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The effects of pyridostigmine bromide, permethrin and DEET alone, or in combination, on fixed-ratio and fixed-interval behavior in male and female rats

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Abstract

Concurrent exposure to pyridostigmine bromide (PB), permethrin (PERM) and/or *N*,*N*-diethyl-*m*-toluamide (DEET) may have contributed to the development of a syndrome that appears to have afflicted military personnel who served during the Gulf War. The present experiment sought to evaluate the behavioral effects of these compounds alone, or in various combinations, in male and female rats. Subjects were exposed to a multiple fixed-ratio (FR) 50, fixed-interval (FI) 2-min schedule of reinforcement. PB dose-dependently decreased FR and FI response rates. FR responding was disrupted by lower doses and there were no differences between the sexes. PERM vehicle administration decreased response rates maintained by both schedules of reinforcement; this was offset by an increase in response rate after the administration of the intermediate dose of PERM. The highest dose of PERM decreased both FR and FI response rates. FR rates in male rats were more disrupted than those in female rats. Only the highest dose of DEET decreased FR and FI response rates in male and female rats. FR rates were more disrupted in female rats than in male rats. Synergistic effects were only observed when FI response rates decreased in male rats upon exposure to half the low dose of PB with half the low dose of PERM or half the low dose of PB with half the low dose of DEET. The results of this experiment thus show that small doses of PB, PERM and DEET disrupt well-established, schedule-controlled behavior in male and female rats in a schedule- and gender-dependent manner; schedule-dependent and gender-dependent synergistic effects were also observed. The mechanism by which the compounds exert these behavioral effects remains to be determined.

Keywords: Gulf War syndrome; Pyridostigmine bromide; Permethrin; *N*,*N*-diethyl-*m*-toluamide; Fixed-ratio schedule; Fixed-interval schedule; Lever press; Male rat; Female rat

1. Introduction

Concurrent exposure to pyridostigmine bromide (PB), permethrin (PERM) and/or *N*,*N*-diethyl-*m*-toluamide (DEET) may have contributed to the development of a syndrome that has afflicted military personnel who served during the Gulf War (Coker, 1996; Haley and Kurt, 1997; Haley et al., 1997; Institute of Medicine, 1996; The Iowa Persian Gulf Study Group, 1997).

PB is a quartenary ammonium compound that inhibits the hydrolysis of acetylcholine (ACh) by competitive reversible binding to acetylcholinesterase (AChE). PB decreases nerve gas toxicity by occupying AChE binding sites (Wolthius and van Wersch, 1984). Reportedly, PB was taken prophylactically during the Gulf War when there was a high risk of nerve gas exposure (Institute of Medicine, 1996). PERM, a synthetic pyrethroid, is a widely used insecticide (Vijverberg and van den Bercken, 1990). In animals, toxic PERM exposure is evidenced by aggressive sparring, hyper-

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sensitivity to external stimuli, whole body tremor and prostration (McDaniel and Moser, 1993). These symptoms are thought to originate in the CNS as they correlate with the concentration of unmetabolized pyrethroid in brain tissue (Gray and Rickard, 1982). During the Gulf War, PERM was used to impregnate uniforms in the field, but the extent of its usage is unknown. DEET is the most commonly used insect repellent in the world (Veltri et al., 1994) that also appears to enhance percutaneous absorption of pharmaceutical agents (Windheuser et al., 1982). Pathological findings indicate that DEET is a demyelinating agent that causes spongiform myelinopathy (Verschoyle et al., 1992). DEET was available during the Gulf War, but used infrequently (as observed in McCain et al., 1997).

Some of the behavioral effects of small doses of PB, PERM and DEET have been documented before. Wolthuis and van Wersch (1984) determined in rats that PB decreased two-way shuttle box avoidance efficiency, decreased open-field locomotor activity and produced a dose-dependent decrease in the number of correct steps in a hurdle-stepping task. In this study, PB's effective dose was determined to be 243 μg/kg, approximately 10% of the LD_{50} following intraperitoneal administration. In other studies, Liu (1991, 1992) and Shih et al. (1991) observed that low doses of PB decreased fixed-ratio (FR) 30 response rates. In these studies, PB's behavioral effects were observed at doses of 3–12 mg/kg following administration by gavage; its LD_{50} was determined to be 80 mg/kg. Recently, we have reported that PB dose-dependently decreased locomotor activity in male and female rats, and more so in female rats (Hoy et al., 1999, 2000a,b). We also showed that acute and repeated PB administration affected learning as it decreased response acquisition with immediate and delayed reinforcers (van Haaren et al., 1999, 2000). Small doses of PERM (up to approximately 20% of the LD_{50} following intraperitoneal administration) dose-dependently decreased responding maintained by various VI (Bloom et al., 1983; Peele and Crofton, 1987) or VR schedules of reinforcement (Stein et al., 1987). PERM did not affect response acquisition in male and female rats (van Haaren et al., 2000). Schoenig et al. (1993) evaluated the neurotoxicity of DEET following acute oral administration or after chronic dietary administration in male and female rats. Acute DEET exposure (0, 50, 200 or 500 mg/kg) did not systematically affect the results of a functional observation battery, nociceptive response time was increased and motor activity was generally unaffected. Dietary DEET administration over two generations (0, 500, 2000 or 5000 ppm), followed by 9 months of continuous administration, only slightly reduced exploratory locomotor activity at the 5000 ppm level.

The behavioral effects of PB, PERM and DEET combinations may exceed the effects of the individual compounds. It has been reported (McCain et al., 1997) that different doses of PB in combination with PERM were more harmful to male laboratory rats than would have been expected had the effects of the compounds merely been additive. Abou-Donia et al. (1996) showed in hens that the behavioral and neurotoxicological effects of combined PB and PERM treatment exceeded those observed after administration of the individual compounds. Other studies have also uncovered evidence to argue that small doses of PB, PERM and DEET, when given in combination, may exert differential behavioral effects in male and female rats. Acute co-administration of PB and DEET or PERM did not result in behavioral effects different from those observed after administration of the individual drugs in female rats, but PB with PERM and DEET with PERM produced significant decreases in locomotor activity in male rats (Hoy et al., 2000b). When subjects were repeatedly injected with these combinations for 7 days and tested 24 h after the final administration, PB and DEET decreased locomotor activity in male and female rats. DEET with PERM, on the other hand, increased locomotor activity in male rats only (Hoy et al., 2000a). Unique neurochemical or physiological interactions between compounds could mediate the behavioral effects of drug combinations. For instance, we have recently shown that serum PERM levels are higher when PB is present in the circulation and more so in female rats than in male rats (Hoy et al., 2000b; van Haaren et al., 2000).

The present experiment was designed to assess the effects of behaviorally active doses of PB, PERM and DEET alone, or in combination, on well-established performance, i.e., responding maintained by a multiple (fixed-ratio 50, fixed-interval 2-min) schedule of reinforcement (MULT (FR 50–FI 2 min)). The doses of the different drugs were selected on the basis of previous reports in the literature (Liu, 1992; McDaniel and Moser, 1993; Peele and Crofton, 1987; Schoenig et al., 1993). It is well known that the behavioral effects of drugs and toxicants not only depend on their chemical properties, but also on the baseline against which they are established (Leander, 1975; Sanger and Blackman, 1976; van Haaren, 1992a,c, 1994). The results of the present experiment will extend our knowledge of the behavioral effects of PB, PERM and DEET and their interactions to include female rats in this schedule-dependent context. Male and female rats participated in this experiment because it has been shown that the behavioral consequences of PB, PERM and DEET administration, just like those of other substances, may be altered by gonadal hormones (Hoy et al., 1999, 2000a,b; van Haaren, 1994; van Haaren and Anderson, 1994a,b; van Haaren et al., 1997).

2. Materials and methods

2.1. Subjects

Twelve male and 24 female Sprague–Dawley rats were obtained from a commercial supplier (Zivic-Miller, Zelie-

nople, PA) when they were approximately 70 days old. They were housed in same-sex groups of three under a reversed light–dark cycle (lights on 6:00 p.m.) and allowed free food and water for 1 week. Access to food was then limited for the remainder of the experiment (16 g/day/male rat and 12 g/day/female rat), while tap water remained continuously available. At the conclusion of the experiments, male rats weighed an average of 440 g (range: 402–478 g) and female rats weighed an average of 303 g (range: 276–338 g). Subjects were tested during their dark hours (between 9:00 a.m. and 3:00 p.m.).

2.2. Apparatus

The experiment was conducted in six identical Coulbourn Instruments (Allentown, PA) modular rodent operant conditioning chambers, which were 25 cm wide, 30 cm long and 29 cm high. The sides of each chamber were made of Plexiglas; the back wall and the intelligence panel were made of stainless steel. The floor consisted of 16 rods, spaced 2 cm apart (center to center). Two retractable rodent levers were located symmetrically to the side of the pellet tray, 6.3 cm from the floor of each chamber. When extended, the levers protruded 1.8 cm from the intelligence panel and required a force of more than 0.20 N to be operated. There were three stimulus lights directly above each lever and a house light was located 3 cm from the ceiling in the middle of the intelligence panel. Noyes 45 mg food pellets were used to consequate appropriate behavior. Each experimental chamber was housed in an individual sound-attenuating, ventilated cabinet. The chambers were connected to an IBM-PC-compatible microcomputer (GatorByte, Gainesville, FL) through a LabLinc interface (Coulbourn Instruments) located in the experimental room itself. Experimental contingencies and data acquisition procedures were programmed in L2T2 (Coulbourn Instruments).

2.3. Procedure

Lever pressing was established according to a procedure that has been described in more detail elsewhere (van Haaren, 1992b). Subjects were then first exposed to a MULT (FR 5, FI 15 s) schedule of reinforcement with a component duration of 2 min, followed by a MULT (FR 10, FI 30 s) schedule with a component duration of 4 min. Thereafter, they were exposed to a MULT (FR 20, FI 45 s) schedule of reinforcement with a component duration of 5 min and a MULT (FR 30, FI 60 s) schedule with a component duration of 7 min. The next to the last experimental baseline schedule consisted of a MULT (FR 40, FI 90 s) schedule with a component duration of 10 min. The different MULT schedules were in effect for 10 sessions. During exposure to the final MULT (FR 50, FI 2 min) schedule, the following conventions remained in place. The component to start the session was randomly determined and the house light was illuminated. When the FI schedule was selected, the left lever was extended into the chamber and the stimulus lights above the lever were illuminated. The fixed-ratio schedule was associated with the right lever. Each component was 10 min in duration and was presented twice during an experimental session. A 30-s blackout period, during which all stimulus lights were extinguished and all contingencies were suspended, separated the two components of the schedule. Experimental sessions were conducted from Monday through Friday. Drug administration was initiated once baseline response rates differed little from session to session (as determined by visual inspection of day-to-day data plots).

2.4. Drug preparation and drug administration

PB was obtained from Sigma Chemical (St. Louis, MO) and dissolved in distilled water. Technical grade PERM ([3-phenoxyphenyl methyl (+)-*cis*,*trans*-3-(2,2-dichloroethenyl)-2,2-dimethylchloro-propanecarboxylate], minimum 35% (±*cis*) and maximum 65% (±) *trans*) was obtained from Coulston Products (Easton, PA; procured via Dr. W. McCain, Aberdeen Proving Grounds, MD) and prepared in a vehicle of equal volumes of Emulphor and 95% ethanol (total volume of 0.2 ml/10 mg PERM). This mixture was diluted with 0.9% physiological saline to the desired concentrations. DEET was obtained from Sigma and administered undiluted. White mineral oil was used as the DEET control. The dose–effect curves for the individual compounds were established first (PB, 0, 3, 10 or 30 mg/kg, by gavage, −30 min; PERM, 0, 15, 30 or 60 mg/kg ip, −15 min and DEET, 0, 50, 200 or 500 mg/kg, by gavage, −30 min). The dose–effect curve for each compound was assessed in different orders across subjects. Following, the behavioral effects of different drug mixtures were assessed (PB 1.5 mg/kg with PERM 7.5 mg/kg and PB 5 mg/kg with PERM 15 mg/kg and PB 1.5 mg/kg with DEET 25 mg/kg and PB 5 mg/kg with DEET 100 mg/kg) and compared to the behavioral effects obtained following the original administration of the individual compounds (PB 3 or 10 mg/kg, PERM 15 or 30 mg/kg and DEET 50 or 200 mg/kg). All drug doses were administered at least twice (once in ascending and once in descending order). Additional determinations were made if there were large discrepancies in the two initial determinations. This strategy is standard practice in our laboratory and is implemented to insure that we obtain the most accurate assessment of the behavioral effect of a drug.

2.5. Estrus cycle determination

Originally, the experiment was designed to test the effects of PB, PERM and DEET in female rats during different parts of the estrus cycle. In that context, cycles were monitored and drugs were administered only when

vaginal smears showed that subjects were either in the pro-estrus or estrus part of their cycle. That strategy was abandoned during the course of the experiment when the data showed that estrus cycle did not interact with the different compounds to alter behavioral effects. From then on, vaginal smears were obtained whenever subjects received vehicle or drug administration to monitor cycle status in the context of drug administration.

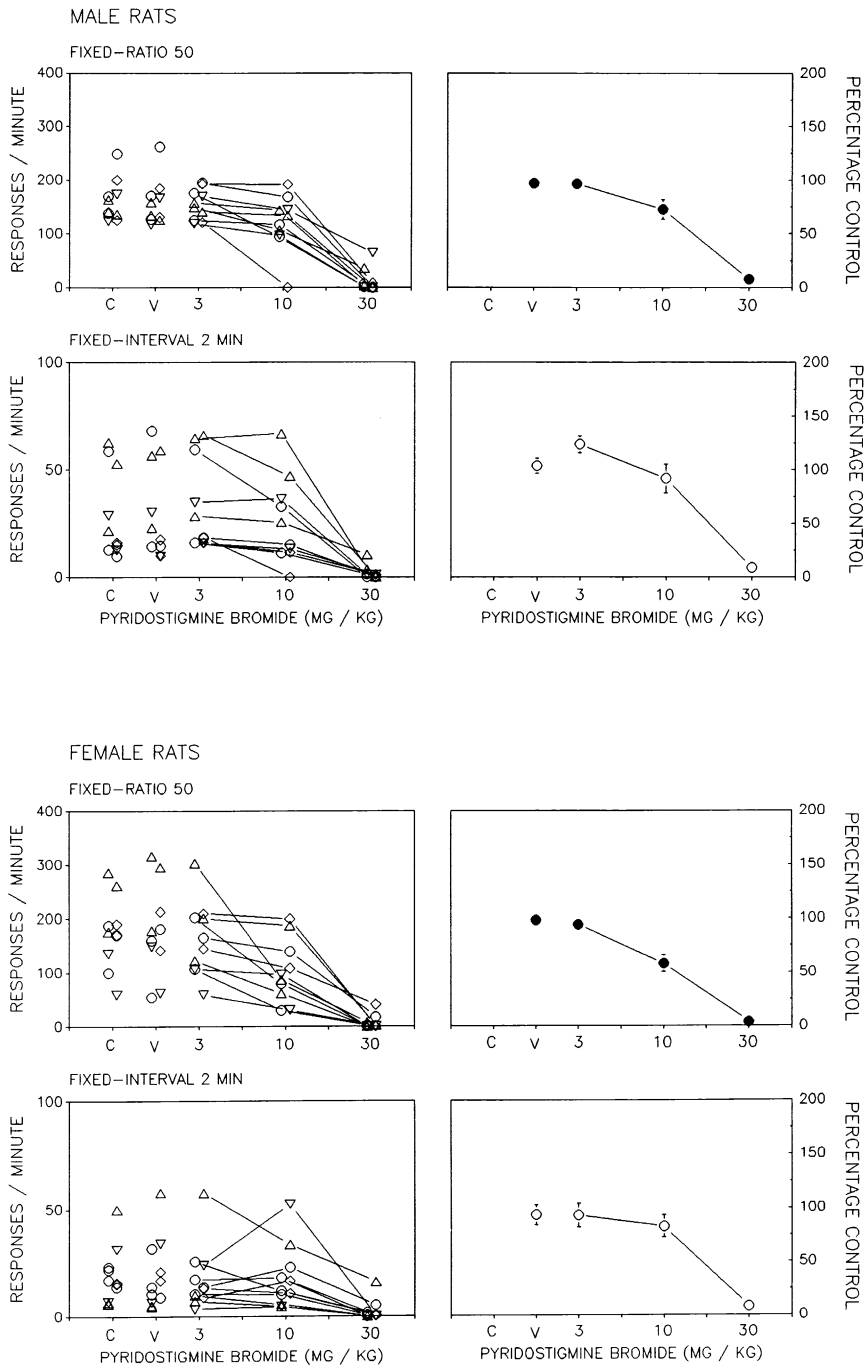

Fig. 1. The effects of different doses of PB (vehicle, 3, 10 or 30 mg/kg) on response rates (responses per minute) maintained by the FR 50 schedule and the FI 2 min schedule in individual male ($n=9$) and female ($n=10$) Sprague–Dawley rats (left hand panels of the figure). Data points plotted above 'C' refer to response rates observed on the days prior to those on which drug was administered (control sessions). These same response rates are plotted as a function of response rates observed during control sessions in the right hand panels of the figure (average ± 1 S.E.M.). In these panels, filled symbols refer to data collected on the FR schedule, open symbols to those collected on the FI schedule.

2.6. Statistical analyses

Analyses of variance including the factors GENDER (male, female) and DOSE (vehicle, low, medium and high) were conducted for each individual drug. When indicated, Duncan's new multiple range tests were used for post-hoc comparisons. Paired *t* tests were used to determine whether the behavioral effects of drug combinations (1/2 Drug 1 + 1/2 Drug 2) differed from half the behavioral effects of the full dose [(Drug 1 + Drug 2)/2].

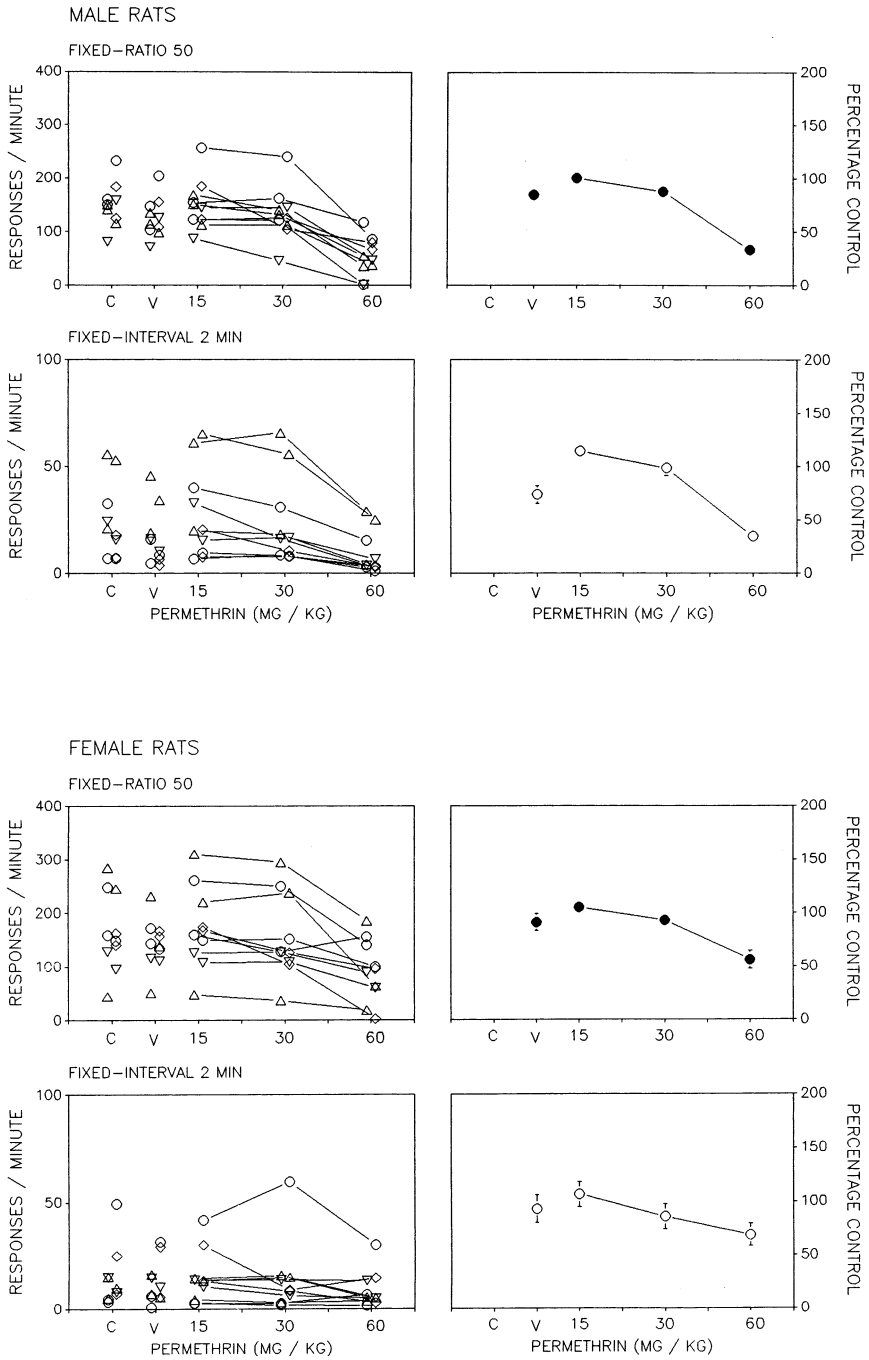

Fig. 2. The effects of different doses of PERM (vehicle, 15, 30 or 60 mg/kg) on response rates (responses per minute) maintained by the FR 50 schedule and the FI 2 min schedule in individual male ($n = 10$) and female ($n = 10$) Sprague–Dawley rats (left hand panels of the figure). Data points plotted above 'C' refer to response rates observed on the days prior to those on which drug was administered (control sessions). These same response rates are plotted as a function of response rates observed during control sessions in the right hand panels of the figure (average ± 1 S.E.M.). In these panels, filled symbols refer to data collected on the FR schedule, open symbols to those collected on the FI schedule.

3. Results

Fig. 1 shows the effects of different doses of PB on response rates maintained by the FR 50 schedule and the FI 2 min schedule in male and female rats. There was considerable attrition in the number of subjects that completed the different dose–effects curves during the course of the experiment. Such probably should not have come as a surprise as it took nearly 18 months to complete the study. Attrition did not appear to be related to any

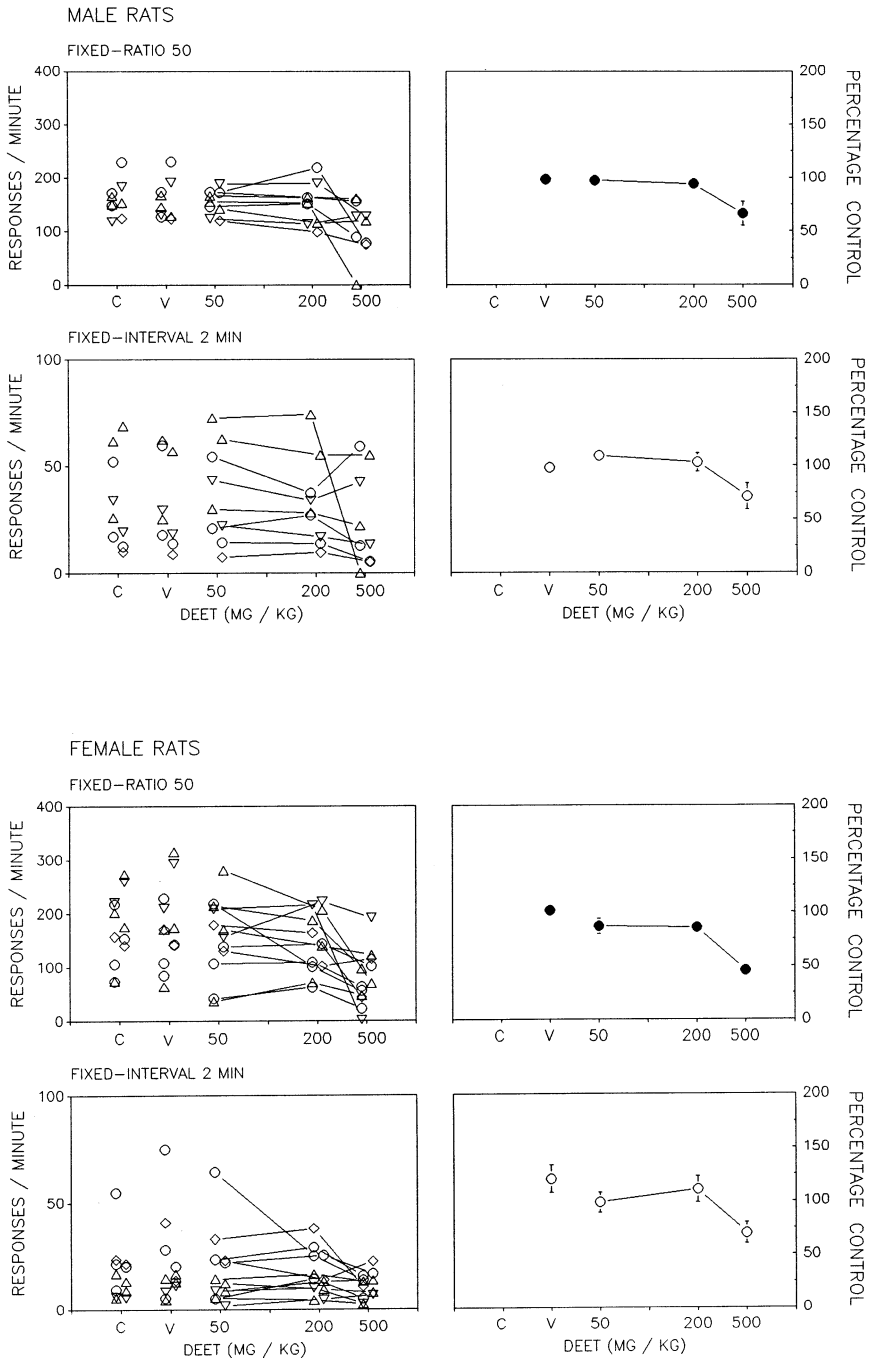

Fig. 3. The effects of different doses of DEET (vehicle, 50, 200 or 500 mg/kg) on response rates (responses per minute) maintained by the FR 50 schedule and the FI 2 min schedule in individual male ($n=9$) and female ($n=12$) Sprague–Dawley rats (left hand panels of the figure). Data points plotted above 'C' refer to response rates observed on the days prior to those on which drug was administered (control sessions). These same response rates are plotted as a function of response rates observed during control sessions in the right hand panels of the figure (average ± 1 S.E.M.). In these panels, filled symbols refer to data collected on the FR schedule, open symbols to those collected on the FI schedule.

experimental treatment. The figures below only include data from subjects who completed the full dose–effect curves. Also, the conclusions that we present are based upon repeated administrations of each dose of each drug. Separately, we determined that these conclusions were consistent with the conclusions that we would have reached had we only analyzed the first administration of each dose of each drug.

The FR 50 schedule maintained much higher response rates than the FI 2 min schedule. In addition, there were considerable differences in baseline response rates between rats exposed to the same schedule of reinforcement. Gender differences in baseline response rates were not observed. To deal effectively with these schedule-related and interindividual differences, absolute response rates were expressed as a percentage of response rates observed during control sessions (right hand panels of the figures). Control sessions were those sessions that took place on the day prior to the day of drug administration. All statistical analyses were conducted on the adjusted data.

FR response rates decreased dose-dependently in male and female rats (DOSE, $F(3,17)=119.55$, $P<.01$; GENDER, $F(1,17)=1.03$, n.s.; DOSE × GENDER, $F(3,17)=0.73$, n.s.). Post-hoc analyses showed that the administration of 10 and 30 mg/kg PB decreased response rates compared to vehicle administration. FI response rates also decreased dose-dependently in male and female rats (DOSE, $F(3,17)=55.47$, $P<.01$; GENDER, $F(1,17)=1.90$, n.s.; DOSE × GENDER, $F(3,17)=1.25$, n.s.). Post-hoc analyses showed that 30 mg/kg PB decreased FI rates

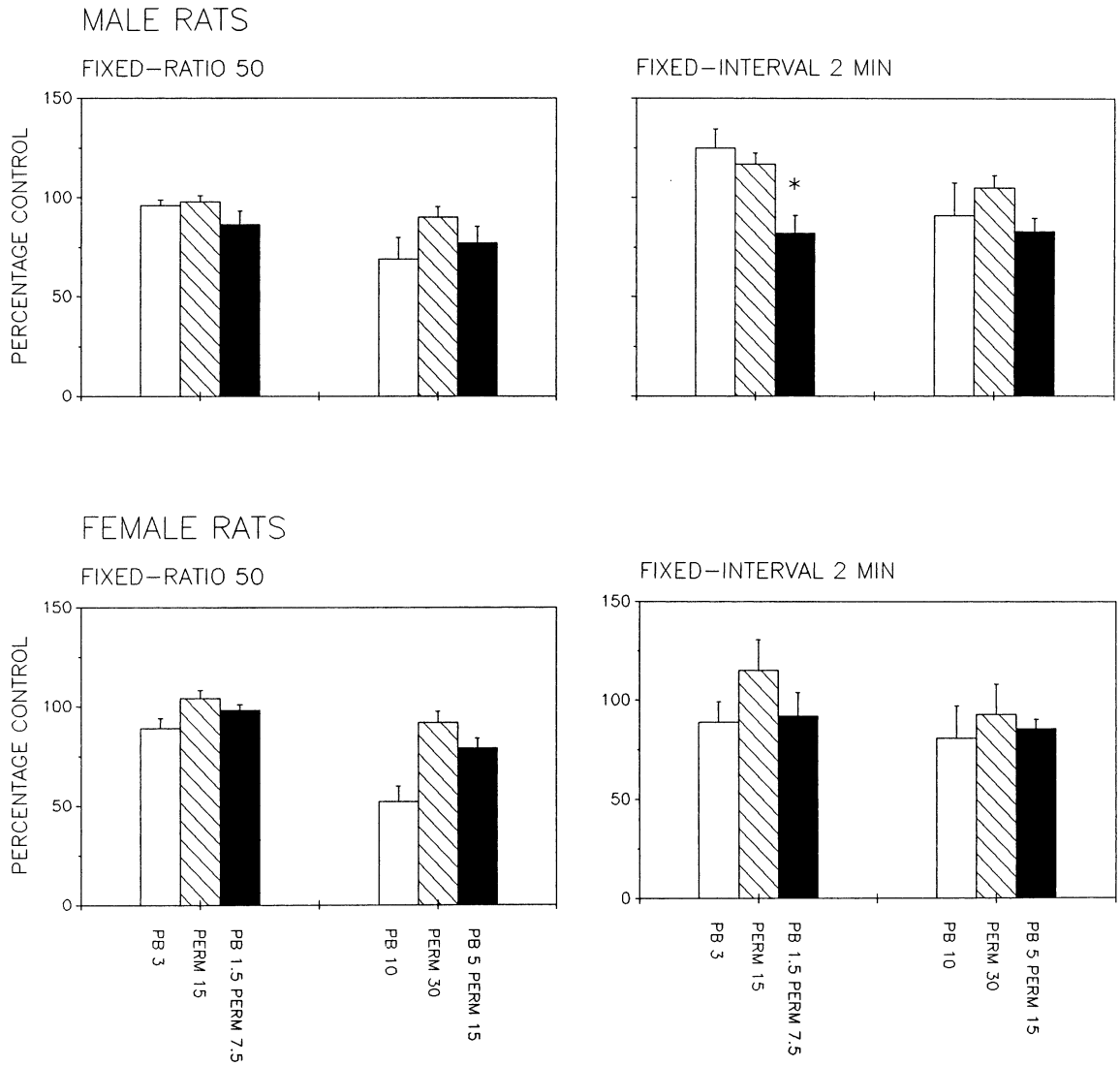

Fig. 4. The effects of PB and PERM alone and in combination on FR response rates (left hand panels) and FI response rates (right hand panels) expressed as a function of control rates in male ($n=8$) and female ($n=7$) Sprague–Dawley rats (average ± 1 S.E.M.). PB and PERM effects are those observed during the initial determination of their dose–effect curves. '*' denotes a significant synergistic decrease in response rate.

compared to vehicle administration. Taken together, these data show that FR responding was disrupted at a lower dose of PB than FI responding and that there were no differences between the sexes.

Fig. 2 shows the behavioral effects of different doses of PERM (vehicle, 15, 30 or 60 mg/kg).

FR response rates were systematically affected by PERM administration (DOSE, $F(3,18)=45.62$, $P<.01$) and differently in male rats than in female rats (GENDER, $F(1,18)=4.51$, $P<.05$). The DOSE × GENDER interaction was not significant ($F(3,18)=1.34$, n.s.). Vehicle administration decreased response rates. Post-hoc analyses revealed significant differences between vehicle rates and those observed after the administration of 15 and 60 mg/kg PERM. Relative to response rates observed after vehicle administration, those observed after 15 mg/kg PERM were higher, whereas those observed after 60 mg/kg PERM were lower. There were no differences between response rates after vehicle administration and the administration of 30 mg/kg PERM. FI response rates were also systematically affected (DOSE, $F(3,18)=12.79$, $P<.01$), but sex differences were not observed (GENDER, $F(1,18)=2.10$, n.s.). There was no significant interaction between DOSE and GENDER ($F(3,18)=2.64$, n.s.). Vehicle administration decreased response rates. There were no differences between FI response rates observed after vehicle administration and administration of 30 mg/kg PERM, but significant differences were observed between response rates after vehicle administration and either 15 or 60 mg/kg PERM. The differences were in the same direction as those observed on FR response rates. In summary then, PERM vehicle administration decreased FR and FI response

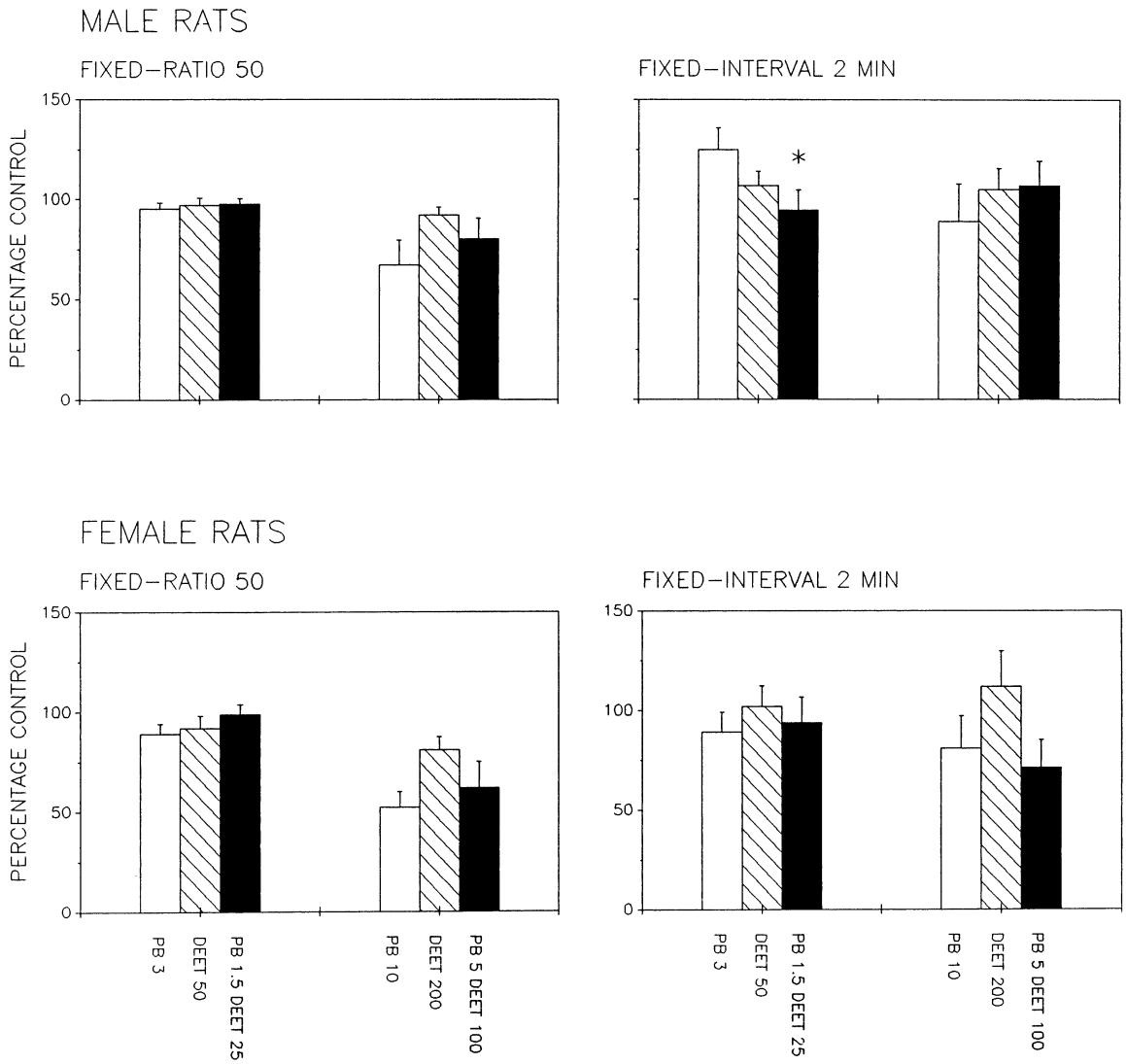

Fig. 5. The effects of PB and DEET alone and in combination on FR response rates (left hand panels) and FI response rates (right hand panels) expressed as a function of control rates in male ($n=7$) and female ($n=7$) Sprague–Dawley rats (average ± 1 S.E.M.). PB and DEET effects are those observed during the initial determination of their dose–effect curves. '*' denotes a significant synergistic decrease in response rate.

rates. The decrease was offset by an increase in rate observed after the administration of 15 mg/kg PERM, while the highest dose of PERM (60 mg/kg) decreased response rates maintained by these schedules of reinforcement.

Fig. 3 shows the behavioral effects of different doses of DEET (vehicle, 50, 200 and 500 mg/kg).

FR response rates decreased dose-dependently after DEET administration (DOSE, $F(3,19) = 20.81$, $P < .01$) and more so in female rats than in male rats (GENDER, $F(1,19) = 5.13$, $P < .05$; DOSE × GENDER, $F(3,19) = 1.30$, n.s.). Post-hoc analyses showed that only 500 mg/kg DEET decreased response rates significantly compared to vehicle administration. FI response rates also decreased after DEET administration (DOSE, $F(3,19) = 6.34$, $P < .01$), but differences between the sexes were not observed (GENDER, $F(1,19) = 0.34$, n.s.; DOSE × GENDER, $F(3,19) = 0.94$, n.s.). FI response rates were significantly lower following 500 mg/kg DEET relative to the behavioral effects of vehicle administration. In summary, only the highest dose of DEET decreased response rates in male and female rats. FR rates decreased more in female rats than in male rats, but sex differences in the behavioral effects of DEET were not observed on the FI schedule.

Figs. 4 and 5 show the effects of PB and PERM alone and in combination (Fig. 4), and of PB and DEET alone, or in combination (Fig. 5), on FR and FI response rates.

We were interested in determining whether the behavioral effect of any of the drug combinations (1/2 Drug 1 + 1/2 Drug 2) differed from half the behavioral effect of the combined individual drug doses, i.e., [(Drug 1 + Drug 2)/2]. Statistical analyses showed that only two of the relevant comparisons were significantly different ($P < .05$). Both involved FI response rates in male rats and revealed a synergetic decrease in response rates after half the low dose of PB with half the low dose of PERM and half the low dose of PB with half the low dose of DEET.

4. Discussion

The present experiment sought to determine the effects of behaviorally active doses of PB, PERM and DEET alone, or in combination, on FR and FI response rates. The results of the experiment confirm and extend those of other studies in a systematic manner.

PB dose-dependently decreased FR and FI response rates. FR rates were disrupted by lower doses than FI rates and there were no differences between the sexes. These data confirm those previously reported by Liu (1991, 1992) and Shih et al. (1991) with respect to the effects of PB on FR response rates. They extend those observations by showing that the response rate-decreasing effects of PB can be schedule-dependent; FR rates were disrupted by lower doses than FI rates. That sex differences were not observed with respect to the effects of PB on schedule-controlled behavior, whereas PB administration sex-dependently disrupted locomotor activity in other experiments (Hoy et al., 1999), merely shows that it is important to evaluate the behavioral effects of a drug under a variety of experimental procedures to appreciate its full behavioral effects.

Some of the behavioral effects of PERM administration were unexpected. PERM vehicle administration decreased response rates maintained by both schedules of reinforcement; this decrease was offset by an increase in response rate following the administration of low dose of PERM. Only the highest dose of PERM significantly decreased FR and FI response rates; FR rates in male rats decreased more than FR rates in female rats. Others have noted that PERM administration decreased VI and VR response rates (Bloom et al., 1983; Peele and Crofton, 1987; Stein et al., 1987). These results thus confirm those previously reported and extend those observations to include different schedules of reinforcement and female subjects. It is interesting to note that there were no schedule-dependent effects as both high and low response rates were equally disrupted by PERM administration. That FR rates were more disrupted in male rats than in female rats should be noted and subject of further evaluation. It is unlikely that differences in PERM bioavailability will account for this finding. We have previously shown that PERM serum levels do not differ between male and female rats following peripheral PERM administration (van Haaren et al., 2000).

Only the highest dose of DEET decreased FR and FI response rates in male and female rats; FR rates were more disrupted in female rats than in male rats. These observations extend those of others as they show that only high doses of acute DEET administration disrupt previously well-established response rates, much as only high doses of DEET decreased locomotor activity (Hoy et al., 2000b; Schoenig et al., 1993).

This experiment was also designed to assess whether or not drug combinations might exert behavioral effects greater than the combined effects of individual doses. In particular, we were interested in assessing the combined effects of PB with PERM and PB with DEET. Much higher doses of these drug combinations had previously been shown to act synergistically in terms of their lethal effect (McCain et al., 1997). In our experiment, only two of the comparisons produced a significant difference in male rats only. Low-dose administration of PB with PERM and PB with DEET decreased FI response rates more than would have been expected on the basis of half the behavioral effects of the full doses. The lack of further synergetic effects of co-administration of these compounds may be related to the observation that in most other cases, administration of single drugs (PB or PERM or DEET) resulted in opposite behavioral effects. These opposite effects cancelled each other out when added to be compared to the behavioral effect of half the combination. Behavioral contingencies may have also played a role as combinations, which synergistically decreased FI response rates, failed to similarly affect behavior maintained by the FR schedule of

reinforcement. Changes in FI response rates, of course, are less likely to affect reinforcement frequency than changes in FR response rates — an observation which may be invoked to partly account for the present findings (cf., Leander, 1975; Sanger and Blackman, 1976; van Haaren, 1992c, 1994).

The results of this experiment thus show that PB, PERM and DEET disrupt well-established, schedule-controlled behavior in male and female rats to varying degrees. Schedule-dependent and gender-dependent synergistic effects were observed when small doses of PERM and DEET were administered in combination with a small dose of PB. We have previously shown that the presence of PB increased PERM serum levels in male and female rats (Hoy et al., 2000b; van Haaren et al., 2000; but see Buchholz et al., 1997 for PERM in brain following PB), whereas DEET serum concentrations decreased (Hoy et al., 2000b). Thus, it appears that PB administration, in addition to having its own behavioral effects, may also alter the behavioral effects of other agents such as PERM and DEET. These results suggest that even very small doses of the compounds that are known to have been used or administered at the time of the Gulf War disrupt performance. It remains to be determined to what extent the disruptive effects of these compounds should be attributed to their central or peripheral actions. Studies in which peripheral cholinergic muscarinic receptors are blocked with the appropriate antagonists should shed further light on these issues (cf., Liu, 1991), as might experiments in which subjects are stressed prior to, or during, drug exposure (cf., Friedman et al., 1996). In addition, we need to point out that soldiers may actually have been exposed to a host of other chemicals and/or stressors that may or may not have affected their health.

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