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Selectivity in generalization to GABAergic drugs in midazolam-trained baboons

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Abstract

When barbiturates have been tested in animals trained to discriminate the intravenous benzodiazepine (Bz) anesthetic midazolam, squirrel monkeys and pigeons did not reliably generalize to barbiturates but rats did. To explore this unexpected phenomenon in another species and to extend the midazolam generalization profile to GABAergic compounds not previously tested, five baboons were trained to discriminate midazolam maleate (0.32 mg/kg iv) from saline under a two-lever procedure. In tests 10 min after dose delivery, the partial agonist imidazenil, the full agonist chlordiazepoxide, and the receptor-subtype-selective hypnotic zolpidem fully shared discriminative effects with midazolam. The barbiturate pentobarbital did so in only one of five baboons, and the intravenous anesthetic propofol failed to do so in the three baboons tested. Testing 1 min after dose delivery shifted midazolam and zolpidem curves to the left and increased generalization to propofol but not pentobarbital. Taken together with previous published data, partial or full agonism at the Bz binding site appears sufficient for midazolam-like discriminative effects in nonhuman primates, pigeons, and rodents, and modulation through the anesthetic site is sufficient in baboons. However, to date, positive modulation of GABA through the barbiturate site is not generally sufficient for this effect in nonhuman primates and pigeons although it is in rodents.

Keywords: Drug discrimination; Benzodiazepines; Anesthetics; Midazolam; Zolpidem; Imidazenil; Chlordiazepoxide; Pentobarbital; Propofol; Baboons; Monkeys

1. Introduction

Midazolam is an imidazo-benzodiazepine that differs from many other classically useful 1,4-benzodiazepines in that it combines high affinity for the benzodiazepine (Bz) binding site with a short duration of action, and the basicity of its molecule makes preparation of water-soluble salts possible (Gerecke, 1983; Garzone and Kroboth, 1989). Peak plasma levels and maximum behavioral effects of midazolam are obtained by the intravenous (iv) route in humans in less than 30 min (Crevoisier et al., 1983; Garzone and Kroboth, 1989). Midazolam's elimination half-life is about 2 h; its main metabolite, 1-hydroxymidazolam, is pharmacologically active but has a shorter elimination half-life than midazolam (Crevat-Pisano et al., 1986). This profile favors midazolam's clinical use for intravenous induction or maintenance of anesthesia.

In drug discrimination studies, midazolam has been one of only two Bz training drugs to show selectivity in its generalization profile with respect to barbiturates; the other is lorazepam. That is, most squirrel monkeys and pigeons trained to discriminate midazolam did not generalize to barbiturates, nor do lorazepam-trained baboons and rats (Spealman, 1985; Evans and Johanson, 1989; Ator and Griffiths, 1989a,b, 1997). However, midazolam-trained rats do generalize to barbiturates (e.g., Garcha et al., 1983; Woudenberg and Slangen, 1989; Ator, 1990, 1999). These results suggest a species difference in midazolam's discriminative effects when it is used as a training drug. This is surprising, because no species differences were found when midazolam served as a test drug in animals trained to discriminate other Bzs (see literature review in Ator, 1999). Although a study that manipulated midazolam training dose in a three-lever discrimination appeared to support functional training dose as the variable that caused apparent "cross-species" differences (Sannerud and Ator, 1995), a later study

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showed that training dose per se was not the critical variable in a two-lever procedure (Ator, 1999).

The present study explored the putative species differences in the midazolam generalization profile in midazolam-trained baboons. Like the Spealman (1985) study in squirrel monkeys, the intravenous route was used, and pentobarbital as well as the classic Bz chlordiazepoxide, were tested. The present study extended the range of drugs tested in midazolam-trained nonhuman primates to include three novel compounds that, like midazolam and pentobarbital potentiate the neurotransmitter γ-aminobutyric acid (GABA) through the $GABA_A$ receptor complex: zolpidem, imidazenil, and propofol. Zolpidem preferentially binds α_1-containing subtypes of the $(GABA)_A$ receptor. It has shared discriminative effects with lorazepam and pentobarbital and, like midazolam and pentobarbital, was reinforcing in baboons (Griffiths et al., 1981, 1992; Ator and Griffiths, 1993; Ator, 2002). Imidazenil is a nonselective benzodiazepine, characterized as a partial agonist (Giusti et al., 1993), but not studied in drug discrimination or self-administration procedures when this study began. Like midazolam and pentobarbital, propofol is clinically useful as an intravenous anesthetic. It increases $GABA_A$ neurotransmission through a site distinct from the barbiturate and Bz sites (Krasowski et al., 1998). Propofol's discriminative effects have not been studied, but subanesthetic doses produced conditioned place preference in animals, were reinforcing in self-administration, and people reported positive effects (see review in Zacny and Galinkin, 1999; Weerts et al., 1999).

2. Method

2.1. Subjects

Five adult male baboons (*Papio hamadryas anubis*) served as subjects. The baboons had histories of intravenous

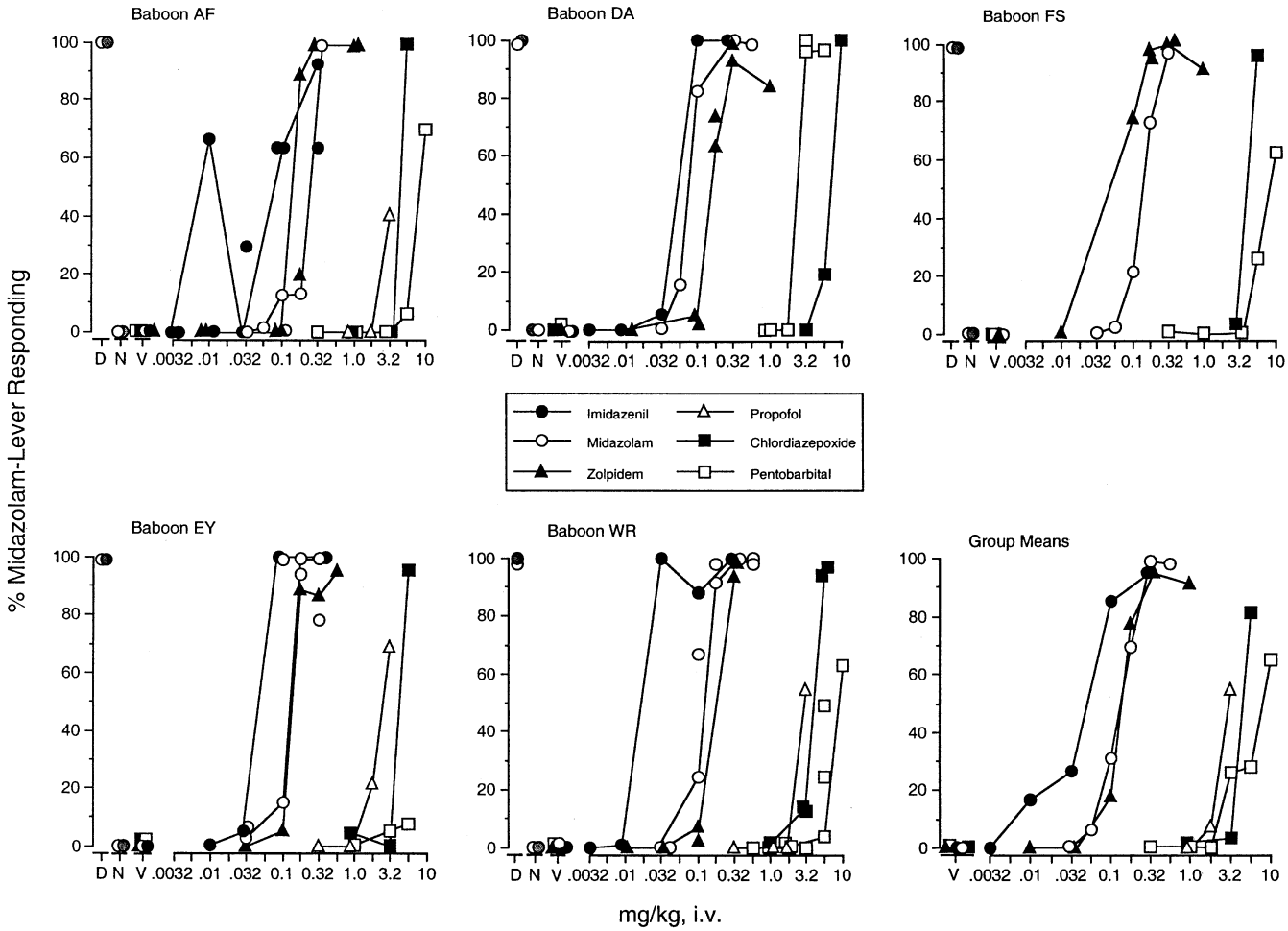

Fig. 1. Percentage of responding on the midazolam-appropriate lever in test sessions preceded by intravenous drug or its vehicle (V) in baboons trained to discriminate midazolam maleate 0.32 mg/kg iv (D). Open points over D represent mean data for all the tests of stimulus control with the midazolam training dose that occurred before study of the dose–effect relation for each drug; shaded points represent the mean of all the control D training sessions during the study. Open and shaded points over N (no drug) represent, respectively, the stimulus control tests with the midazolam vehicle and the control training sessions that were not preceded by any injection. Injections were 10 min before the sessions.

midazolam self-administration and also training to discriminate intravenous midazolam but no testing with other drugs; one (DA) previously served in a study of oral alcohol self-administration. The baboons were housed individually, under a natural light cycle. They had constant access to water, toys, and visual and auditory contact with other baboons. Feeding was between 1100 and 1200 h each day, 1–2 h after the experimental session; it consisted of commercial monkey diet, a multivitamin, and fresh fruit. Silastic catheters had been surgically implanted in a jugular or a femoral vein (Ator, 2000). Ketamine hydrochloride (HCl), preceded by atropine sulfate, was given every 2 weeks to permit weighing, physical examination, and care of the catheter exit site. Body weights were not reduced from those under free-feeding conditions that preceded the study; the chow that supplemented food pellets earned was based on kilocalories needed to maintain initial weights or permit them to increase. Weight ranges were 21–30 kg for baboon FS; 29–37 kg for AF, DA, WR; and 35–41 kg for EY. The experimental protocol was approved by the Johns Hopkins Institutional Animal Care and Use Committee.

2.2. Apparatus

The upper half of the back wall of the home cage was a custom-made stainless steel intelligence panel. Two identical custom-made levers were mounted 15 cm apart in the lower left quadrant; a cue light with a white cap was mounted above each lever. In the panel's center was a recessed hopper into which 1-g banana-flavored pellets (Bio-Serv, Frenchtown, NJ) were delivered. Feeder operation was accompanied by 1-s illumination of the hopper. A white Plexiglas panel was mounted in the upper right quadrant of the panel. A speaker was mounted behind the panel. The baboons were fitted with a vest and tether to protect the catheter. The tether was connected to a stainless steel liquid swivel above which a three-way valve permitted fluid delivery through each port: one for drug, one for vehicle flush, and one for continuous delivery of heparinized (5 units/1000 ml) 0.9% saline. Other details on the catheter system and on control of experimental conditions and data collection are in Lukas et al. (1982) and Ator (2002), respectively.

2.3. Procedure

2.3.1. Design

A single-subject design was used in which each animal serves as his own control and is studied under all conditions (Bordens and Abbott, 1996; Sidman, 1960). Replications within subject demonstrate reliability of a result and replication across subjects its generality.

2.3.2. Training

The baboons had been trained to discriminate midazolam maleate 0.32 mg/kg iv from the no drug (N) condition as in Ator and Griffiths (1993). Sessions were conducted about the same time each morning 5 to 7 days a week; drug (D) and N sessions usually alternated. Before D sessions, the midazolam was injected, followed by vehicle to flush all drug solution into the vein. When the flush was completed, a 10-min time-out began, which preceded a 15-min opportunity to obtain food pellets. White noise was turned on at the beginning of time-out and continued through the session. In time-out, the Plexiglas panel was transilluminated; lever presses were recorded but had no programmed consequences. After time-out, both cue lights over the levers were illuminated; operation of either lever produced a 0.1-s tone. Completing a fixed number of consecutive responses on one of the levers produced a pellet. A response on the inappropriate lever reset the number of responses required on the appropriate lever. Completion of the response requirement turned off the cue lights, operated the feeder and hopper light, and began a 6-s time-out. For baboons EY, FS, and WR, the left lever was paired with pellet delivery in the D condition and the right in the N; the reverse was true for baboons AF and DA. The consecutive response requirement was the same on both levers and ranged, in increments of 5, from 10 to 35 across animals. The value for each baboon was chosen because criterion performance was well maintained at that value. Criterion performance was defined as (1) completing the required consecutive number of responses on the appropriate lever before the first pellet delivery of the session and (2) completing at least 95% of the session's total responses on the appropriate lever.

2.3.3. Testing

Before study of each drug, reliable stimulus control was demonstrated. First four consecutive training sessions (two D and two N) in which performance met criterion had to occur. Second stimulus control by midazolam 0.32 mg/kg

Table 1
ED_{50s} (mg/kg iv) for discriminative and response rate effects of intravenous test drugs delivered 10 min before the session

	Baboons					
	AF	DA	EY	FS	WR	Mean (S.D.)
Discriminative effects						
Midazolam	0.23	0.072	0.13	0.13	0.13	0.14 (0.055)
Imidazenil	0.078	0.056	0.056	NT	0.018	0.052 (0.052)
Zolpidem	0.13	0.13	0.13	0.032	0.18	0.12 (0.054)
Propofol	–	NT	–	NT	–	–
Chlordiazepoxide	4.3	7.8	4.3	4.3	4.3	5.0 (1.56)
Pentobarbital	–	0.23	–	–	–	–
Response rate effects						
Midazolam	–	0.43	–	–	0.43	0.43 (0)
Imidazenil	–	–	–	NT	–	–
Zolpidem	0.56	0.23	–	0.56	–	0.45 (0.19)
Propofol	–	NT	–	NT	–	–
Chlordiazepoxide	–	<3.2	–	–	–	–
Pentobarbital	7.8	7.8	7.8	7.8	7.8	7.8 (0)

Data are shown only when the drug occasioned >80% responding on the midazolam-appropriate lever and reduced rates <50%, respectively.

compared to its vehicle had to be shown in test sessions. Test sessions were identical to training except that a dose of drug or its vehicle preceded the initial time-out, and a food pellet was delivered if the consecutive response requirement was completed on either lever (switching levers still reset the requirement). In the stimulus control tests, the percentage of responses on the D lever had to be 80% after midazolam 0.32 mg/kg and >80% on the N lever after vehicle (see Section 2.3.5 for rationale). These results had to occur in consecutive midazolam and vehicle (or the reverse order) tests. If the percentage was below 80 in either the first or the second test, then D and N training sessions recommenced until four consecutive training sessions with criterion level performance occurred; then the tests were repeated. If the percentage was >80 in the first test, but the baboon obtained pellets from responding on both levers, a D and an N training session at criterion level were required before the next stimulus control test; otherwise the other test session occurred next.

Dose–effect determinations were made with midazolam, pentobarbital, chlordiazepoxide, zolpidem, and imidazenil, in that order, for baboons AF and FS; chlordiazepoxide and zolpidem were reversed for DA; for EY the order was pentobarbital, chlordiazepoxide, imidazenil, midazolam, and zolpidem; for WR, it was imidazenil, pentobarbital, midazolam, chlordiazepoxide, and zolpidem. Propofol was studied last for all baboons. Each test with a novel midazolam dose or novel drug was preceded by at least one criterion-level D and N training session. Failure to show criterion performance in any training session required that it then be shown in four training consecutive training sessions before the next test. Order of D and N sessions was counterbalanced so the type of training session preceding consecutive tests alternated.

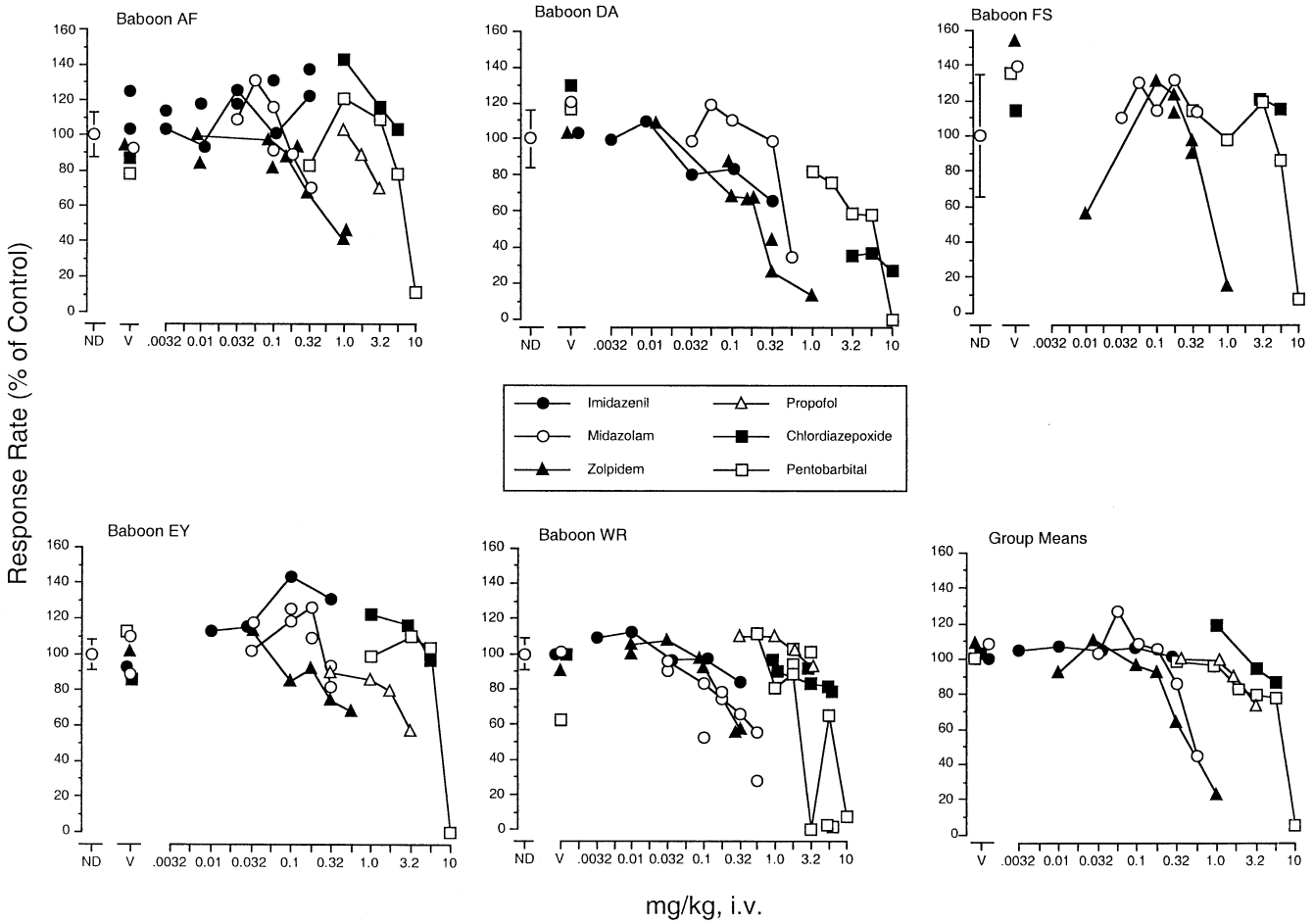

Fig. 2. Response rate as a percentage of the mean rate in control training sessions not preceded by any injection (i.e., no drug, N, sessions). Only the control sessions during the dose–effect determinations for a given drug were used in the calculations for that drug and its vehicle (V). Mean response rates (responses/s) and their S.D.s in N control sessions fell within the following ranges: Baboon AF (1.8–2.1; ±0.1–0.4), DA (1.2–1.7; ±0.1–0.4), EY (1.3–2.8; ±0.1–0.3), FS (1.7–2.2; ±0.4–1.1), WR (2.2–3.5; ±0.1–0.5). Mean responses per second and S.D.s in D control sessions fell within the following ranges: AF (1.3–1.7; ±0.1–0.3), DA (0.62–0.92; ±0.07–0.48), EY (1.3–2.7; ±0.1–0.3), FS (1.8–2.1; ±0.1–0.6), WR (1.7–2.6; ±0.1–0.3). Bars around the 10% N value encompass the grand mean S.D. for all six drugs. The test session data are from the same ones as in Fig. 1.

Drugs first were studied with a 10-min pretreatment interval; some dose–effect curves later were determined when the pretreatment interval was 1 min; propofol also was studied with a 5-min pretreatment interval. The pre-session time-out began right after the injection was completed. The dose range was adjusted for each baboon to encompass at least one low dose that did not occasion >20% D lever responding and, if practicable, at least one high dose that decreased response rates below those in N training sessions. Under the single-subject design, the goal was to study all five baboons with all drugs at all pretreatment times; where they were not, it was because a baboon could no longer be tested via the intravenous route.

2.3.4. Drugs

Doses were calculated as the form given below and prepared within the hour of being administered. Midazolam maleate (Hoffmann-LaRoche, Summit, NJ), chlordiazepoxide HCl, pentobarbital sodium (both from Sigma, St. Louis, MO), and zolpidem tartrate (Research Biochemicals, Natick, MA) were dissolved in 0.9% sterile saline. Imidazenil (Drs. A. Guidotti and E. Costa) was delivered in a vehicle of 20% propylene glycol, 30% polyethylene glycol 200, and 50% 0.9% saline (powder was dissolved in propylene glycol; the other two components were added sequentially). Propofol (as Diprivan, Zeneca Pharmaceuticals, Wilmington, DE) was used as the sterile 10-mg/ml commercial stock in intralipid vehicle. Drugs other than propofol were filter-sterilized (0.22 μM, Millipore, Bedford MA) prior to administration as was imidazenil vehicle. Volume of injection was 5 ml, except imidazenil 0.01 mg/kg and above required 10 ml; chlordiazepoxide above 1.0 mg/kg required 10 ml for the heaviest baboon (EY); and volumes of propofol ranged from 1 to 12 ml depending on total dose. Rate of fluid delivery was 75 to 90 s per 5 ml depending upon the baboon (i.e., 150–180 s for most drug and flush deliveries); propofol and intralipid vehicle flush delivery was 140 to 495 s, depending on dose and baboon weight, to hold rate of delivery constant.

2.3.5. Data analysis

Distribution of responding across levers is reported as percentage on the D lever if a "lever choice" was made, that is, the response requirement was completed at least once. Because 95% accuracy was required in training sessions, 80% can be considered significantly different from chance in a two-choice conditional discrimination (Sidman, 1980). By convention in drug discrimination studies, the discriminative effect of a test dose was not considered qualitatively different from the midazolam training dose if percentage of D-lever responding was >80%; conversely <20% D-lever responding was not seen

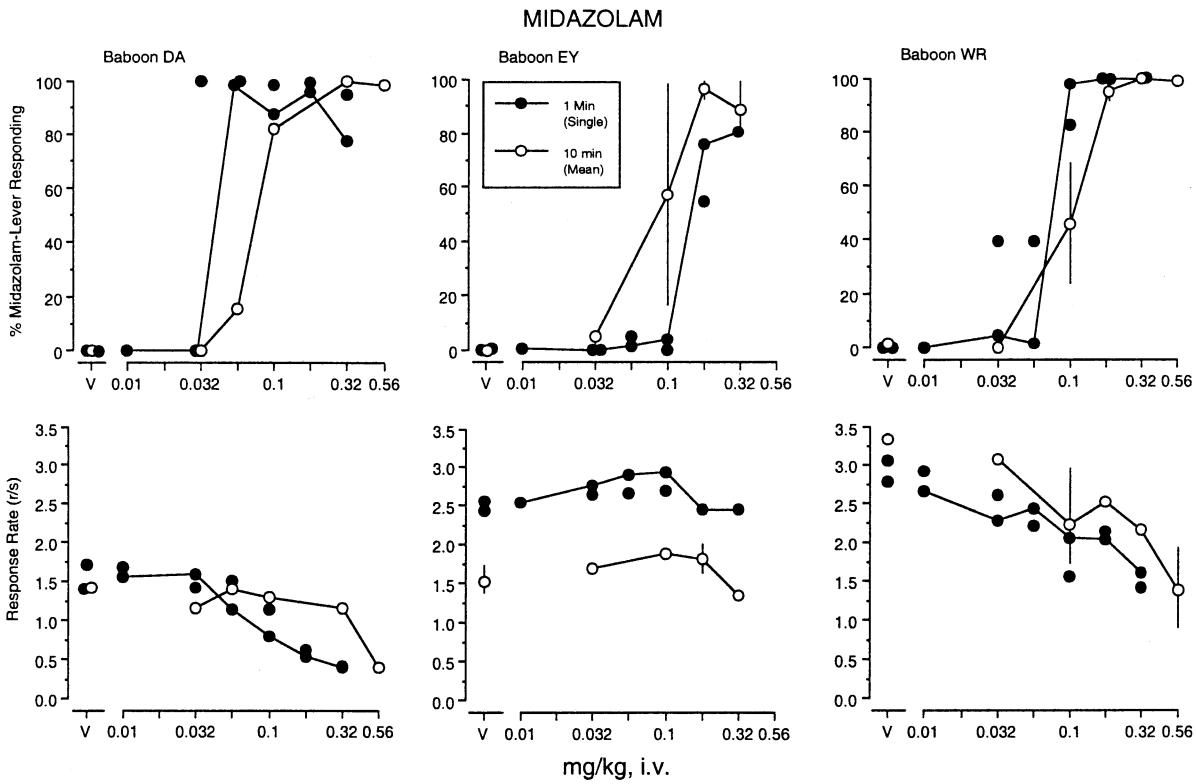

Fig. 3. Percentage of responding on the midazolam-appropriate lever (upper panels) and response rate (lower panels) in test sessions preceded by intravenous midazolam or its vehicle (V). Tests were conducted 1 or 10 min after completion of the injection sequence (i.e., drug followed by saline flush). The control N data fell within the ranges given for Fig. 2.

as different from N. ED_{50}s were determined by interpolation to the nearest quarter log_{10}-unit dose at which a generalization gradient reached 50%. Response rates were calculated by dividing total responses on both levers by session duration, excluding time and responses in time-outs. Rates are given as percentages of the mean rate in the control N sessions during the dose–effect determination for that drug.

3. Results

3.1. Discriminative effects

In control D and N training sessions and in the tests for stimulus control conducted before determination of each dose–effect curve, virtually all responding was on the appropriate lever (Fig. 1).

Tests with midazolam yielded monotonically increasing generalization gradients, which peaked at 100% at 0.1 or 0.32 mg/kg (Fig. 1). For every baboon, some doses above 0.032 mg/kg produced substantial responding on both levers. Even though pellets were received following responding on both levers, performance in the next training sessions met the training criteria in all but one instance (baboon WR made only 91% appropriate responses after the first 0.18 mg/kg midazolam test due to bursts of responses on the incorrect lever between Pellets 11 and 12 and 33 and 34).

Imidazenil, zolpidem, and chlordiazepoxide fully and dose-dependently shared discriminative effects with midazolam in every baboon tested, and some doses occasioned responding on both levers (Fig. 1). Order of potency was imidazenil>midazolam = zolpidem>chlordiazepoxide (ED_{50}s, Table 1).

Although propofol and pentobarbital occasioned dose-dependent increases in midazolam-appropriate responding, neither produced full generalization in most baboons (Fig. 1). Only one fully generalized from midazolam to pentobarbital. The highest percentage of midazolam-appropriate responding after pentobarbital in the other four baboons ranged from 8% to 70% at 5.6 or 10 mg/kg. The highest propofol dose occasioned a maximum of 40% to 69% midazolam-appropriate responding in the three baboons tested (Fig. 1).

Repeating tests with various doses yielded the same conclusion of no (0–19%), partial (20–80%), or full (81–100%) generalization as had the previous test (unconnected symbols, Fig. 1). Including repetitions of tests with midazolam, categorical replication occurred 26 of 33 times. Exceptions typically were at the intermediate doses.

Distributions of responding across levers in tests that yielded partial generalization to imidazenil or zolpidem did not indicate that the intermediate percentages represented

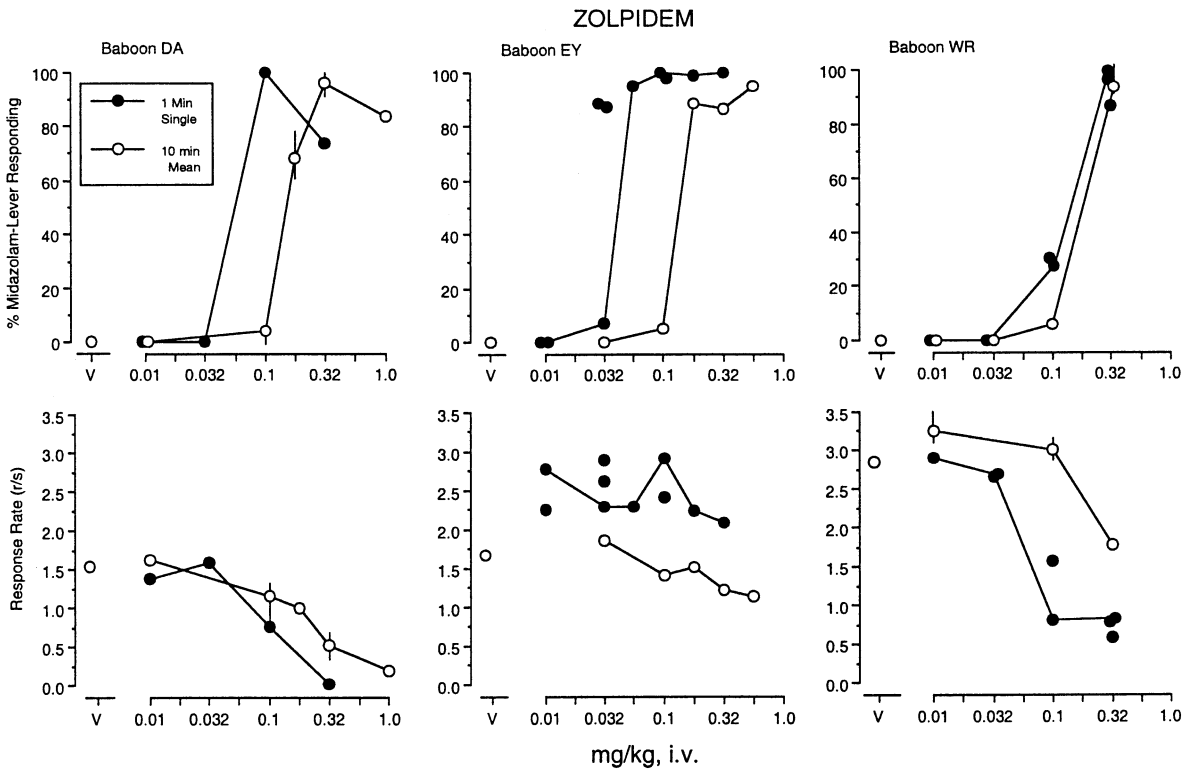

Fig. 4. Percentage of responding on the midazolam-appropriate lever (upper panels) and response rate (lower panels) in test sessions preceded by intravenous zolpidem or its vehicle (V). Tests were conducted 1 or 10 min after completion of the injection sequence (i.e., drug followed by saline flush). The control N data fell within the ranges given for Fig. 2.

onset or offset of the drug effects in the course of the 15-min test sessions; nor did it represent switching back and forth between pellets. Rather, a pattern of obtaining several pellets on one lever before switching to the other predominated. For example, at 0.1 mg/kg imidazenil, baboon AF obtained Pellets 1–11, 17–19, and 27–29 by responding entirely on the N lever and obtained the rest of the 29 total pellets by responding on the D lever. (Responding in the next two training sessions was 100% on the appropriate lever.)

In most pentobarbital tests that yielded partial generalization, response rates were low. The baboons usually were ataxic after 5.6 mg/kg and apparently anesthetized after 10 mg/kg pentobarbital (cf., Table 1). In those instances, responding was distributed across both levers before completing the response requirement for the first pellet, or there was about equal responding on both levers in a context of few pellets being received. Examples are that at 10 mg/kg baboon WR made 98 responses on the D lever and 71 on the N lever before the first pellet; baboon AF received Pellets 1–3 for responding on the D lever and Pellets 4 and 5 (of 5 total) for responding on the N lever.

3.2. Response rates

Midazolam dose-dependently reduced overall response rates below those in control N sessions and below those in saline test sessions for all baboons except FS (Fig. 2). The 0.32 mg/kg training dose itself reduced rates for baboons AF and WR. Imidazenil and chlordiazepoxide did not affect rates except that chlordiazepoxide decreased them for baboon DA, and both drugs increased rates for AF (Fig. 2). Zolpidem and pentobarbital both dose-dependently decreased response rates below the control range and below that after vehicle in all baboons. The highest dose of pentobarbital virtually or completely eliminated responding in every baboon (Fig. 2). The highest propofol dose reduced responding to 60% of control for AF and EY but not at all for WR. The order of potency for the drugs that reduced rates was zolpidem>midazolam>propofol>chlordiazepoxide>pentobarbital (Table 1).

3.3. Shorter pretreatment time

With one exception, generalization gradients for midazolam and zolpidem 1 min after injection were shifted to the left of those 10 min after injection (Figs. 3 and 4). This was true also for propofol; but in addition, gradients determined 1 min after propofol represented full generalization in two baboons (EY, WR) whereas gradients determined 10 min after injection had not for any of those three baboons (Fig. 5). By 5 min after propofol injection, however, full generalization to propofol was lost (Fig. 5). For

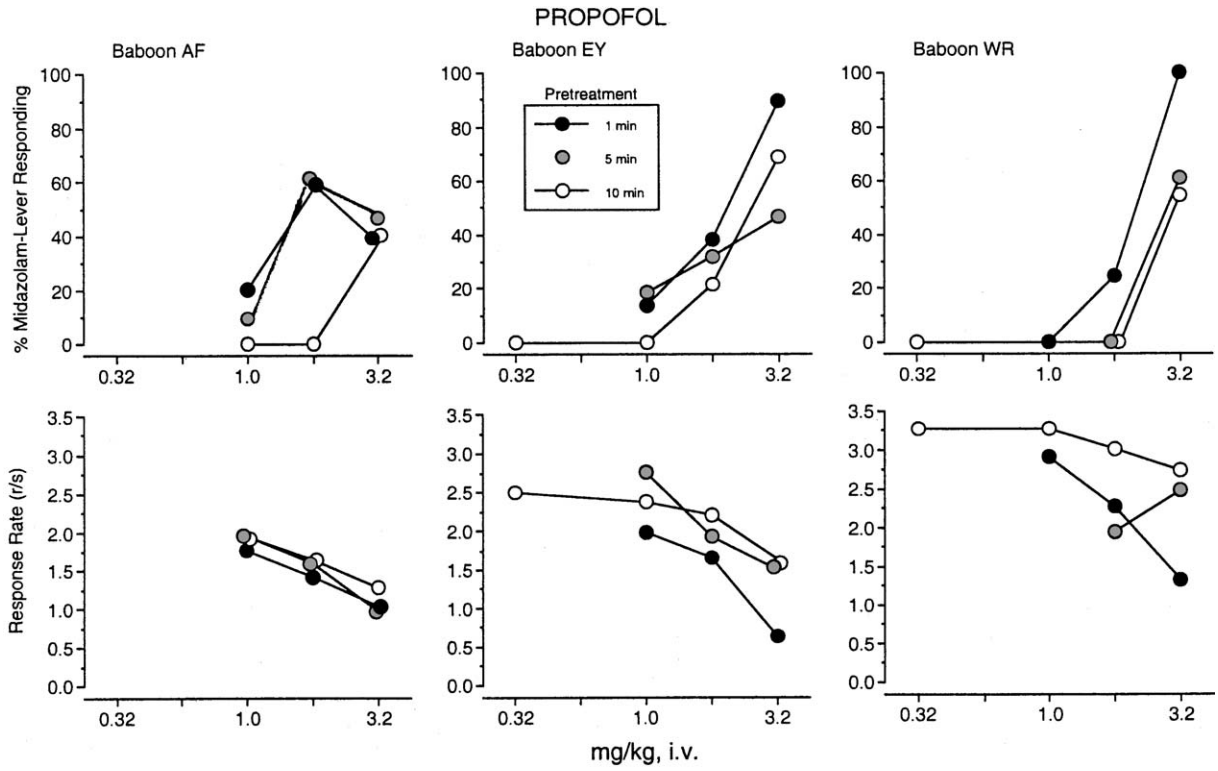

Fig. 5. Percentage of responding on the midazolam-appropriate lever (upper panels) and response rate (lower panels) in test sessions preceded by intravenous propofol or its vehicle (V). Tests were conducted 1, 5, or 10 min after completion of the injection sequence (i.e., drug followed by the intralipid vehicle flush). The control N data fell within the ranges given for Fig. 2.

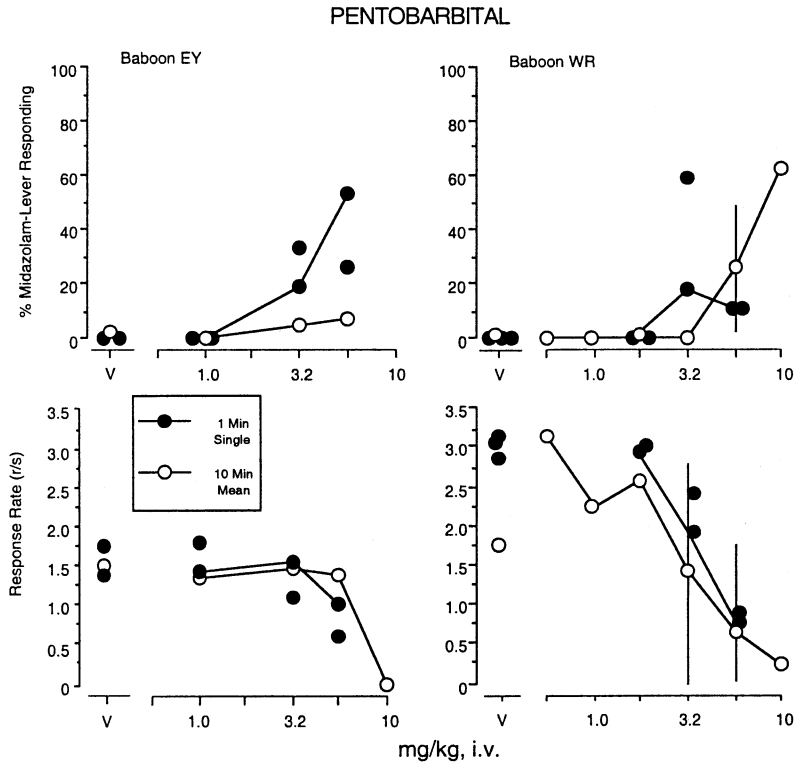

Fig. 6. Percentage of responding on the midazolam-appropriate lever (upper panels) and response rate (lower panels) in test sessions preceded by intravenous pentobarbital or its vehicle (V). Tests were conducted either 1 or 10 min after completion of the injection sequence (i.e., drug followed by saline flush). The control N data fell within the ranges given for Fig. 2.

pentobarbital, testing 1 min after injection produced somewhat more midazolam-appropriate responding, but not reliably more than 50% (Fig. 6). Response rate dose–effect curves for the shorter pretreatment time(s) were either shifted to the left of those for 10 min or were unaffected (Figs. 3–6).

4. Discussion

For baboons trained to discriminate the intravenous Bz anesthetic midazolam after a 10-min pretreatment interval, three test drugs dose-dependently shared midazolam-like discriminative effects when also delivered 10 min after intravenous injection. Those three, ligands for the Bz binding site on the $GABA_A$ receptor complex, are the full agonist chlordiazepoxide, the partial agonist imidazenil, and the selective agonist zolpidem. Two other GABAergic drugs, pentobarbital and propofol, which each act through a distinct non-Bz site on the $GABA_A$ complex, did not fully share discriminative effects with midazolam 10 min after intravenous injection, although there was partial generalization.

The partial generalization produced by propofol and pentobarbital 10 min after dosing could be interpreted as representing an effect qualitatively but not quantitatively similar to that of midazolam 0.32 mg/kg, which carries the implication that under some other condition, a fully midazolam-like effect could be achieved. With a shorter, 1-min pretreatment time, dose–effect functions for discriminative and rate effects shifted to the left for drugs that previously produced full generalization, and propofol produced >90% midazolam-appropriate responding in two of the three baboons tested. This manipulation did not produce generalization to pentobarbital, however.

This effect of pretreatment interval is consistent with intravenous propofol pharmacokinetics. Equilibration of propofol levels between plasma and brain is rapid in humans, as is onset of anesthesia. Plasma levels decline quickly due to rapid distribution and high metabolic clearance (50% of the maximum in 5 min; 25% by 10 min), and there is rapid awakening (*Physicians' Desk Reference*, 2003). In this context, it is notable that testing propofol 5 min after dosing yielded a negative result as had testing with a 10-min interval. Given the positive results for two baboons at 1 min, testing a propofol dose higher than 3.2 mg/kg in the third baboon might also have produced full generalization. To put the tested dose range in context, the recommended intravenous anesthetic induction propofol dose without premedication is 2.5–3.5 mg/kg for children aged 3 to 16, 2.0–2.5 mg/kg for adults, and 1.0–1.5 mg/kg for elderly adults (*Physicians' Desk Reference*, 2003).

Practical considerations (high volume and already lengthy administration duration with the available formulation) precluded testing higher than 3.2 mg/kg in this study, however.

Response rates often are of interest in relation to interpretation of discriminative effects. Midazolam itself dose-dependently reduced response rates, which was true for the training dose in some baboons. However, generalization from midazolam to a test drug was unrelated to the test drug's effect on response rate. Chlordiazepoxide and imidazenil shared discriminative effects with midazolam in every baboon even though no dose reduced response rates. With propofol, intermediate percentages of responding occurred at the highest doses but response rates were over 1.0 response/s. This latter result is of interest in relation to speculation in the drug discrimination literature that intermediate responding may be due to an effect extraneous to a discriminative effect, such as ''intoxication.'' For propofol, however, switching levers 5 or 10 min after dosing occurred when rates were at control levels. When propofol pretreatment time was shortened to 1 min, and response rates were indeed decreased (but still were >0.5 response/s) orderly full generalization was obtained. Thus, intoxication seems an unlikely concept to account for the intermediate responding with propofol. The ''intoxication'' concept may be relevant to the intermediate percentages of responding at the highest pentobarbital doses, however. In those tests, very few pellets were obtained; and control by the reinforcement schedule was disrupted in that baboons switched back and forth across the levers before obtaining individual pellets, which is an unusual pattern when a fixed number of consecutive responses has been required for reinforcement.

Another variable of interest is pharmacological history. Only one baboon generalized completely to pentobarbital, and he had a history of oral alcohol self-administration. A history of intravenous midazolam self-administration increased sensitivity to intravenous midazolam discriminative effects (Ator and Griffiths, 1993). However, in a study of imidazenil with the same baboons that served in the present study, conducted after the present data were collected, imidazenil failed to maintain intravenous self-administration and only partially shared discriminative effects with midazolam when the baboons self-administered their own imidazenil test doses intravenously (Ator, 2002). Self-administration history with respect to drug discrimination has yet to be explored systematically.

In relation to self-administration data described above, midazolam, zolpidem, chlordiazepoxide, propofol, and pentobarbital all served as reinforcers in self-administration procedures in the baboon under intravenous dosing parameters similar to those used in the present study but did not all share discriminative effects with intravenous midazolam; imidazenil did share discriminative effects with midazolam in the present study but did not later reliably serve as a reinforcer. These data further indicate that drug discrimination likely trains a stimulus that is not necessarily isomorphic with the one that subserves drug reinforcement (cf., Ator, 2002).

The present results with the full Bz agonist chlordiazepoxide and the selective Bz site agonist zolpidem in the baboon replicate, respectively, drug discrimination results in midazolam-trained squirrel monkeys and rats in two-lever procedures (Spealman, 1985; Ator, 1999). The partial Bz agonist imidazenil shared discriminative stimulus effects with pentobarbital in rats and rhesus monkeys and partially shared them with lorazepam in rats and baboons (Ator and Kautz, 1997, 2000; Rowlett and Woolverton, 1998; Ator, 2002). Propofol has not previously been tested in drug discrimination.

The present data with pentobarbital also replicate those of Spealman (1985), who found that squirrel monkeys trained to discriminate intravenous midazolam did not generalize to intravenous pentobarbital or barbital. The present results also are similar to those from a study with pigeons trained to discriminate intramuscular midazolam, in which only two of five pigeons generalized to pentobarbital (Evans and Johanson, 1989). A study in midazolam-trained rhesus monkeys did find generalization to pentobarbital (Lelas et al., 1999). However, interpretation of those results in relation to the generalization profile for midazolam is compromised, because the same monkeys previously were trained to discriminate triazolam. Generalization to pentobarbital did occur when they were triazolam trained as it had in triazolam-trained rats (Ator and Griffiths, 1989b; Lelas et al., 1999). The importance of this is that history of training with another drug has been shown to expand the generalization profile for a subsequently trained drug to include all the ones that previously occasioned drug-appropriate responding (Overton et al., 1983; McMillan et al., 1996). Thus, an unequivocal test of whether midazolam-trained macaques would generalize to pentobarbital has yet to be carried out.

In contrast to squirrel monkeys, baboons, and pigeons, rats generalized to pentobarbital under two-lever procedures regardless of rat strain (Ator, 1990, 1999; Garcha et al., 1983; Woudenberg and Slangen, 1989). When a midazolam dose vs. dose vs. vehicle discrimination was trained in rats, full dose-dependent generalization to pentobarbital occurred in relation to the lower (0.32 mg/kg) but not the higher (3.2 mg/kg) training dose (Sannerud and Ator, 1995). Thus, functional training dose seemed to be the basis for earlier species differences. In a follow-up study, rats were trained to discriminate 3.2 mg/kg midazolam under a two-lever procedure, and full generalization to pentobarbital did occur (Ator, 1999). At least for two-lever procedures, the species used continues to be the most prominent variable in accounting for differential generalization to pentobarbital by midazolam-trained animals.

Species differences in pharmacokinetics that could contribute to the differential discriminative effects for pentobar-

bital have been considered, but no basis for these has been found in the literature. Furthermore they would have to occur in the time between dosing and the end of the experimental session for midazolam and/or pentobarbital, which seems unlikely given the half-lives and metabolic pathways of these drugs (Crevat-Pisano et al., 1986). In addition, the use of multiple routes of administration within and/or across species and the multiple experimental time frames used in the studies to date make pharmacokinetic differences an unlikely, but not impossible, source of the differential generalization to pentobarbital in midazolam-trained animals.

Thus, pentobarbital continues to appear to be a qualitatively different stimulus for midazolam-trained nonhuman primates, although other parametric manipulations are warranted. That pentobarbital binds a site on the $GABA_A$ complex that is distinct from those for either propofol or the Bzs remains a clearly relevant mechanism for the lower probability of pentobarbital's sharing discriminative effects with midazolam. The present results also show that neither potentiation of GABA per se nor effectiveness as an intravenous anesthetic is sufficient for a GABAergic compound to share discriminative effects with midazolam in all species.

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References

Ator NA. Drug discrimination and drug stimulus generalization with anxiolytics. Drug Dev Res 1990;20:189–204.

Ator NA. High-dose discrimination training with midazolam: context determines generalization profile. Pharmacol Biochem Behav 1999;64:237–43.

Ator NA. Zaleplon and triazolam: drug discrimination, plasma levels, and self-administration in baboons. Drug Alcohol Dep 2000;61:55–68.

Ator NA. Relation between discriminative and reinforcing effects of midazolam, pentobarbital, chlordiazepoxide, zolpidem, and imidazenil in baboons. Psychopharmacology 2002;163:477–87.

Ator NA, Griffiths RR. Asymmetrical cross-generalization in drug discrimination with lorazepam and pentobarbital training conditions. Drug Dev Res 1989a;16:355–64.

Ator NA, Griffiths RR. Differential generalization to pentobarbital in rats trained to discriminate lorazepam, chlordiazepoxide, diazepam, and triazolam. Psychopharmacology 1989b;98:20–30.

Ator NA, Griffiths RR. Differential sensitivity to midazolam discriminative stimulus effects following self-administered versus response-independent midazolam. Psychopharmacology 1993;110:1–4.

Ator NA, Griffiths RR. Selectivity in the generalization profile in baboons trained to discriminate lorazepam: benzodiazepines, barbiturates and other sedative/anxiolytics. J Pharmacol Exp Ther 1997;282:1442–57.

Ator NA, Kautz MA. Discriminative stimulus and reinforcing effects of the $GABA_A$ partial allosteric modulator imidazenil. Behav Pharm 1997;8:639–40.

Ator NA, Kautz MA. Differentiating benzodiazepine- and barbiturate-like discriminative stimulus effects of lorazepam, diazepam, pentobarbital, imidazenil and zaleplon in two- versus three-lever procedures. Behav Pharm 2000;11:1–14.

Bordens KS, Abbott BB. Research design and methods: a process approach. 3rd ed. Mountain View (CA): Mayfield; 1996.

Crevat-Pisano P, Dragna S, Granthil C, Coassolo P, Cano JP, Francois G. Plasma concentrations and pharmacokinetics of midazolam during anesthesia. J Pharm Pharmacol 1986;38:578–82.

Crevoisier C, Ziegler WH, Eckert M, Heizmann P. Relationship between plasma concentration and effect of midazolam after oral and intravenous administration. Br J Clin Pharmacol 1983;16:51S–61S.

Evans SM, Johanson CE. Discriminative stimulus effects of midazolam in the pigeon. J Pharmacol Exp Ther 1989;248:29–38.

Garcha HS, Rose IC, Stolerman IP. Midazolam cue in rats: generalization tests with anxiolytic and other drugs. Psychopharmacology 1983;87:233–7.

Garzone PD, Kroboth PD. Pharmacokinetics of the newer benzodiazepines. Clin Pharmacokinet 1989;16:337–64.

Gerecke M. Chemical structure and properties of midazolam compared with other benzodiazepines. Br J Clin Pharmacol 1983;16:11S–6S.

Giusti P, Ducic I, Puia G, Arban R, Walser A, Guidotti A, et al. Imidazenil: a new partial positive allosteric modulator of aminobutyric acid (GABA) action at $GABA_A$ receptors. J Pharmacol Exp Ther 1993;266:1018–28.

Griffiths RR, Lukas SE, Bradford LD, Brady JV, Snell JD. Self-injection of barbiturates and benzodiazepines in baboons. Psychopharmacology 1981;75:101–9.

Griffiths RR, Sannerud CA, Ator NA, Brady JV. Zolpidem behavioral pharmacology in baboons: self-injection, discrimination, tolerance and withdrawal. J Pharmacol Exp Ther 1992;260:1199–208.

Krasowski MD, Koltchine VV, Rick CE, Ye Q, Finn SE, Harrison NL. Propofol and other intravenous anesthetics have sites of action on the g-aminobutyric acid type A receptor distinct from that for isoflurane. Mol Pharmacol 1998;53:530–8.

Lelas S, Gerak LR, France CP. Discriminative-stimulus effects of triazolam and midazolam in rhesus monkeys. Behav Pharmacol 1999;10:39–50.

Lukas SE, Griffiths RR, Bradford LD, Brady JV, Daley L, Delorenzo R. A tethering system for intravenous and intragastric drug administration in the baboon. Pharmacol Biochem Behav 1982;17:823–9.

McMillan DE, Sun W-L, Hardwick WC. Effects of drug discrimination history on the generalization of pentobarbital to other drugs. J Pharmacol Exp Ther 1996;278:50–61.

Overton DA, Merkle DA, Hayes ML. Are "no drug" cues discriminated during drug-discrimination training? Anim Learn Behav 1983;11:295–301.

Physicians' Desk Reference. 57th ed. Montvale (NJ): Thomson PDR; 2003.

Rowlett JK, Woolverton WL. Discriminative stimulus effects of benzodiazepine agonists and partial agonists in pentobarbital-trained rhesus monkeys. Behav Pharmacol 1998;9:81–92.

Sannerud CA, Ator NA. Drug discrimination analysis of midazolam under a three-lever procedure: I. Dose-dependent differences in generalization and antagonism. J Pharmacol Exp Ther 1995;272:100–11.

Sidman M. Tactics of scientific research. New York: Basic Books; 1960.

Sidman M. A note on the measurement of conditional discrimination. J Exp Anal Behav 1980;33:285–9.

Spealman RD. Discriminative-stimulus effects of midazolam in squirrel monkeys: comparison with other drugs and antagonism by Ro 15-1788. J Pharmacol Exp Ther 1985;235:456–62.

Weerts EM, Ator NA, Griffiths RR. Comparison of the reinforcing effects of propofol and methohexital in baboons. Drug Alcohol Depend 1999; 57:51–60.

Woudenberg F, Slangen JL. Discriminative stimulus properties of midazolam: comparison with other benzodiazepines. Psychopharmacology 1989;97:466–70.

Zacny JP, Galinkin JL. Psychotropic drugs used in anesthesia practice. Anesthesiology 1999;90:269–88.