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Effects of Various Drugs Including Organophosphorus Compounds (OPC) and Therapeutic Compounds Against OPC on DRL Responding

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BIZOT, J. C. *Effects of various drugs including organophosphorus compounds (OPC) and therapeutic compounds against OPC on DRL responding.* PHARMACOL BIOCHEM BEHAV **59**(4) 1069–1080, 1998.—The effects of various drugs were assessed in rats responding under a Differential-Reinforcement-of-Low-Rate 30-s (DRL 30-s) schedule. Atropine, scopolamine, and CEB-1957 (a new muscarinic blocker) increased response rate and decreased reinforcement rate, while methylatropine only decreased reinforcement rate. Physostigmine decreased response and reinforcement rates, when pyridostigmine had few effect on DRL responding. The irreversible acetylcholinesterase (AChE) inhibitors organophosphorus compounds (OPC) soman and sarin, injected at one-third of the LD_{50} did not consistently alter DRL performance, suggesting that they produce few behavioral effects in the rat when administered at subtoxic doses. Three oximes—pralidoxime, pyrimidoxime, and HI-6—decreased both response and reinforcement rates. Mecamylamine had few consistent effects on performance, and nicotine, *d*-amphetamine, diazepam, and the wakening drug modafinil increased response rate and decreased reinforcement rate. These two latter drugs also increased the number of very premature responses. These results, taken together, indicate that a DRL schedule is a useful tool to bring to light the existence of psychotropic effects of a drug. The explanation of drug-induced alterations of DRL performance, in terms of effects on cognition or on mood, is also discussed. © 1998 Elsevier Science Inc.

Acetylcholine Acetylcholinesterase inhibitors Muscarinic blockers Soman Sarin Oximes Benzodiazepine Operant behavior Rat

ORGANOPHOSPHORUS compounds (OPC) are irreversible acetylcholinesterase (AChE) inhibitors. They produce excessive cholinergic stimulation in the central nervous system and in peripheral effector muscles and organs that leads to bronchoconstriction, laryngospasm, muscle weakness, convulsion, and death (16,50). The current therapy against OPC is generally a combination of a muscarinic blocker, an oxime that reactivates AChE and an anticonvulsant. A reversible AChE inhibitor could also be used for pretreatment, and the effectiveness of the nicotinic blocker mecamylamine has also been demonstrated (14). Because OPC, as well as some therapeutic compounds against OPC, act on the central nervous system (CNS), they could induce behavioral impairments. Therefore, there is a risk of incapacitation in subjects intoxicated by sign-free doses of OPC or in the event of an untimely self-administration of the therapy. Animal behavior studies would help to better delineate such a risk of incapacitation.

The differential-reinforcement-of-low-rate (DRL) schedule is an operant schedule that requires the subject to pause for a specified minimum period between responses to obtain a reward. Therefore, efficient DRL performance depends on various components of cognition, such as vigilance, short-term memory, waiting ability, and time estimation, and DRL responding could also be sensitive to alterations of motivation or excitation, or to sedation. Thus, it is likely that a great number of psychoactive drugs including current or potential antidepressants (17,28,32), antipsychotics (17,32,33,40), benzodiazepines (37,40,41,48), glutamate antagonists (39,47), or amphetamine-like drugs (36,38,41) alter performance under DRL schedules, while the explanations of their effects may remain uncertain. Because DRL responding seems to be altered by most of the drugs that alter mood or cognition in humans, it can be considered as a poorly specific but highly sensitive tool to predict if a drug that has never been administered to humans may induce psychotropic effects.

The first purpose of this study was to determine the effects on DRL performance of drugs currently or potentially used for the treatment or pretreatment of OPC poisoning. These drugs were two classical muscarinic blockers, atropine and scopolamine, a new muscarinic blocker CEB-1957 (Trouiller, Derrien, Garrigue, and Christin, unpublished results), two reversible AChE inhibitors, pyridostigmine and physostigmine, three oximes, pralidoxime, pyrimidoxime and HI-6, the ganglionic blocking agent mecamylamine, and the anticonvulsant benzodiazepine, diazepam. The second aim of the study was to assess the effects of two OPC at low doses, soman and sarin. For comparison, we also studied the effects of the peripherally acting muscarinic blocker methylatropine, of nicotine, of *d*-amphetamine and of modafinil, a wakening drug (19).

METHOD

Animals

The subjects were 85 experimentally naive male Wistar rats (C.E.R.J, Le Genest, France) weighing 160 g (range: 150–170 g) at the beginning of the experiments and 490 g (range: 410–650 g) at the end. They were maintained at 80–85% of their free-feeding weight by daily feeding with measured rations. They were housed four per cage under standard conditions (12 L:12 D cycle, lights on at 0600 h; room temperature: 21 ± 1°C) with water freely available in the home cage.

Apparatus

Four operant chambers (Campden Instruments Ltd) enclosed in sound-attenuating chambers were used. They were connected to a microcomputer IBM PC XT via an computer interface (Paul Fray Ltd, UK). The experiments were controlled, and data recorded, using a program written under Spider Basic language (Paul Fray, UK).

Training Procedure

A response was a downward force on the right lever of the chamber equivalent to approximately 15 g. Presses on the left lever had no effect throughout the experiment. Rats were subjected to a 30-min session, 5 days per week, during which they were required to make responses to obtain food pellets (45 mg, Campden, UK). After five sessions of acquisition on a fixed-ratio 1 schedule, a DRL 30-s schedule was instituted and maintained during the experiment. On this schedule, only responses occurring at least 30 s after the previous one are reinforced.

The following data were collected after each session: 1) response rate, 2) number of very premature responses = responses occurring less than 6 s after previous one, 3) number of premature responses = responses occurring between 6 and 30 s after previous one, and 4) reinforcement rate.

When performance had stabilized, the drug treatments were initiated.

Drug Testing

Four batches of rats were used for this study. Batch 1 (n = 36) received, in order, atropine (IM, 30 min before testing), scopolamine (IM, 30 min before testing), CEB-1957 (IM, 30 min before testing), methylatropine (IM, 30 min before testing), and soman (SC, 15 min before testing). Batch 2 (n = 13) received, in order, pyridostigmine (SC, 30 min before testing), physostigmine (SC, 10 min before testing), diazepam (IP, 30 min before testing), and modafinil (IP, 30 min before testing). Batch 3 (n = 24) received, in order, nicotine (IP, 15 min before testing), mecamylamine (SC, 15 min before testing), *d*-amphetamine (IP, 30 min before testing), and sarin (SC, 15 min before testing). Batch 4 (n = 12) received, in order, pralidoxime (IM, 15 min before testing), pyrimidoxime (IM, 15 min before testing), and HI-6 (IM, 15 min before testing). The doses used are indicated in the result section. At least 1 week elapsed between testing two doses of the same drug on the same animals, and at least 2 weeks elapsed between testing two different drugs. The animals were subjected to at least two training-session (without injection) under the DRL 30-s schedule between two drug studies.

Drug studies were conducted only on well-performing rats, defined as animals that obtained at least 10 reinforcements during the four training sessions preceding the test sessions. For each dose of each drug (except OPC), a drug study was performed over two consecutive daily sessions according to a crossover design. At each drug study, one dose of a given drug (batches 2 an 4), or two or three doses of a given drug (batches 1 and 3) were studied. For each drug study conducted on batches 1 and 3, the well-performing rats were randomly assigned to two or three groups on which two or three doses of the drug were tested. All the animals of one group received the same dose of the drug studied. Each animal of the batches 1 and 3 received four different doses of the same drug at the most. For each drug study conducted on batches 2 and 4 the well-performing rats were included in one group on which one dose of the drug studied was tested. Each animal of the batches 2 and 4 received six different doses of the same drug at the most. A group of animals was subdivided in two subgroups, matched according to their performance recorded during training. One subgroup of rats was given the drug under study at the same dosage before the first session (drug session) and the vehicle before the second session (control session); rats of the other subgroup received the vehicle and the drug in the reverse order. Thus, the performance of a rat during a control session was used as the control for the adjacent drug session.

For soman and sarin testing, animals were assigned to two subgroups, matched according to their performance. One subgroup of rats was given the OPC under study before the test session; rats of the other subgroup received saline. Performance was examined 15 min after treatment and during the five subsequent days.

Atropine sulfate, scopolamine hydrobromide, atropine methyl bromide, pyridostigmine, physostigmine, nicotine, and mecamylamine were obtained from Sigma, France. (S)-[3-(*N,N*-Dimethylamino) prop-1-yl] cyclohexyl (3-thienyl) glycolate (CEB-1957), 1-(methyl-imidazolium)-3(4-carbaldoxime-pyridinium)propane dibromide (pyrimidoxime), *d*-amphetamine, pinacolyl methylphosphonofluoridate (soman; purity >97%), and isopropyl methylphosphonofluoridate (sarin, purity >97%) were synthesized, in the Chemistry Department of the Centre d'Etudes du Bouchet. Soman was diluted at a concentration of 4 mg/ml in propanol 2, and sarin was diluted at a concentration of 4 mg/ml in methyl-ethyl-cetone. Soman and sarin were stored at −30°C. Pralidoxime methyl sulfate (contrathion®) was purchased from S.E.R.B., France. 4-carbamoyl-2′-hydroximinoethyl-1-1′-oxidimethylendi(pyridinium) chloride (HI-6) was generously made available by Dr. J. G. Clement, Defence Research Establishment Suffield, Canada. Modafinil (Modiodal®) was generously made available by Dr. Laurent, Laboratoires L. LAFON, Maisons-Alfort, France. Diazepam base was generously made available by Dr. Haefely, Hoffmann–La Roche, Switzerland. All the drugs except diazepam were dis-

solved in saline (NaCl 0.9%). Diazepam was suspended in acacia gum and saline. Drugs or vehicle were administered either intramuscularly (IM) in the hind leg, subcutaneously (SC), or intraperitoneally (IP), in a volume of 1 ml/kg of body weight.

Statistics

For each treatment except soman and sarin, the overall effects of the drug on response and reinforcement rates, on the number of very premature responses and on the number of premature responses were analyzed by a two-way ANOVA using repeated measure (drug session vs. control session) and doses as factors. In case of a drug producing a significant effect (at least $p \leqslant 0.05$ for repeated measure and/or for the interaction dose $\times$ repeated measure), the effects of each dose were analyzed by a paired Student's *t*-test. For soman and sarin, comparisons between treated and control groups were made using a two-tailed Student's *t*-test.

RESULTS

Control Performance

More than 85% of the rats exhibited good performances (i.e., at least 10 reinforcements/session) after 12 weeks of DRL 30-s schedule. Performance during control sessions varied widely between subjects (response rate: mean = 67.6, range = 39–125; very premature responses: mean = 8.6, range = 0–55; premature responses: mean = 30.1, range = 4–78; reinforcement rate: mean = 28.9, range = 9–46).

Muscarinic Blockers

Table 1 gives results of the ANOVA for overall drug effects. Atropine increased the number of premature responses and decreased the reinforcement rate. Post hoc comparisons indicated that the doses of 0.25, 0.5, 1, and 4 mg/kg of atropine increased the number of premature responses, and that the doses from 0.25 to 4 mg/kg decreased the reinforcement rate (see Fig. 1A). Scopolamine increased the response rate and the number of premature responses and decreased the reinforcement rate. These effects were significant with the doses from 0.015 to 0.12 mg/kg (see Fig. 1B). Scopolamine also increased the number of very premature responses, but post hoc comparisons did not reveal a statistically significant effect on this parameter at any dose tested. CEB-1957 increased the response rate, the number of very premature responses and the number of premature responses and decreased the reinforcement rate. As indicated in Fig. 1C, the increase in reinforcement rate and in the number of premature responses and the decrease in response rate were significant with doses from 0.25 to 2 mg/kg. Only 2 mg/kg of CEB-1957 significantly increased the number of very premature responses. The only significant effect of methylatropine was a decrease in reinforcement rate. This effect reached a significant level at the dose of 2 mg/kg (see Fig. 2A).

Reversible and Irreversible AChE Inhibitors

The results of ANOVA for overall effects of physostigmine and pyridostigmine are summarized in Table 2. Physostigmine decreased both response and reinforcement rates and the number of premature responses. Post hoc comparisons revealed that 0.12 mg/kg of physostigmine decreased the reinforcement rate, and that the 0.25 mg/kg dose decreased the response rate and the number of premature responses (see Fig. 2B). Pyridostigmine (0.25–2 mg/kg) significantly altered the reinforcement rate, but post hoc comparisons did not reveal any significant difference with any dose tested (data not shown).

TABLE 1

STATISTICAL ANALYSIS OF THE EFFECTS OF MUSCARINIC BLOCKERS: OVERVIEW OF *F* RATIOS

	Repeated Measures			Dose × Repeated Measure		
Drug	*df*	*F* ratio	*p*	*df*	*F* ratio	*p*
Atropine						
Response rate	1,67	0.678	NS	6,67	1.339	NS
Very premature responses		1.524	NS		0.748	NS
Premature responses		21.511	<0.0001		1.574	NS
Reinforcement rate		116.791	<0.0001		9.151	<0.0001
Scopolamine						
Response rate	1,60	34.249	<0.0001	5,60	5.234	0.0005
Very premature responses		4.528	0.038		1.547	NS
Premature responses		48.321	<0.0001		5.700	0.0002
Reinforcement rate		28.119	<0.0001		4.217	0.0024
CEB-1957						
Response rate	1,39	74.112	<0.0001	4,39	15.722	<0.0001
Very premature responses		10.880	0.0021		5.435	0.0014
Premature responses		66.136	<0.0001		7.391	0.0002
Reinforcement rate		47.249	<0.0001		3.427	0.017
Methyl-atropine						
Response rate	1,15	1.441	NS	1,15	0.936	NS
Very premature responses		0.131	NS		0.435	NS
Premature responses		0.243	NS		0.253	NS
Reinforcement rate		26.496	0.0001		0.972	NS

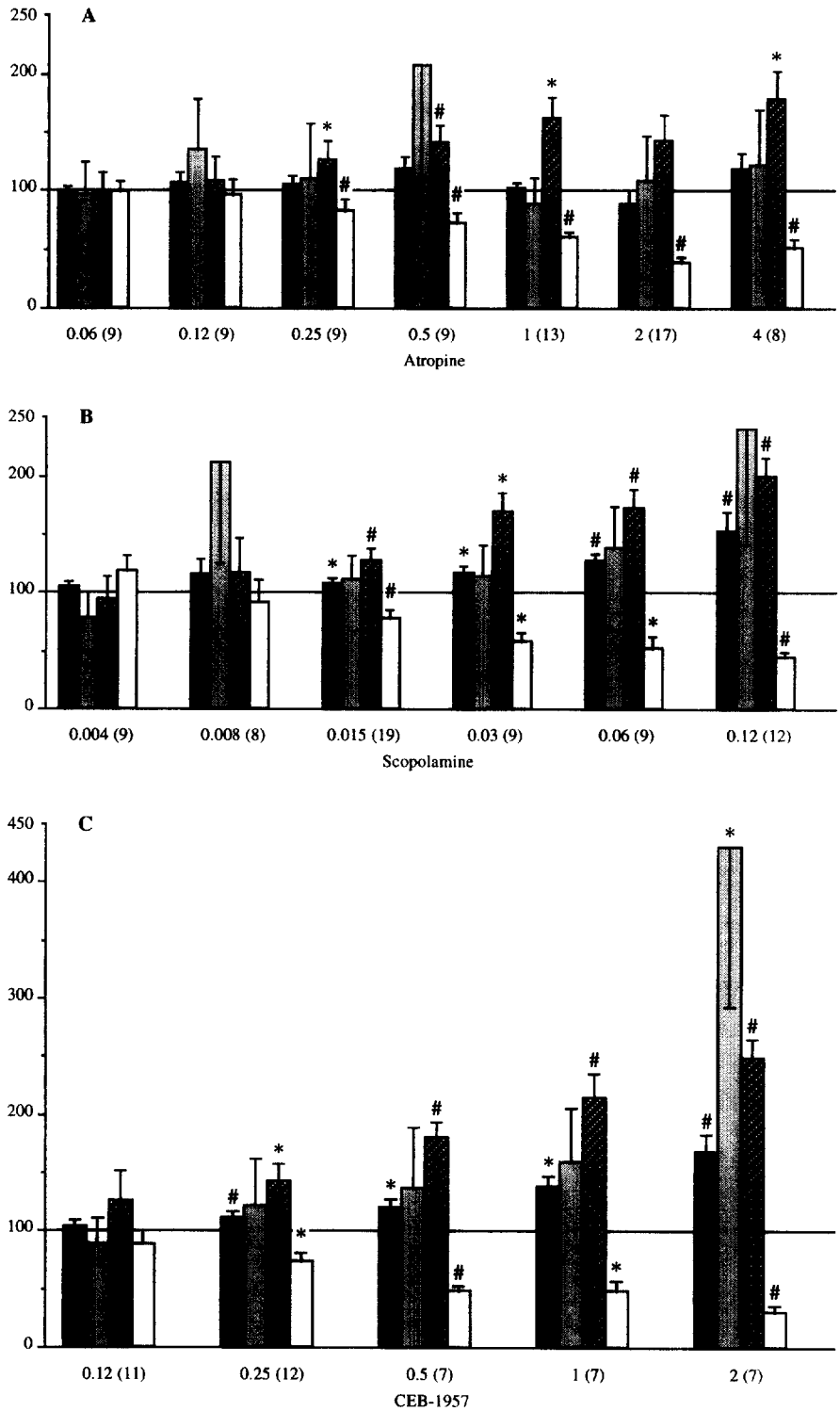

FIG. 1. Effect of atropine (A), scopolamine (B) and CEB-1957 (C) on DRL responding. The doses are expressed in mg/kg. The number of animals used for each dose studied is indicated in parentheses. Values (mean ± SEM) are expressed in percent of performance on control session. Solid bars: response rate; gray bars: number of very premature responses; striped bars: number of premature responses; open bars: reinforcement rate. Significantly different from control performance, $*p < 0.05$, $\#p < 0.01$.

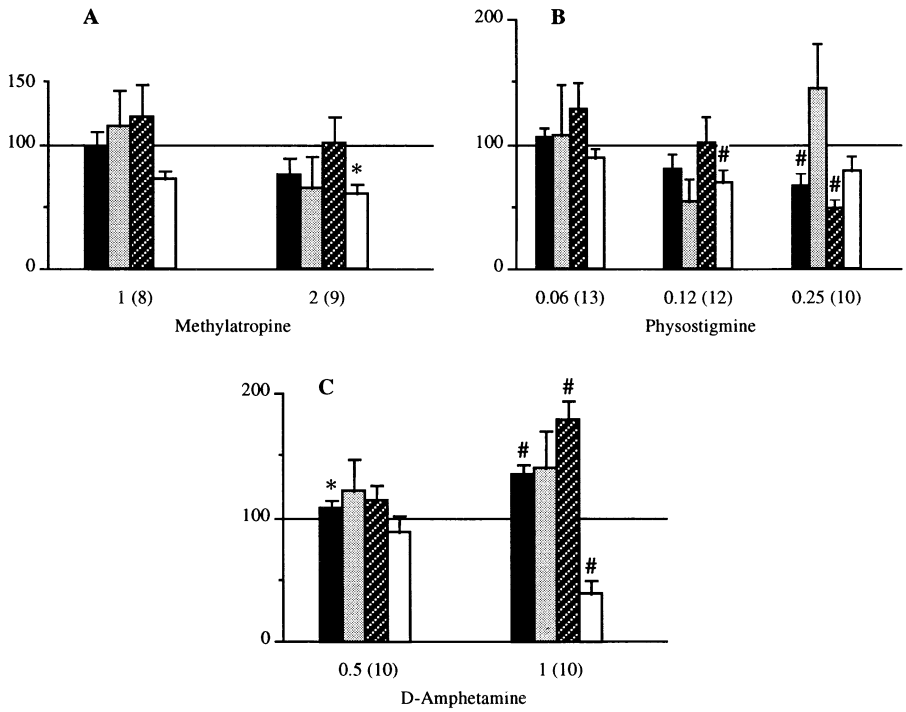

FIG. 2. Effect of methylatopine (A), physostigmine (B), and *d*-amphetamine (C) on DRL responding. For details, see legend of Fig. 1.

As shown in Fig. 3, there were no significant effects of soman and sarin on any measure. These drugs did not induce overt signs of poisoning, and the performance during the 5 days after treatment remained unaltered (data not shown).

Oximes

Table 3 gives results of the ANOVA for overall drug effects. Pralidoxime decreased response and reinforcement rates and the number of premature responses. Figure 4A shows that these effects were statistically significant only at the dose of 200 mg/kg. Pyrimidoxime decreased response and reinforcement rates and the number of premature responses. The decrease in response and reinforcement rates was significant at the doses from 75 to 150 mg/kg and the decrease in the number of premature responses was significant at the doses of 100 and 150 mg/kg (see Fig. 4B). HI-6 decreased response and reinforcement rates. Figure 4C shows that the decrease in reinforcement rate was statistically significant at doses from 200 to 400 mg/kg; the decrease in response rate was significant at the dose of 400 mg/kg.

Nicotinic Ligands

Table 4 gives results of the ANOVA for overall drug effects. Nicotine increased the number of premature responses and the response rate and decreased the reinforcement rate. Figure 5A shows that the increase in the number of premature responses was significant at doses from 0.12 to 1 mg/kg; the increase in response rate was significant at 0.5 and 1 mg/kg; the

TABLE 2

STATISTICAL ANALYSIS OF THE EFFECTS OF REVERSIBLE ACHE INHIBITORS: OVERVIEW OF *F* RATIOS

	Repeated Measures			Dose × Repeated Measure		
Drug	*df*	*F* ratio	*p*	*df*	*F* ratio	*p*
Physostigmine						
Response rate	1,32	4.216	0.048	2,32	2.305	NS
Very premature responses		0.454	NS		1.272	NS
Premature responses		0.490	NS		4.363	0.021
Reinforcement rate		11.772	0.0017		0.695	NS
Pyridostigmine						
Response rate	1,35	1.445	NS	3,35	2.267	NS
Very premature responses		0.015	NS		1.451	NS
Premature responses		0.211	NS		2.506	NS
Reinforcement rate		1.040	NS		3.409	0.028

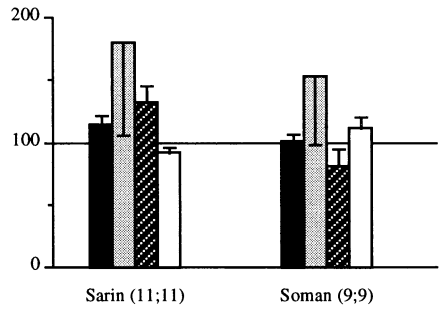

FIG. 3. Effect of sarin (0.03 mg/hg) and soman (0.03 mg/hg) on DRL responding. Numerals in parentheses indicate the number of control rats and treated rats. Values (mean ± SEM) are expressed in percent of performance of control group. Solid bars: response rate; gray bars: number of very premature responses; striped bars: number of premature responses; open bars: reinforcement rate.

decrease in reinforcement rate was significant at all the doses tested except 0.06 mg/kg. Mecamylamine increased the number of very premature responses and decreased the reinforcement rate, but these effects were statistically significant only at the dose of 2 mg/kg for very premature responses and at 4 mg/kg for reinforcement rate (see Fig. 5B).

Benzodiazepine

Because diazepam (16 mg/kg) totally suppressed responding (see Fig. 6A), this dose has been left out of the ANOVA. The results of ANOVA for overall drug effects are summarized in Table 5. Diazepam significantly altered response and reinforcement rates, the number of premature responses, and the number of very premature responses. Figure 6A shows that 2 and 4 mg/kg of diazepam increased the number of premature responses, the number of very premature responses, and the response rate; 2 mg/kg of diazepam also decreased the reinforcement rate; responding was totally suppressed at the dose of 16 mg/kg.

Psychostimulant and Wakening Drug

Table 5 gives results of the ANOVA for overall drug effects. *d*-Amphetamine increased the response rate and the number of premature responses and decreased the reinforcement rate. These effects were significant at the dose of 1 mg/kg, and at 0.5 mg/kg of *d*-amphetamine, there was a slight but significant increase in response rate (see Fig. 2C). Modafinil did increase the response rate, the number of very premature responses, and the number of premature responses and decreased the reinforcement rate. Post hoc comparison showed that 128 mg/kg of modafinil altered the four parameters; the dose of 64 mg/kg increased the number of very premature responses and the number of responses and decreased the reinforcement rate (see Fig. 6B).

DISCUSSION

The muscarinic blockers atropine, scopolamine, and CEB-1957 increased responding and decreased the reinforcement rate. These effects are consistent with those found by other authors in classical DRL schedules (22,28) or in a two-lever DRL schedule (45). They are probably due to an action of these compounds within the CNS, because the noncentrally acting muscarinic blockers methylatropine did not increase response rate while scopolamine, which is very centrally active (3), had a very marked effect. The possibility that methylatropine becomes effective at higher doses cannot be totally excluded, but it does not seem plausible because it has been demonstrated that another noncentrally acting muscarinic blocker, methylscopolamine, is ineffective in DRL schedule (45). The number of very premature responses was also significantly increased by scopolamine and CEB-1957, but this effect was less prominent (except at the highest dose of CEB-1957) than with other drugs such as diazepam (see below). In the present study scopolamine induced a significant effect at lower doses than in studies of other authors, who found a significant effect with doses from 0.06 mg/kg (28) or from 0.5 mg/kg (45). A difference in the method of administering the drug (intraperitoneal in these author's studies and intramuscular in the present study) could explain this difference in the dose–

TABLE 3

STATISTICAL ANALYSIS OF THE EFFECTS OF OXIMES: OVERVIEW OF *F* RATIOS

	Repeated Measures			Dose × Repeated Measure		
Drug	*df*	*F* ratio	*p*	*df*	*F* ratio	*p*
Pralidoxime						
Response rate	1,30	15.075	0.0005	3,30	3.096	0.042
Very premature responses		0.385	NS		1.210	NS
Premature responses		5.278	0.029		0.980	NS
Reinforcement rate		16.780	0.003		3.087	0.042
Pyrimidoxime						
Response rate	1,40	41.679	<0.0001	4,40	8.680	<0.0001
Very premature responses		3.699	NS		1.573	NS
Premature responses		16.743	0.0002		4.668	0.0035
Reinforcement rate		32.352	<0.0001		3.631	0.013
HI-6						
Response rate	1,50	4.774	0.034	5,50	4.517	0.0018
Very premature responses		0.557	NS		0.809	NS
Premature responses		0.570	NS		2.060	NS
Reinforcement rate		41.169	<0.0001		6.219	0.0001

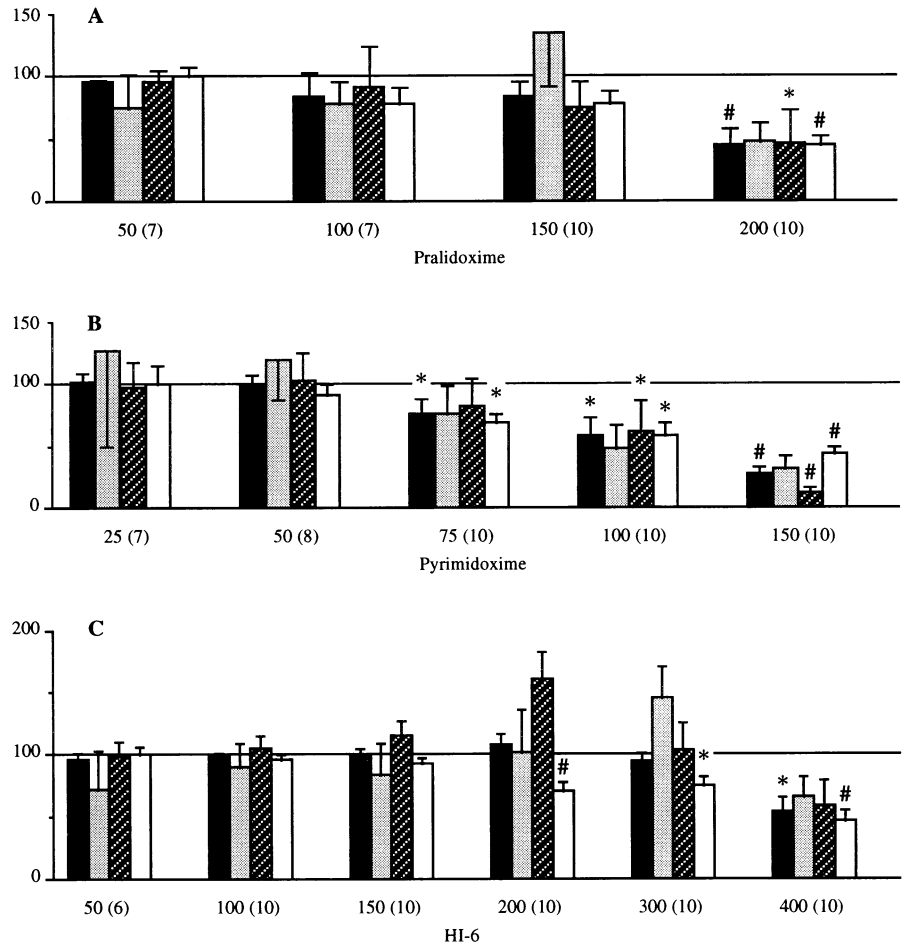

FIG. 4. Effect of pralidoxime (A), pyrimidoxime (B), and HI-6 (C) on DRL responding. For details, see legend of Fig. 1.

effect relationships. Generally speaking, disruptions of DRL performance were induced by doses of muscarinic blockers that are very low in comparison with doses that are generally used in studies of therapy against OPC. Numerous studies of the efficacy of drugs against OPC have been made with doses of atropine of about 10 or 20 mg/kg, and sometimes 60 mg/kg [see (7) for review]. Thus, these doses of atropine, if they are therapeutically effective in rats, would also be very incapacitating, because they are at least 40 times higher than the lowest psychoactive dose in the DRL schedule.

TABLE 4

STATISTICAL ANALYSIS OF THE EFFECTS OF NICOTINIC LIGANDS: OVERVIEW OF *F* RATIOS

	Repeated Measures			Dose × Repeated Measure		
Drug	*df*	*F* ratio	*p*	*df*	*F* ratio	*p*
Nicotine						
Response rate	1,74	11.765	0.0010	5,74	2.950	0.018
Very premature responses		0.596	NS		1.589	NS
Premature responses		61.790	<0.0001		3.282	0.0099
Reinforcement rate		55.060	<0.0001		2.876	0.020
Mecamylamine						
Response rate	1,48	0.045	NS	4,48	1.915	NS
Very premature responses		6.480	0.014		2.883	0.032
Premature responses		0.003	NS		0.294	NS
Reinforcement rate		5.327	0.025		2.285	NS

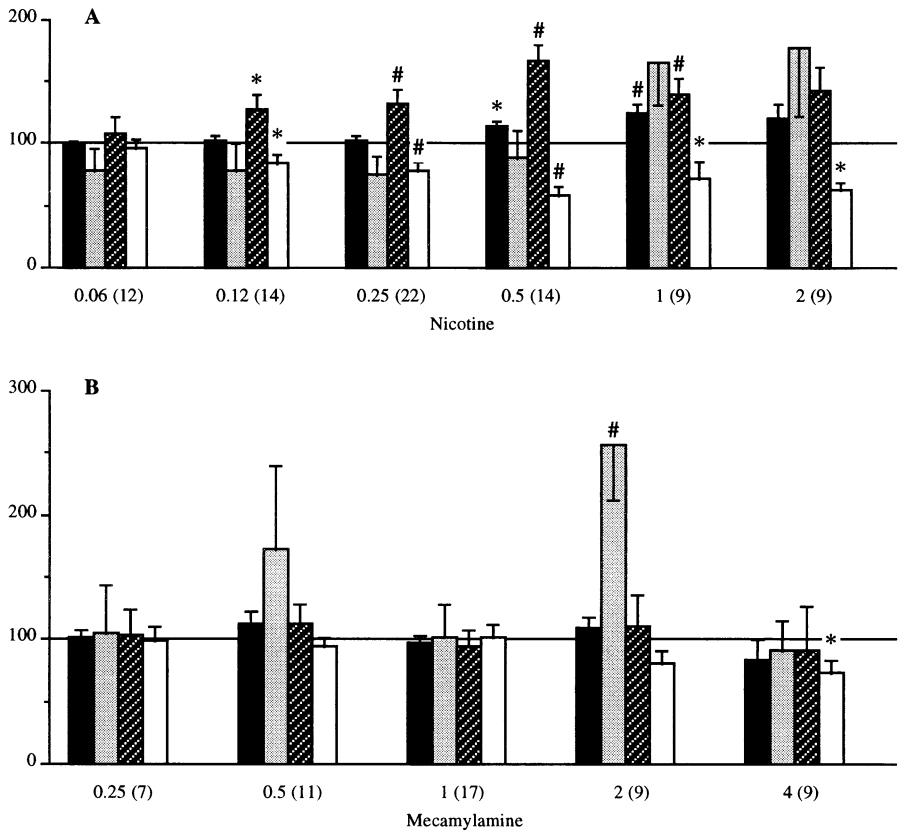

FIG. 5. Effect of nicotine (A) and mecamylamine (B) on DRL responding. For details, see legend of Fig. 1.

Physostigmine decreased the number of premature responses and both the response and reinforcement rates. This effect was probably due to a central inhibition of AChE, because the AChE inhibitor pyridostigmine, which does not penetrate the blood–brain barrier (50), did not significantly alter the reinforcement rate. Soman and sarin were devoid of effects at the doses studied, so approximately 1/3 LD_{50} (personal unpublished results). The effect of OPC on DRL performance has been poorly investigated. A past experiment showed that an exposure to soman resulted in an inability to learn the DRL task in surviving rats, but the soman was administered at a high dose that induced convulsions, and the observed effect was induced by neuropathology resulting from seizures (27). Thus, because DRL performance may be altered by most of the psychotropic drugs (in my experience, no centrally acting drug exists that alter rats' behavior and that does not alter DRL responding), our results suggest that these two OPC, at low doses are devoid of behavioral effects in rats. This point remains controversial. For example, some authors (31,56) found that very low doses (from 2 to 30% of the LD_{50}) of soman or sarin decreased locomotor activity, while other studies indicated that a decrease in locomotor activity in rats was induced by either soman or sarin only at doses of at least 50% of the LD_{50} (13,20,35). In addition, soman induced behavioral alterations in other tests (conditioned avoidance, acoustic startle response) only at doses 60% of the LD_{50} (13). Our results are in agreement with those of authors that found few behavioral effects of soman and sarin in rats. Because in rodents a very little proportion of soman or sarin penetrates the brain (4,21,34), the absence of effects of nontoxic doses of these drugs on DRL performance is consistent with the near absence of the effects of pyridostigmine described above.

Relatively moderate doses of pyrimidoxime, and high doses (from 200 mg/kg) of pralidoxime and HI-6 decreased reinforcement rate. This effect followed a decrease in response rate and/or in the number of premature responses for pyrimidoxime, pralidoxime, and the highest dose of HI-6. These results suggest that pralidoxime and HI-6, at the doses generally used in studies of therapy against OPC (7), have no behavioral toxicity. On the other hand, pyrimidoxime would induce behavioral effects at lower doses than the two other oximes. The pharmacological action that underlies the effects of oximes on DRL performance remains unknown. Because, in vitro, oximes exhibit an antimuscarinic activity (5,11,42), it could be postulated that their effects on DRL responding result from a blockade of peripheral muscarinic receptors. HI-6 (5)—but neither pyrimidoxime nor pralidoxime (11)—binds on nicotinic receptors. HI-6 also has ganglion-blocking effects (23). Therefore, to compare the effects of HI-6 with those of nicotinic ligands, we studied the effects on DRL performance of nicotine and mecamylamine, an agonist and an antagonist, respectively, of nicotinic receptors. The effect of nicotine—an increase in response rate and in the number of premature responses and a decrease in reinforcement rate—and the effect of mecamylamine—an increase in the number of very premature

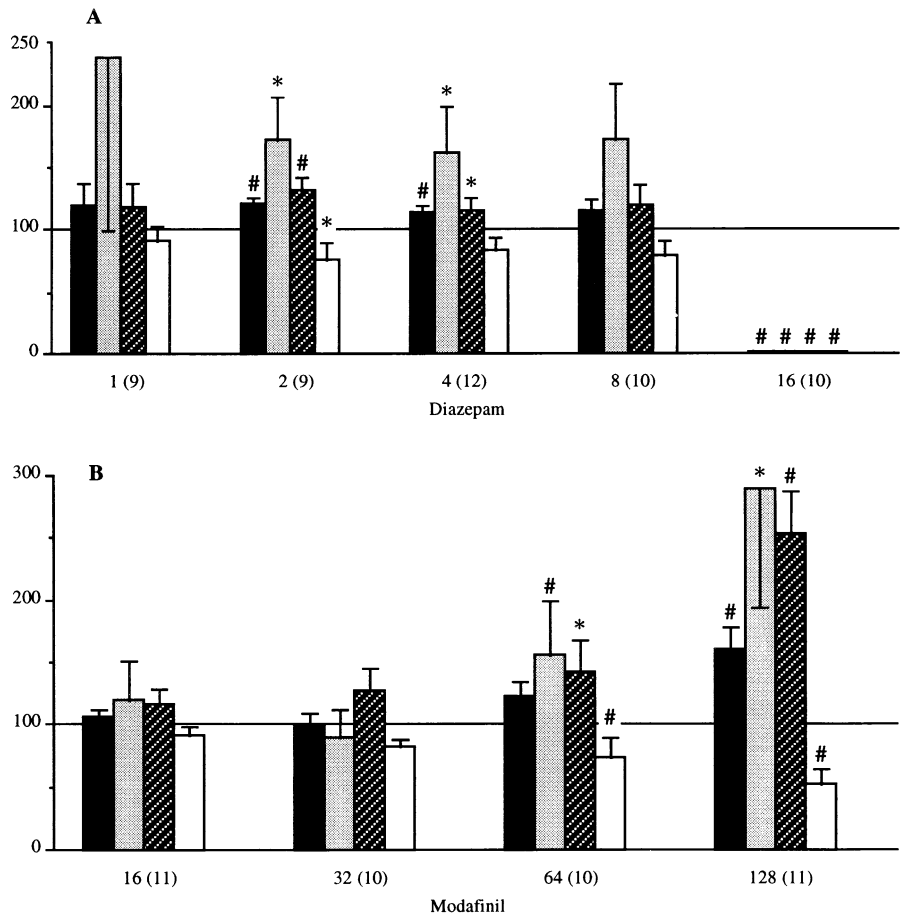

FIG. 6. Effect of diazepam (A) and modafinil (B) on DRL responding. For details, see legend of Fig. 1.

responses at the dose of 2 mg/kg and a decrease in reinforcement rate at the dose of 4 mg/kg—were quite different from the effect of HI-6. Thus, it is implausible, from these results, that the effects of HI-6 on DRL performance could be due either to a stimulation or to a blockade of nicotinic receptors.

The anticonvulsant drug diazepam increased response rate and decreased reinforcement rate. This effect was statistically significant with doses of 2 and 4 mg/kg, which are commonly used for treatment of OPC poisoning [see (7) for review]. A sedative effect was induced by the highest dose, which totally disrupted the animals' ability to make responses. Our results are in agreement with those of numerous authors (37,40, 41,48) who reported the same effects after the injection of agonists of benzodiazepine receptors. Diazepam, like other benzodiazepines, increased the number of very premature responses, which are frequently called burst responses (37,40, 41,48). This effect was less prominent after injections of muscarinic blockers, or of stimulant drugs such as nicotine or amphetamine (18), as it has been shown in the present study and in others (36,41). Interestingly, the awakening drug modafinil, unlike amphetamine and nicotine, increased the number of very premature responses. This result is consistent with the assumption that modafinil does not induce the same behavioral effects as amphetamine (44). Although the exact mechanism of action of modafinil remains not fully elucidated yet, an effective central α_1-adrenergic tone seems to be required for the awakening and motor stimulant effect of modafinil (9). It has also been shown that modafinil decreases cortical GABA release (49). It is implausible that such an alteration of GABA release could be responsible for the effect of modafinil on DRL responding, because it is well established that such an effect (an increase in the number of very premature and premature responses and a decrease in reinforcement rate) is induced by drugs that, on the contrary, increase GABA neurotransmission, such as benzodiazepines or barbiturates, as shown in the present study and in others (37,40,41). Thus, it can be assumed that an effective central α_1-adrenergic tone would be required for the modafinil's effects on DRL responding, because the blockade of these receptors produces an opposite effect: a decrease in response rate (24).

Various explanations of the effects of drugs on DRL performance can be made. We will propose four that are not mutually exclusive: alteration of waiting ability, alteration of time perception, disruption of short-term memory, and an attenuation of the punishment effect of nonreward.

Modifications of waiting ability, which is a major component of the control of impulsivity (15), could, at least partly, account for the effects of some drugs on DRL performance, particularly antidepressants and benzodiazepines. Thus, current or potential antidepressants, which decrease response

TABLE 5

STATISTICAL ANALYSIS OF THE EFFECTS OF A BENZODIAZEPINE, A PSYCHOSTIMULANT DRUG, AND A WAKENING DRUG: OVERIVEW OF *F* RATIOS

	Repeated Measures			Dose × Repeated Measure		
Drug	*df*	*F* ratio	*p*	*df*	*F* ratio	*p*
Diazepam						
Response rate	1,36	16.747	<0.001	3,36	0.132	NS
Very premature responses		8.435	<0.01		0.201	NS
Premature responses		19.154	<0.0001		0.999	NS
Reinforcement rate		9.625	<0.01		0.194	NS
D-Amphetamine						
Response rate	1,18	22.605	0.0002	1,18	8.884	0.0080
Very premature responses		2.911	NS		0.466	NS
Premature responses		40.331	<0.0001		19.289	0.0004
Reinforcement rate		20.463	0.0003		10.589	0.0044
Modafinil						
Reponse rate	1,38	21.334	<0.0001	3,38	6.862	0.0008
Very premature responses		11.168	0.0019		4.128	0.013
Premature responses		32.178	<0.0001		6.718	0.0010
Reinforcement rate		36.235	<0.0001		4.796	0.0063

rate and increase reinforcement rate (28,32), also enhance the rats' ability to wait for food reward (2,52). Conversely, benzodiazepines, that increase responses—and especially very premature responses—and that decrease reinforcements (37,40,41) also reduce the tolerance to reward delay (52), an effect that could contribute to their antipunishment effect (53).

It has been postulated that dopamine (DA) neurotransmission plays a major role in time perception (29). Thus, the effect of amphetamine-like drugs on DRL performance (i.e., an increase in the number of premature responses) could be due to an overestimation of time. This explanation is consistent with results of other authors (25), who found that alterations of time estimation induced by methamphetamine in the rat could relate to an acceleration of an internal clock. Such an hypothesis is also corroborated by a recent study that demonstrated that DA agonists induced effects in a peak-time procedure, that could reflect an overestimation of time (10). ACh neurotransmission also seems to play a major role in time perception (29), and physostigmine has been shown to disrupt time discrimination in the rat (43). Such an effect could account for the physostigmine-induced disruption of the performance in DRL schedules. However, an alteration of time perception cannot explain the effects of muscarinic blockers on DRL performance, because the effects of scopolamine on time-related operant behaviors (1,43) could be interpreted as an underestimation of time. Such underestimation of time could induce a lengthening of the intervals of time between lever presses in DRL schedule, while scopolamine induced a shortening of intervals of time between presses, as assessed by the increase in the number of premature responses.

Alterations of short-term memory (which could be secondary to vigilance disruption) could also explain the effects of some drugs on DRL performance. In the DRL schedule, the rat has to remember the moment when the last response has been emitted, so as to respond opportunely, to be rewarded. Forgetting the last response, induced by a drug that disrupts short-term memory, could be responsible for the emission of a premature response. Therefore, drugs that increase premature responses and decrease reinforcements, such as muscarinic blockers, NMDA antagonists or benzodiazepines, also disrupt short-term memory in operant tasks such as delayed matching tasks (6,8,47).

It also can be assumed that an increase in nonreinforced responses could be secondary to an attenuation of the punishment effect of nonreward. Thus, drugs that induce antipunishment effects in conflict procedures, such as benzodiazepines, NMDA antagonists, or barbiturates (12,26,51,53–55) also increase response rate, and particularly the number of very premature responses in DRL schedules, as demonstrated in the present study and in others (37,39,41,47). Interestingly, compounds that increase premature responses and/or response rate, but which do not reliably increase very premature responses, such as amphetamine, nicotine, or scopolamine [present study; (36,41)] have no antipunishment effect in conflict procedures (12,26,30,51,54). Thus, it can be postulated that the very premature responses could result from the same psychopharmacological effect as punished responses in conflict tests, and could be indicative of an anxiolytic effect.

In conclusion, the results of this study showed the different effects of drugs on rats' performance under DRL-30 s schedule. Three centrally acting muscarinic blockers (atropine, scopolamine, and CEB-1957), nicotine, and *d*-amphetamine increased responding and decreased reinforcement rate, with no effect (or only a marginal increase) on the number of very premature responses. Others, like diazepam and modafinil, increased response rate (both premature and very premature responses) and decreased reinforcement rate. Physostigmine and three oximes (pralidoxime, pyrimidoxime, and HI-6) decreased responding and reinforcement rate, while pyridostigmine and two OPC, soman and sarin, had few effects, and methylatropine decreased reinforcement rate. As discussed above, the alterations of DRL performance by these drugs could bring to light various psychotropic effects. Therefore, our results suggest that in animal studies of the treatment of OPC poisoning [see (7,46) for review], pyridostigmine, phys-

ostigmine, pralidoxime, and HI-6 are given at doses that probably have few behavioral effects, while diazepam is given at doses that could induce behavioural impairments, and muscarinic blockers (atropine in most cases) are given at doses that induce very dramatic cognitive impairments.

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