ELSEVIER

Pharmacology, Biochemistry and Behavior 68 (2001) 81–85

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

The effects of pyridostigmine bromide on progressive ratio performance in male and female rats

Frans van Haaren*, Stephen C. Haworth, Shonnie M. Bennett, Bethany A. Cody

Department of Psychology, University of Florida, Gainesville, FL 32611-2250, USA

Received 24 April 2000; received in revised form 10 August 2000; accepted 4 September 2000

Abstract

Small doses of pyridostigmine bromide (PB) affect the acquisition and maintenance of food-maintained behavior in laboratory animals. The present experiment was designed to investigate the effects of this drug on food motivation. Male and female rats were trained to respond on a progressive-ratio schedule of reinforcement and treated with different doses of PB. PB dose-dependently decreased breaking points and response rates in male and female rats. Gender differences were not observed. The results indicate that decreased food motivation may be a factor that contributes to the behavioral effects of PB administration. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Pyridostigmine bromide; Gulf War; Progressive ratio schedule; Breaking point; Male and female rats

Pyridostigmine bromide (PB) is a quatenary ammonium compound that inhibits the hydrolysis of acetylcholine (ACh) by competitive reversible binding to acetylcholinesterase (AchE). PB may decrease nerve gas toxicity by occupying AChE binding sites (Dirnhuber et al., 1979; Wolthuis and van Wersch, 1984). Reportedly, PB was taken prophylactically during the Gulf War when there was a high risk of nerve gas exposure (Hoy et al., 2000a). PB exposure has been implicated in the development of Gulf War syndrome (Abou-Donia et al., 1996; Coker, 1996; Haley and Kurt, 1997; Haley et al., 1997a; Haley et al., 1997b). It should be noted, however, that soldiers may have been exposed to a host of other chemicals and/or stressors that may or may not have affected their health (Institute of Medicine, 1996; The Iowa Persian Gulf Study Group, 1997).

Previous research has shown that even small doses of PB can have serious behavioral effects. Wolthuis and van Wersch (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 other studies, Liu et al. (Liu, 1991; Liu, 1992; Shih et al., 1991) observed that low doses of PB that did not produce any overt signs of toxicity decreased response rates maintained by a fixed-ratio (FR) schedule of reinforcement. We have recently reported that PB dose-dependently decreased locomotor activity in male and female rats, and more so in female rats (Hoy et al., 1999; Hoy et al., 2000a; Hoy et al., 2000b). We also showed that acute and repeated PB administration affected learning as it impeded response acquisition with immediate and delayed reinforcers (van Haaren et al., 1999; van Haaren et al., 2000a). In yet another study, we observed that PB dose-dependently decreased responding in male and female rats that was maintained by a fixed-interval (FI) 2-min schedule and a FR 50 schedule of reinforcement. Gender differences were not observed in this experiment, but FR rates were decreased by lower doses of PB than FI rates (van Haaren et al., 2000b).

Previous experiments have shown that PB administration decreases locomotor activity in male and female rats (Hoy et al., 1999; Hoy et al., 2000a; Hoy et al., 2000b), which could account for PB's effects on avoidance behavior and hurdle-stepping. It could also account for the effects of PB on food-maintained responding, but other variables may have also played a role. One of the variables that has not yet received experimental attention is the notion that PB administration may alter the reinforcing efficacy of food presentation. This

* Corresponding author. Tel.: +1-352-392-0601 ext. 292; fax: +1-352-392-7985.

E-mail address: haaren@psych.ufl.edu (F. van Haaren).

PII: S0091-3057(00)00438-X

question appears opportune as it has previously been shown that PB administration decreased voluntary water consumption in water-deprived rats without affecting locomotor activity (Liu, 1992). The present experiment was designed to investigate to what extent PB administration affects food motivation as measured with a progressive-ratio (PR) schedule of reinforcement. On PR schedules, the response requirement is systematically increased by (usually) a fixed number of responses after presentation of each reinforcer. The breaking point (in our case, the penultimate ratio that a subject completed before he/she failed to complete the ultimate ratio within a specific period of time) is the major dependent variable of interest. This breaking point can be assumed to reflect motivational variables inasmuch as it has been shown to vary systematically with increases in food deprivation and the volume and concentration of a liquid reinforcer (Hodos, 1961; Hodos and Kalman, 1963). The procedure has previously been used in our laboratories to show that there are no gender differences in food motivation in the absence of drug administration (van Hest et al., 1988). Male and female rats participated in the present experiment because previous studies have shown that the behavioral effects of drug administration (van Haaren, 1994; van Haaren and Anderson, 1994a; van Haaren and Anderson, 1994b; van Haaren et al., 1997), including PB (Hoy et al., 1999; Hoy et al., 2000a; Hoy et al., 2000b), may be a function of the subjects' gender.

1. Method

1.1. Subjects

Twelve male and twelve female Wistar–Hanover rats were obtained from a commercial supplier (Harlan Sprague–Dawley, Indianapolis, IN) 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 per male rat and 12 g/day per female rat), while tap water remained continuously available. Male rats weighed an average of 369 g (range: 331–407 g) and female rats weighed an average of 245 g (range: 230–261 g) at the conclusion of the experiment. Experimental sessions were conducted during the subjects' dark hours between 9:00 a.m. and 3:00 p.m.

1.2. Apparatus

The experiment was conducted in six identical Coulbourn Instruments (Allentown, PA) modular rodent operant-conditioning chambers, that 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. The pellet tray was illuminated by a white light bulb during the delivery of a food pellet (Noyes, 45 mg purified rodent formula). 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).

1.3. Procedure

Lever pressing was first established according to a procedure that has been described in detail elsewhere (van Haaren, 1992). Subjects were then trained to respond on a PR 5 schedule of reinforcement. At the beginning of the session, the left lever was extended into the chamber and the stimulus lights above the lever as well as the house light were illuminated. The subjects were required to complete a PR 5 schedule to obtain the first food pellet. The response requirement was then increased by five responses after every food pellet presentation (i.e. PR 5, PR 10, PR 15, and so on) until the subject failed to complete the scheduled PR requirement within a 5-min period of time. Baseline sessions were conducted until the breaking point reached stability as indicated by visual inspection of day-to-day data plots. Individual stability was deemed to be present when there were no increasing or decreasing trends in breaking points across 10 sessions.

1.4. Drug preparation and drug administration

PB was obtained from Sigma (St. Louis, MO) and dissolved in distilled water. Different doses of PB (vehicle, 3, 10, or 30 mg/kg) were administered by gavage, 30 min prior to the start of an experimental session. These different doses of PB were selected because they had been used in previous experiments (e.g. Hoy et al., 1999; van Haaren et al., 2000b). Drug administration took place on Tuesdays and Fridays of each week, provided that baseline control rates were stable on Mondays and Thursdays. The different doses were administered at least twice (once in ascending and once in descending order). As is customary in our laboratory, additional doses were administered when there was a substantial difference in the behavioral effects of the initial two determinations. Frequently, the behavioral effects of intermediate doses need to be re-evaluated as their effects may change following exposure to higher doses of a drug.

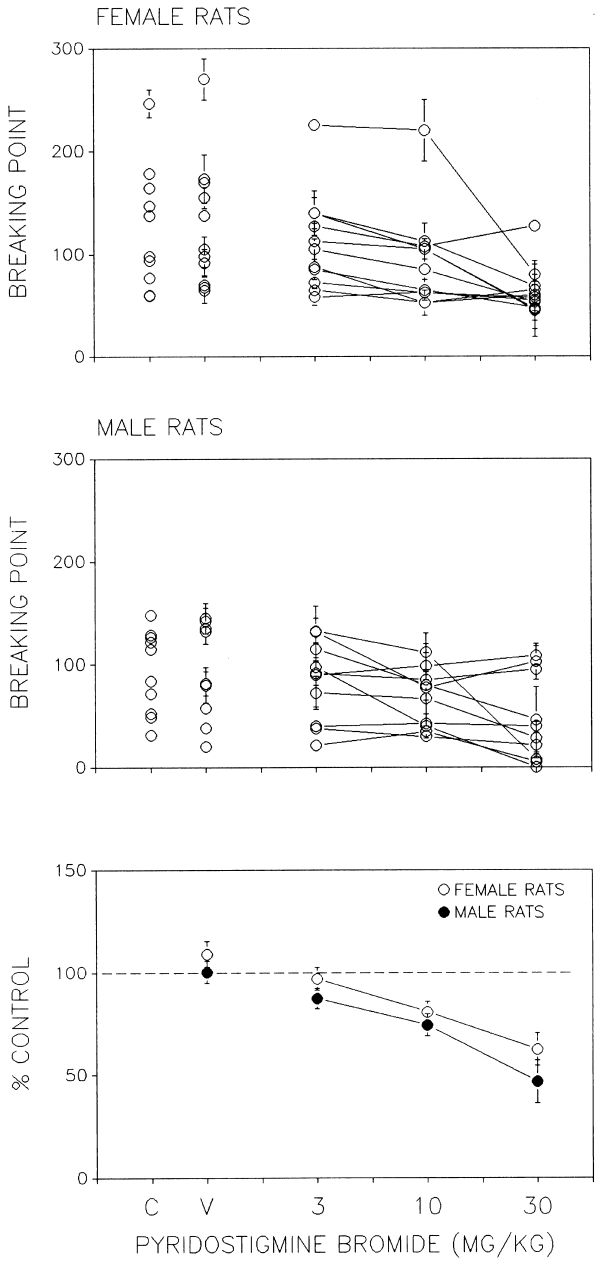

Fig. 1. Breaking points (failure to complete a specific ratio within 5 min, average ± 1 S.E.M.) as a function of the dose of PB in individual female rats (top panel) and individual male rats (middle panel). The data points depicted above 'C' refer to observations collected on the days immediately preceding those on which vehicle or drug was administered (control days). The bottom panel of the figure shows the data from the top and middle panels expressed as a percentage of these control values.

1.5. Statistical analyses

Analysis of variance including the factors Gender (male, female) and Dose (vehicle, 3, 10, or 30 mg/kg) was used to analyze breaking points and response rates. Duncan's new multiple range tests were used for post hoc comparisons. Significance levels were set at $P < .05$.

2. Results

Fig. 1 shows the breaking point as a function of the dose of PB in individual female rats (top panel) and individual male rats (middle panel). The data of one female rat and two male rats are not included because they failed to complete

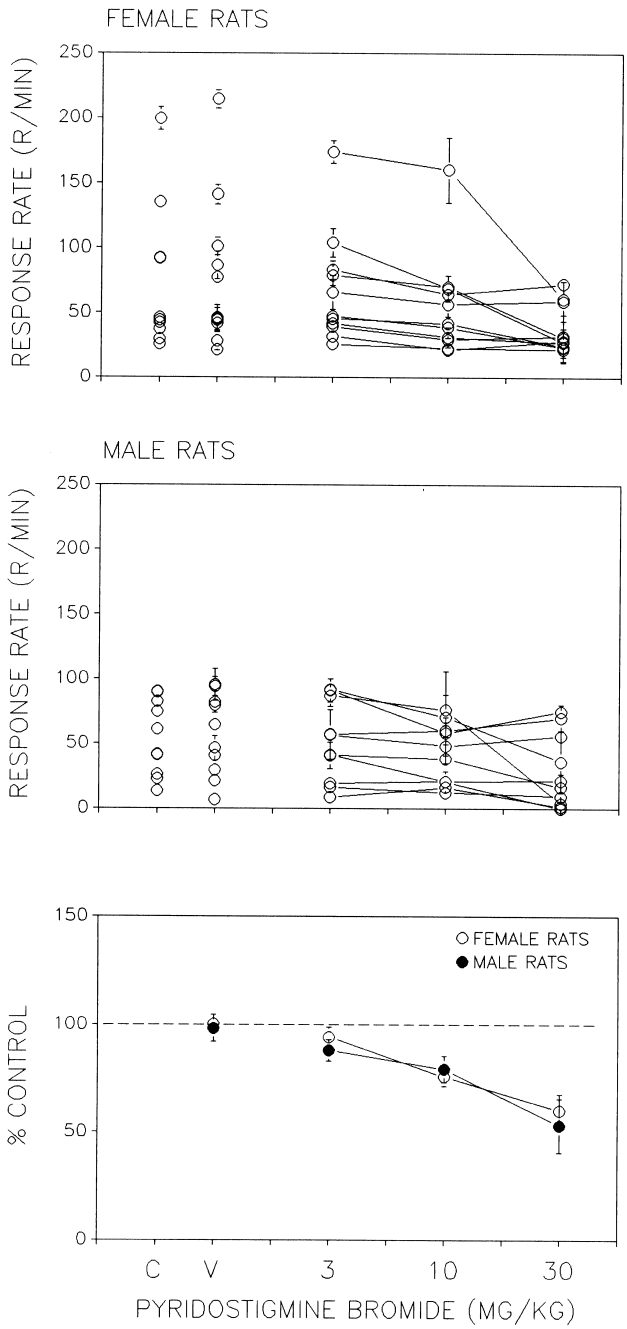

Fig. 2. Response rates (response per minute average ± 1 S.E.M.) as a function of the dose of PB in individual female rats (top panel) and individual male rats (middle panel). The data points depicted above 'C' refer to observations collected on the days immediately preceding those on which vehicle or drug was administered (control days). The bottom panel of the figure shows the data from the top and middle panels expressed as a percentage of these control values.

the assessment of the full dose–effect curve. Breaking points varied considerably within groups of male and female subjects. They were thus expressed as a function of nondrug control values (shown above 'C' in the top and middle panels of the figure) to facilitate comparisons. These data are shown in the bottom panel of the figure. Statistical analyses were performed on these normalized observations. Fig. 1 shows that breaking points decreased as a function of the dose of PB [$F(3,19) = 27.95$, $P < .01$]. Differences between male and female rats were not observed [Gender, $F(1,19) = 2.75$, n.s.] nor was there a significant interaction between drug administration and the gender of the experimental subjects [$F(3,19) = 0.24$, n.s.]. Post hoc analyses, which combined the data for male and female rats across doses, showed that all three doses of PB decreased breaking points as compared to vehicle administration and that the differences between doses were also significant.

Fig. 2 shows response rates (responses per minute) as a function of the dose of PB in individual female rats (top panel) and individual male rats (middle panel). Response rates (which varied considerably within groups of male and female subjects) were also expressed as a function of nondrug control values (shown above 'C' in the top and middle panels of the figure) to facilitate comparisons. These data are shown in the bottom panel of the figure. As before, statistical analyses were performed on these normalized observations. Fig. 2 shows that response rates also decreased as a function of the dose of PB administration [PB, $F(3,19) = 16.94$, $P < .01$]. Differences between male and female rats were not observed [Gender, $F(1,19) = 0.32$, n.s.], nor was there an interaction between PB administration and the gender of the experimental subjects [$F(3,19) = 0.28$, n.s.]. Post hoc analyses, which combined the data for male and female rats across doses, showed that, compared to vehicle administration, response rates were significantly lower after the administration of 10 and 30 mg/kg PB, but not after the administration of 3 mg/kg PB.

3. Discussion

The present experiment was designed to investigate whether or not PB administration would differentially affect responding maintained by a PR 5 schedule of reinforcement. Our results indicate that PB administration dose-dependently decreased breaking points and response rates maintained by a PR 5 schedule of reinforcement in male and female rats. The lowest dose of PB did not decrease response rates, but slightly altered food motivation as reflected by a small decrease in breaking points. Sex differences were not observed.

When drug administration decreases schedule-controlled responding, it can be due to a variety of factors. For instance, drug administration may affect motor activity making it more difficult for the subject to execute the operant response, or drug administration may increase responses incompatible with the operant response (such as locomotor activity). Alternatively, drug administration may alter the palatability of the reinforcer, or drug administration may induce nausea or such to decrease the efficacy of the reinforcer used to maintain the operant response. Or it may be that changes in motivation are a consequence of drug administration. The results of the present experiment suggest that the latter may be the case when subjects are injected with a low dose of PB. Our data indicate that breaking points were decreased by a lower dose of PB than response rates. Thus, it appears reasonable to suggest that PB administration may have altered the reinforcing efficacy of food presentation in experimental subjects that had limited access to food in the home cage. It should be noted, however, that this was a very small effect, which will have to be corroborated in other experiments employing other reinforcers (access to water, reinforcing brain stimulation). Our data argue against explanations that make reference to changes in motor function, such as an increase in the frequency of responses incompatible with the reinforced response. The lowest dose of PB decreased breaking points, but not response rates. This observation precludes an explanation in terms of an increase in incompatible responses, especially in view of the fact that previous studies conducted in our laboratory have indicated that PB administration tends to decrease locomotor activity (Hoy et al., 1999; Hoy et al., 2000a; Hoy et al., 2000b). Previous experiments have shown that PB administration may induce observable signs of cholinergic challenge following doses that exceed 20 mg/kg (Liu, 1992; Shih et al., 1991). In this context, to explain the differential effect of PB's low dose on breaking points and response rates in terms of drug-induced nausea or such appears an untenable position as well. In summary, these data and those of others who measured voluntary water consumption in water-deprived rats following PB administration support a role of PB in affecting motivational variables (cf. Liu, 1992).

Sex differences were not observed in the present experiment. As such these observations confirm those of other studies in which we also showed that PB administration decreases response rates maintained by different schedules of reinforcement, but that it does so equally in both male and female rats (van Haaren et al., 2000b). It should be noted, however, that we observed gender-dependent effects of PB administration when we studied locomotor activity in other experiments (Hoy et al., 1999). Apparently, PB's behavioral effects are different when evaluated against a baseline of spontaneous or unlearned behavior then when evaluated against a baseline of schedule-controlled or learned behavior. These observations again emphasize the importance of evaluating the behavioral effects of a drug under a variety of experimental procedures to appreciate its full range of behavioral effects.

The results of this experiment suggest that even a small dose of a compound that may have been used or administered at the time of the Gulf War can disrupt operant

performance. It remains to be determined to what extent the disruptive effects should be attributed to its central of peripheral actions. Studies in which peripheral cholinergic muscarinic receptors are blocked with the appropriate antagonists should shed further light on these issues (Liu, 1991), as might experiments in which subjects are stressed prior to or during drug exposure (Friedman et al., 1996). Such shall be the charge for investigators who want to further elucidate the behavioral effects of PB in future experiments.

Acknowledgments

This research was supported by a grant from the Department of Defense (DAMD17-96-1-6036, F. van Haaren, PI). It was conducted in accordance with the guidelines described in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication 86-23, revised 1985). The opinions and assertions expressed herein are the views of the authors and are not to be construed as official views of the Department of Defense. The authors thank Laura Tucker for expert assistance with the statistical analyses.

References

Abou-Donia MB, Wilmarth KR, Jensen KF, Oehme FW, Kurt TL. Neurotoxicity resulting from coexposure to pyridostigmine bromide, deet and permethrin: implications of Gulf War chemical exposures. J Toxicol Environ Health 1996;48:35–56.

Coker WJ. A review of Gulf War Illness. J R Navy Med Serv 1996; 82:141–6.

Dirnhuber P, French MC, Green DM, Leadbeater L, Stratton JA. The protection of primates against soman poisoning by pretreatment with pyridostigmine. J Pharm Pharmacol 1979;31:295–9.

Friedman A, Kaufer D, Shemer J, Hendler I, Soreq H, Tur-Kaspa I. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. Nat Med 1996;2:1382–5.

Haley RW, Kurt TL. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. J Am Med Assoc 1997;277:231–7.

Haley RW, Hom J, Roland PS, Bryan WW, Van Ness PC, Bonte FJ, Devous D, Mathews D, Fleckenstein JL, Wians FH, Wolfe GI, Kurt TL. Evaluation of neuralgic function in Gulf War veterans. J Am Med Assoc 1997a;277:223–30.

Haley RW, Kurt TL, Hom J. Is there a Gulf War Syndrome? J Am Med Assoc 1997b;277:215–22.

Hodos W. Progressive ratio as a measure of reward strength. Science 1961;134:943–4.

Hodos W, Kalman G. Effects of increment size and reinforcer volume on progressive ratio performance. J Exp Anal Behav 1963;6:387–92.

Hoy JB, Cody BA, Karlix JL, Schmidt CJ, Tebbett IR, Toffolo S, van Haaren F, Wielbo D. Pyridostigmine bromide alters locomotion and thigmotaxis of rats: gender effects. Pharmacol, Biochem Behav 1999; 63:401–6.

Hoy JB, Cornell JA, Karlix JL, Schmidt CJ, Tebbett IR, van Haaren F. Interactions of pyridostigmine bromide, DEET, and permethrin alter locomotor behavior of rats. Vet Hum Toxicol 2000a;42:65–71.

Hoy JB, Cornell JA, Karlix JL, Tebbett IR, van Haaren F. Repeated coadministrations of pyridostigmine bromide, DEET, and permethrin alter locomotor behavior of rats. Vet Hum Toxicol 2000b;42:72–6.

Institute of Medicine. Health consequences of service during the Persian Gulf War: recommendations for research and information systems Washington DC: National Academy Press, 1996.

Liu W-F. Cholinolytic antagonism to the disruptive effects of oral low doses of pyridostigmine on simple discrimination performance in rats. Pharmacol, Biochem Behav 1991;40:745–9.

Liu W-F. Acute effects of oral low doses of pyridostigmine bromide on simple visual discrimination and unconditioned consummatory acts in rats. Pharmacol, Biochem Behav 1992;41:251–4.

Shih J-H, Liu W-F, Lee SF, Dong Lee J, Ma C, Lin C-H. Acute effects of oral pyridostigmine bromide on conditioned operant performance in rats. Pharmacol, Biochem Behav 1991;38:549–53.

The Iowa Persian Gulf Study Group. Self-reported illness and health status among Gulf War veterans. J Am Med Assoc 1997;277:238–45.

van Haaren F. Response acquisition with fixed and variable resetting delays of reinforcement in male and female Wistar rats. Physiol Behav 1992; 52:767–72.

van Haaren F. The effects of acute and chronic cocaine administration on paced responding in intact and gonadectomized male and female Wistar rats. Pharmacol, Biochem Behav 1994;48:265–73.

van Haaren F, Anderson KG. Behavioral effects of acute and chronic cocaine administration in male and female rats: effects of fixed-ratio schedule parameters. Behav Pharmacol 1994a;5:607–14.

van Haaren F, Anderson KG. Effects of cocaine on fixed-interval behavior and schedule-induced alcohol consumption in male and female rats. Pharmacol, Biochem Behav 1994b;47:997–1002.

van Haaren F, Katon E, Anderson KG. The effects of chlordiazepoxide on low-rate behavior are gender-dependent. Pharmacol, Biochem Behav 1997;58:1037–43.

van Haaren F, de Jongh R, Hoy JB, Karlix JL, Schmidt CJ, Tebbett IR, Wielbo D. The effects of acute and repeated pyridostigmine bromide administration on response acquisition with immediate and delayed reinforcement. Pharmacol, Biochem Behav 1999;62:389–94.

van Haaren F, Cody B, Hoy JB, Karlix JL, Schmidt CJ, Tebbett IR, Wielbo D. The effects of pyridostigmine bromide and permethrin, alone or in combination, on response acquisition in male and female rats. Pharmacol, Biochem Behav 2000;66:739–46.

van Haaren F, Haworth SC, Cody BA, Bennett SM, Hoy JB, Karlix JL, Tebbett IR. The effects of pyridostigmine bromide, permethrin and DEET alone, or in combination, on fixed-ratio and fixed-interval performance in male and female rats, submitted for publication, 2000.

van Hest A, van Haaren F, van de Poll NE. The behavior of male and female Wistar rats pressing a lever for food is not affected by sex differences in food motivation. Behav Brain Res 1988;27:215–21.

Wolthuis OL, van Wersch RAP. Behavioral changes in the rat after low doses of cholinesterase inhibitors. Fundam Appl Toxicol 1984;4: S195–208.