumulative doses of dizocilpine, the results were similar to the effects of dizocilpine alone (compare Figures 3 and 2); that is, the two lowest doses of dizocilpine produced saline-appropriate responding and the two highest doses produced dizocilpine-appropriate responding when combined with 1.0 mg/kg pentobarbital. At higher doses of pentobarbital (3.0 and 5.6 mg/kg) administered in combination with cumulative doses of dizocilpine, there was a dose-dependent increase in the number of subjects responding on the pentobarbital-appropriate key after lower doses of dizocilpine, and a dose-dependent increase in the number of subjects responding on the drug-combination key after higher cumulative doses of dizocilpine. Finally, the effects of 10.0 mg/kg pentobarbital combined with cumulative doses of dizocilpine were similar to the 5.6 mg/kg pentobarbital plus dizocilpine combinations, except that there was a dose-dependent increase in the number of cumulative doses of dizocilpine that had to be administered before the subjects switched from the pentobarbital-key to the drug-combination key, an effect often characterized as overshadowing, in this case by the higher pentobarbital dose. The results for the experiments with drug combinations suggest that the four-key drug discrimination procedure can be used to reliably differentiate among pentobarbital-like effects, dizocilpine-like effects, effects reflecting a combination of pentobarbital- and dizocilpine-like effects, and effects lacking either pentobarbital- or dizocilpine effects. These discriminations presumably reflect the actions of the training drugs at $GABA_A$ receptors (pentobarbital) or NMDA receptors (dizocilpine), or in the case of the combined-drug discrimination, both of these receptors. The procedure was then applied to study the discriminative stimulus effects of drugs that have been purported to act at one or both of these receptors.

The two lower doses of phencyclidine produced responding primarily on the saline-appropriate key. It was anticipated that higher doses of phencyclidine would produce responding on the dizocilpine-appropriate key, since phencyclidine interacts with NMDA receptors in a manner similar to dizocilpine. In previous studies, pigeons trained in a two-choice procedure to discriminate dizocilpine from vehicle responded on the dizocilpine-appropriate key after phencyclidine (Butelman et al., 1991). In our own laboratory, we have found that pigeons trained to discriminate between phencyclidine and saline respond on the phencyclidine-appropriate key when given dizocilpine (McMillan & Wenger, 1983). The present experiments showed an unexpected effect of phencyclidine. At the 1.0 mg/kg dose of phencyclidine, 2 subjects responded on the pentobarbital key, 2 responded on the dizocilpine key, and 2 subjects responded on the drug-combination key. At the highest dose, 1.8 mg/kg, 5 subjects responded on the drug-combination key and only one responded on the dizocilpine key. These findings suggest that $GABA_A$ receptors contribute to the discriminative stimulus effects of phencyclidine in addition to the expected effects of phencyclidine at NMDA receptors. Indeed, we have reported previously that pentobarbital partially substitutes for phencyclidine in pigeons (McMillan & Wenger) and rats (Snodgrass & McMillan, 1991) which is consistent with phencyclidine having at least partial pentobarbital-like effects. In other two-choice drug-discrimination experiments in pigeons, phencyclidine has been shown to substitute for dizocilpine (Butelman et al., 1991). In a three-choice discrimination in rats trained to discriminate among dizocilpine, ethanol, and water, phencyclidine also substituted for dizocilpine (Bowen & Grant,

1998), although in monkeys trained to discriminate between dizocilpine and saline, phencyclidine only partially substituted for dizocilpine (France, Moerschbaecher, & Woods, 1991). There is also evidence of overlap of the discriminative stimuli produced by dizocilpine and pentobarbital. Several studies in rats have shown partial substitution of dizocilpine for pentobarbital (Bowen et al., 1997; Snodgrass & McMillan, 1991; Willetts & Balster, 1989), although pentobarbital does not appear to substitute for dizocilpine in pigeons (Butelman et al., 1991), perhaps because it does not interact appreciatively with NMDA receptors. These data support the idea that the phencyclidine discriminative stimulus has some overlap with the discriminative stimuli produced by pentobarbital, but stronger overlap with discriminative stimuli produced by dizocilpine. In the present experiments, having a response key associated with the combined stimulus properties of pentobarbital and dizocilpine revealed effects of phencyclidine that were less apparent using conventional two-choice discrimination procedures.

The effects of ethanol were similar to those of phencyclidine in that at lower discriminable doses of ethanol subjects responded on either the pentobarbital and dizocilpine key, while at higher doses responding shifted to the drug-combination key in all subjects except one. There was good reason to expect responding to occur on the drug-combination key after the highest dose of ethanol, since the ethanol discriminative stimulus has been hypothesized to have components mediated by both NMDA and $GABA_A$ receptors (Bowen et al., 1997; Fleming et al., 2001; Shelton & Balster, 1994). The present studies confirm these observations and suggest a slight predominance of $GABA_A$ effects at low doses relative to the effects of ethanol at NMDA receptors because more pigeons switched from responding on the saline key to responding on the pentobarbital key as the dose of ethanol increased. A predominance of $GABA_A$ effects at lower doses of ethanol in some subjects might explain why there is only partial substitution of ethanol for dizocilpine in pigeons (Butelman et al., 1993). Previous drug discrimination studies have shown that phencyclidine will substitute for ethanol (Grant, Knisely, Tabakoff, Barrett & Balster, 1991; Grant, Columbo, Grant & Rogawski, 1996; Järbe & McMillan, 1983), although ethanol does not reliably substitute for phencyclidine (McMillan, 1982; McMillan & Wenger, 1983). These differences may reflect quantitative differences between the NMDA and $GABA_A$ receptor populations activated by phencyclidine and ethanol. Alternatively, there may be other mechanisms involved in the discriminative stimulus properties of these two drugs that are unrelated to either $GABA_A$ or NMDA receptors.

As the dose of diazepam was increased, responding shifted from the saline- to the pentobarbital-appropriate key. Benzodiazepines are known to substitute for barbiturates in drug discrimination experiments in pigeons (Herling et al., 1980), so these results were expected. There was no evidence for dizocilpine-like responding after diazepam. Morphine was not expected to produce discriminative stimuli similar to those of pentobarbital, dizocilpine or their combination, so it was employed as a negative control. As anticipated, subjects responded predominantly on the saline-appropriate key after morphine administration. These data suggest that drugs without discriminative stimulus characteristics of pentobarbital, dizocilpine, or a combination of these drugs, produce a ''none of the above'' response (i.e., saline-key responses).

After low doses of dextrorphan, responding occurred predominantly on the saline key, but as the dose increased, 5 subjects switched to the dizocilpine key, and 2 of these later responded on the drug-combination key after the highest dose. One subject responded only on the saline key at all doses up to those that eliminated responding. The substitution of dextrorphan for dizocilpine is consistent with previous reports (Butelman et al., 1991) and it seems likely that these stimulus properties are mediated via noncompetitive blockade of the NMDA receptor since dextrorphan binds to the same site as dizocilpine with moderately high affinity (Sun & Wessinger, 2004). The responding on the drug-combination key after the highest dose of dextrorphan may indicate some weak pentobarbital-like stimulus effects of dextrorphan; partial generalization of pentobarbital to dextrorphan has been reported in two-choice drug discrimination experiments (Herling et al., 1980) which is consistent with this idea. Likewise, the discrimination is symmetrical in that pentobarbital only partially substitutes for dextrorphan in pigeons trained

to discriminate dextrorphan (Herling, Solomon, & Woods, 1983).

After dextromethorphan, 4 of 6 subjects responded on the dizocilpine-appropriate key after the highest dose, while 2 subjects responded primarily on the saline key after all doses. The potency of dextromethorphan in eliciting dizocilpine-appropriate responding in some subjects was somewhat surprising, given its relatively low affinity at the dizocilpine-binding site (Sun & Wessinger, 2004). In mammals, dextromethorphan is rapidly metabolized to its active metabolite, dextrorphan (Barnhart, 1980), which could contribute to the substitution pattern observed; however, its metabolism in pigeons has not been characterized to our knowledge. Both dextrorphan and dextromethorphan appear to have much stronger dizocilpine-like effects and to lack the pentobarbital-like effects that occurred in some subjects following ethanol and phencyclidine.

In the present study, some of the individual differences among subjects in dose-dependent discrimination patterns are difficult to explain. For example, with phencyclidine, all subjects responded on the saline key after the 0.3 and 0.56 mg/kg doses of phencyclidine. At the 1.0 mg/kg dose 2 subjects switched to the pentobarbital key, 2 to the dizocilpine key, and 2 to the drug-combination key. At the highest doses, 5 of 6 subjects ended up responding on the drug-combination key. The effects of the highest dose suggests that phencyclidine has both dizocilpine-like and pentobarbital-like effects, but does not explain why, at an intermediate dose, some subjects appeared to detect pentobarbital-like effects first, others detected the dizocilpine-like effects first and others detected drug-combination effects. Similarly, the lowest dose of ethanol (0.25 g/kg) elicited saline-appropriate responding in all subjects; the highest dose (1 g/kg) elicited drug-combination key responding in 5 of 6 subjects. Yet, intermediate doses resulted in a variety of results with some subjects responding predominately on the pentobarbital key and others selecting the dizocilpine key. We considered the possibility that these differences might be related to the original sensitivity of the individual subjects to the training drugs. For example, we looked to see if the subjects that switched from responding on the saline key to the pentobarbital key at lower doses when pentobarbital was tested alone were the same ones that responded on the pentobarbital key before the dizocilpine key in the pentobarbital–dizocilpine combination experiments; or were the same subjects that responded on the pentobarbital-appropriate key after some doses of phencyclidine and other drugs were administered. However, we could not find consistent substitution patterns to support this hypothesis; thus, the reasons for the individual differences in response patterns at intermediate doses remain obscure.

The four-choice drug-discrimination procedure using a drug combination as one of the choices should be a useful tool for studying the discriminative stimuli produced by other drugs with mixed actions. The availability of a drug-combination key allowed the subjects to choose among pentobarbital-like effects, dizocilpine-like effects, pentobarbital-like plus dizocilpine-like effects, or ''saline-like'' effects, in a way not possible in experiments where fewer choices are provided. When two mechanisms contribute to the discriminative stimulus properties of a drug, procedures with fewer response choices do not permit subjects to choose among these three drug states. The present study showed that such discriminations between two drugs, a combination of the two drugs, and the absence of drug effects, can be established with a high degree of stimulus control albeit following a great deal of effort in training. Subsequently, the procedure can be employed to study the effects of a range of doses of single drugs to determine at each dose whether their discriminative stimulus properties overlap with either or both of the training drugs.

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Received: May 20, 2008
Final Acceptance: June 17, 2009

Table A1

Overall rates of responding (responses/sec) following combinations of pentobarbital and dizocilpine.

	Dizocilpine			
Pentobarbital	0.03	0.056	0.1	0.18
1.0	0.64	0.89	0.36	0.32
3.0	0.23	0.66	0.31	0.21
5.6	0.14	0.29	0.23	0.18
10.0	0.11	0.10	0.10	0.10

Note. The first row shows the dose of dizocilpine and the first column shows the dose of pentobarbital. Doses are mg/kg as the salt. Values are means in responses/sec from single observations in 6 pigeons.

Table A2
Effects of individual drug doses on overall rates of responding (responses/sec).

mg/kg Dose	Pentobarbital	Dizocilpine	Phencyclidine	Ethanol	Diazepam	Morphine	Dextrorphan	Dextromethorphan
0.03		0.78						
0.056		0.34						
0.1		0.14			1.16			
0.18		0.10						
0.3			0.66		0.83			0.95
0.56			0.35					
1.0	0.95		0.27		0.94	1.05	0.97	0.78
1.8			0.13					
3.0	0.51				0.21	0.76	0.58	0.65
5.6	0.26					0.42		0.52
10	0.24					0.27	0.55	0.30
18							0.29	
32							0.12	
250				0.65				
500				0.56				
750				0.42				
1000				0.33				

Note. Each value is a mean of single observations in 6 pigeons.