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Effects of Pesticides and Drugs on Working Memory in Rats: Continuous Non-Match

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HEISE, G. A. and J. D. Hudson. *Effects of pesticides and drugs on working memory in rats: Continuous non-match.* PHARMACOL BIOCHEM BEHAV **23**(4) 599–605, 1985.—Effects of four pesticides (carbaryl, propoxur, chlordimeform, and deltamethrin) and two reference drugs, physostigmine and chlordiazepoxide, were measured on the performance of rats trained on a continuous non-match (CNM) delayed comparison, working memory procedure. These same compounds were also tested in analogous, large and small stimulus difference discrimination (i.e., non working-memory) procedures. The effects of the pesticides and physostigmine on CNM performance were qualitatively similar, and also similar to their effects on discrimination performance. As dosage of these compounds increased, only small effects on accuracy were observed, followed at still larger doses by an abrupt and non-selective decrease in all responding. The pesticides and physostigmine did not selectively affect working memory: the magnitude of their effects did not increase with intertrial interval, and the compounds were equally effective in disrupting discrimination and CNM performance. Effects of chlordiazepoxide on performance in the CNM and discrimination control procedures differed qualitatively from those of the pesticides and physostigmine.

Continuous non-match Working memory Discrimination Pesticides Carbaryl Propoxur Chlordimeform Deltamethrin Physostigmine Chlordiazepoxide

ALTHOUGH "memory impairment" has often been listed as an effect of exposure to toxicants (e.g., [17]) few studies have investigated the effects of toxic substance specifically on memory in animals [9]. Recently, however, the present authors reported [11] that carbaryl, propoxur, chlordimeform, and deltamethrin did not selectively disrupt working memory as measured in three continuous delayed response procedures. In general, as doses of these compounds increased, only small decrements in accuracy were observed, followed at higher doses by an abrupt decline in all responding. In contrast, the prototypic amnesic agent, scopolamine, did selectively disrupt working memory under the same test conditions.

The generality of the study just cited was limited by its exclusive use of delayed response procedures. In delayed response procedures the stimulus events presented prior to the delay (retention) interval completely determine which post-delay response will be correct: thus it is theoretically possible for the animal to "bridge the gap" by means of orienting responses [6]. Furthermore, only a single (5 sec) intertrial (retention) interval was employed: consequently any specific effects of treatments on retention could not have been detected.

The present research examined the same pesticides and reference drugs as in the previous study, but measured their effects on working memory in a delayed comparison (sometimes called delayed discrimination) procedure, continuous non-match (CNM). In a delayed comparison procedure the correct post-delay response is determined by post-delay as well as by pre-delay stimuli [11]; hence, the likelihood of coding by means of orienting responses is less than in delayed response. In addition, the CNM schedule included three different intertrial (retention) intervals—2.5, 5, and 10 sec—thus making possible the assessment of treatment effects on the time-dependent process of retention.

The CNM procedure is described in detail elsewhere [18,22]. In the CNM schedule, sequences of one or more trials with a "bright" panel light alternate with sequences of one or more trials with a "dim" panel light. A single lever press on a "change" or non-match trial (i.e., a trial on which the intensity of the panel light is different from that on the immediately preceding trial) is rewarded; lever presses on "match" trials (trials on which the intensity of the panel light is the same as on the immediately preceding trial) are never rewarded. Rats trained on this procedure attain a stable baseline of performance in which they typically respond on approximately 70 percent of their non-match trials ("hits") and on about 30 percent of their match trials ("False Alarms": FA's).

The CNM is a continuous discrete trial, delayed comparison, working memory procedure. It is continuous in that each trial is both the occasion for responding with respect to the stimuli presented on the preceding trial and also the occasion for presentation of the stimulus ("sample") to be remembered on the next trial. It is a delayed comparison (rather than delayed response) procedure since the correct response on a trial is determined by the relationship ("same" or "different") between the stimuli on the current trial and

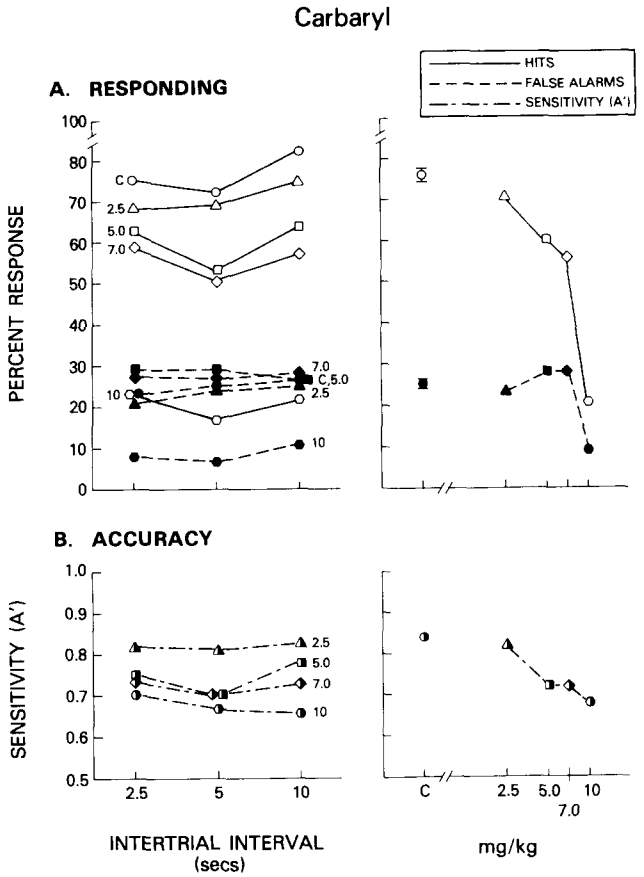

FIG. 1. Effects of graded doses of carbaryl on CNM performance. Part A. (Top): Percentage of hits and false alarms, shown separately for the three intertrial (retention) intervals (left) and for the three intertrial intervals combined (right). Brackets indicate ± 1 S.E.M. Part B. (Bottom): Sensitivity (A') for each of the three intertrial intervals (left) and for the three intertrial intervals combined (right).

the stimuli presented on the preceding trial. It measures working memory (rather than reference memory) since the correct response on a trial with a particular stimulus ("bright" or "dim") varies depending upon which of the alternative stimulus events occurred on the preceding trial (cf. [12,13]).

Obviously, all defects in CNM performance produced by the pesticides and reference drugs are not necessarily defects in working memory (cf. [10]): the treatments could affect sensory discrimination, motor coordination, or other non-working memory aspects of performance. In order to evaluate such performance effects in the CNM, rats in the present study were also tested with the pesticides and reference drugs in an analogous discrimination procedure in which a response in the presence of one trial stimulus (e.g., "bright") was always reinforced, whereas a response in the presence of the other trial stimulus (e.g., "dim") was never reinforced. Thus, the discrimination was a reference rather than working memory procedure: correct responding on a trial depended only on whether the current trial stimulus was bright or dim and did not also depend (as in the CNM) upon which stimulus had been presented on the preceding trial.

Four pesticides were examined for effects on CNM performance: carbaryl (Sevin: 1-naphthyl N-methyl carbamate); propoxur (Baygon: 1-isopropoxyphenyl N-methyl carbamate); deltamethrin, a synthetic pyrethroid; and chlordimeform, a formamidine. Considerable evidence implicates central cholinergic system involvement in memory processes (e.g., [2]); three of the four pesticides have anticholinesterase or anticholinesterase-like activity. The two carbamates, carbaryl and propoxur, inhibit acetylcholinesterase [5,7]; and deltamethrin has some cholinergic-like effects on behavior [19]. Chlordimeform inhibits monoamine oxidase in addition to other pharmacological actions but does not inhibit acetylcholinesterase [16].

Two comparison drugs were selected for possible similar action to that of the pesticides. Physostigmine is a central and peripheral inhibitor of acetylcholinesterase. Chlordiazepoxide was included because it was expected to have a different type of behavioral action than the other compounds and also because, like chlordimeform, it stimulates food intake and (unlike chlordimeform) also stimulates water intake [23].

METHOD

Animals

The subjects were 16 male Sprague-Dawley derived (CD) rats received from Charles River Breeding Laboratories at approximately 70 days of age. Eight of these animals were originally trained on the continuous non-matching schedule, and eight were originally trained on a corresponding discrimination control procedure. One animal from each group died before testing with toxic substances was completed. The animals were housed individually and maintained on a 12-hour light-dark cycle. The animals were maintained on Purina rat chow ad lib, and deprived of water for approximately 23 hours prior to the 5-days-a-week experimental sessions. The animals received water as reinforcement during experimental sessions and also for approximately 20 min following each experimental session. Water was freely available on weekends.

Apparatus

Each animal was trained and tested in one of eight identical operant chambers (25 × 24 × 18.5 cm) constructed at Indiana University. Two frosted glass response levers, requiring 25–30 g force for activation, were mounted on the front wall of each chamber. The levers were 10 cm above the grid floor and displaced 6.5 cm on either side of the midline. The rat had access to only the right lever during all experiments described here; the left lever was always covered with a metal shield.

A brass spout, calibrated to deliver 0.05 cc water/drop, protruded 2.7 cm into the chamber and was located 5.5 cm above the floor on the centerline of the front panel. Three recessed white 6-W panel lights were mounted 15 cm above the floor, one over each lever and one on the centerline. Only the center light was used in the present experiments.

Each of the operant chambers was enclosed in a heavy, sound-attenuating shell. A Texas Instruments 980A minicomputer, located in a room adjoining the experimental room, controlled the experiments and recorded the data.

Behavioral Procedures

a. Continuous non-match (CNM). In the CNM schedule, a variable number of trials signalled by a "bright" panel

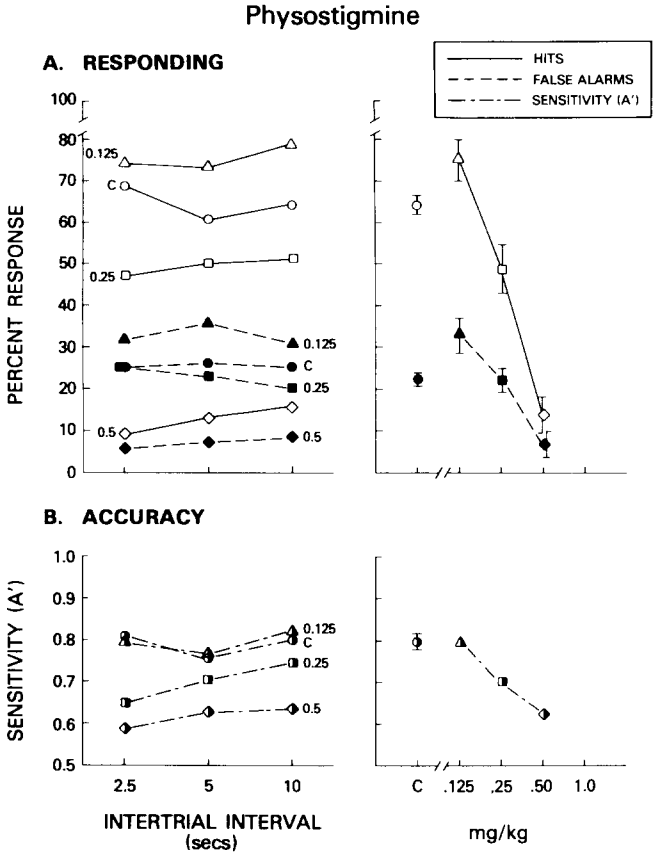

FIG. 2. Effects of graded doses of physostigmine on CNM performance. Same notation as Fig. 1.

light alternated with a variable number of trials signalled by a "dim" panel light. Maximum trial duration was 5 sec. The ratio of intensities of the bright and dim lights was approximately 56:1. The first lever press on a "non-match" trial—a trial on which the light intensity was different from the intensity on the preceding trial—terminated the trial and produced water reinforcement. A lever press on a non-match trial was termed a "hit." Lever presses on "match" trials—trials on which the panel light intensity was the same as that on the preceding trial—did not terminate the trial and did not produce reinforcement. A lever press on a match trial was termed a "false alarm" (FA). Correction trials were presented following all match trials on which responses occurred; correction trials were not presented following failures to respond on non-match trials.

Not counting correction trials, an experimental session consisted of 50 non-match trials and 179 match trials: the ratio of opportunities for a false alarm to the number of opportunities for a hit was thus approximately 3.6:1. Each trial stimulus (bright or dim) appeared on approximately one-half of the match and one-half of the non-match trials. In the final schedule, used for testing of chemicals, each of three intertrial (retention) intervals—2.5, 5, and 10 sec—was presented equally often in semi-random order. Experimental sessions were one to 1½ hours long. A pretrial delay contingency was in effect during the last one sec of each intertrial interval: each response during this interval postponed the onset of the next trial by one sec from the time of the response.

Performance on the CNM schedule was scored in two ways: (1) in terms of the proportion of hits and false alarms (p(Hit) and p(FA) respectively), and (2) in terms of the Theory of Signal Detection (TSD) measure of sensitivity. For this latter measure, a non-parametric measure of sensitivity (A′) was calculated from the observed p(Hit) and p(FA) according to the formula given by Grier [8]. A value for A′ of 0.50 indicates chance accuracy, and a value for A′ of 1.0 indicates perfect accuracy.

Training on the CNM schedule consisted of first, lever press training on discrete trials, then CNM sessions with the intertrial interval fixed successively at 1 sec, 2.5 sec, and 5 sec, and, finally, the terminal schedule comprised of 2.5, 5, and 10 sec intertrial intervals. Approximately 15 training sessions with the final schedule were required for stable baseline performance.

b. Discrimination. The distribution of intertrial intervals, patterning of go and no-go trials, density of possible reinforcements, trial durations, etc., were the same for the discrimination procedures as for the CNM. However, in the discrimination procedures, responses ("hits") in the presence of one stimulus ("bright" for one-half, "dim" for the other half of the animals) were always reinforced, and responses ("false alarms") in the presence of the other trial stimulus were never reinforced.

It is well established that the magnitude of drug effects on discrimination performance varies inversely with level of stimulus control (accuracy) ([14], see [12] for review). Therefore, in the first series of discrimination experiments the intensities of the bright and dim stimuli were reduced and increased respectively relative to those used in the CNM, until the accuracy of performance was approximately the same as in the CNM. The resulting ratio between the intensities of the bright and dim stimuli for this "Small Difference" discrimination was approximately 2:1. After testing of all the pesticides and reference drugs on performance of this discrimination had been completed, the same animals were tested with carbaryl, physostigmine, and chlordiazepoxide on performance of a Large Difference discrimination, in which the difference in intensity between the two stimuli was the same as in the CNM (i.e., intensity ratio approximately 56:1). The proportion of hits, FA's, and the Theory of Signal Detection measure of Sensitivity, A′, were calculated in the same way for the discriminations as for the CNM.

Testing Procedures

Typically, squads of four animals were tested concurrently with a particular treatment. Each of the eight animals trained on CNM was injected twice with each of the various doses of chlordimeform and deltamethrin. For all other determinations, each animal was tested once with each dose of each substance. The order of administration of the various doses of a compound was counterbalanced among the various animals. Treatments were ordinarily given on Tuesdays and Fridays, with control sessions recorded on Mondays and Thursdays.

Compounds were administered by IP injection 20 min prior to the experimental session. Carbaryl, deltamethrin, and propoxur were given dissolved in warm corn oil; all other compounds were dissolved in water. The following compounds were generously donated by their manufacturers: carbaryl 99.9% analytical grade from Union Carbide, Jacksonville, FL; propoxur 97% technical grade from Mobay

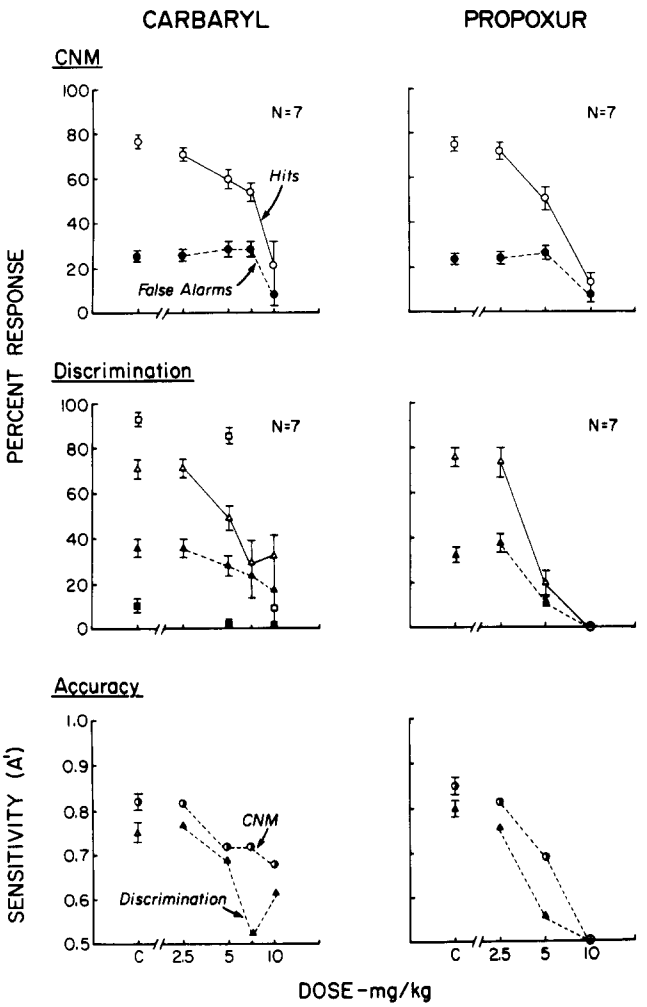

FIG. 3. Effects of carbaryl and propoxur on performance on the CNM and discrimination controls, for all three intertrial intervals combined. Top: Effects on CNM performance. Middle: Effects on the Small Difference Discrimination (triangles). The square symbols show effects of carbaryl on performance of the Large Difference discrimination. Bottom: Comparison of effects on accuracy (A') effects in the CNM and Small Difference discrimination procedures.

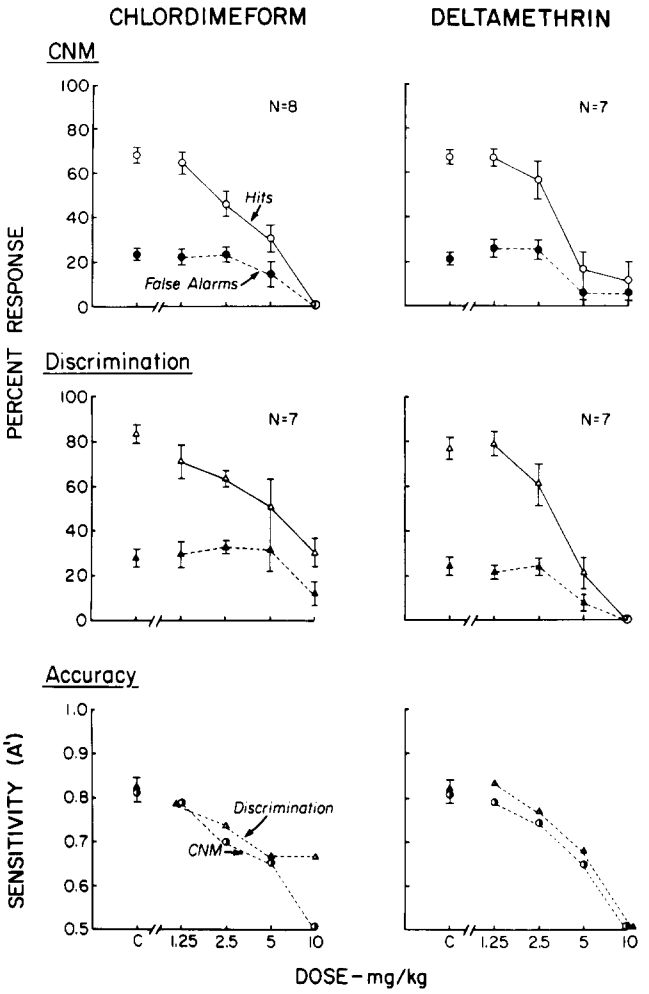

FIG. 4. Effects of chlordimeform and deltamethrin on performance on the CNM and Small Difference discrimination. Same notation as Fig. 3.

Chemical Corp., Kansas City, MO; chlordimeform 97% pure from Nor-Am, Napierville, IL; deltamethrin from Roussel UCLAF, Romainville, France; and chlordiazepoxide HCl from Hoffman LaRoche, Inc., Nutley, NJ. Physostigmine (eserine) was purchased from Sigma Chemical Co., St. Louis, MO.

RESULTS

Figure 1 shows time-response and dose-response curves for the effects of a representative anticholinesterase pesticide, carbaryl, on CNM performance; Fig. 2 shows the curves obtained with the anticholinesterase comparison drug, physostigmine.

The left sides of Figs. 1 and 2 show that performance did not vary systematically with intertrial (retention) interval under treatment conditions, and the magnitude of treatment effects did not increase with increasing intertrial interval. Thus carbaryl and physostigmine did not impair retention. A similar absence of time-related effects was also observed with all the other compounds tested. Consequently, results for the three intertrial intervals were combined in further analyses of the data.

Dose-response curves obtained with carbaryl and physostigmine for the three intertrial intervals combined are presented on the right sides of Figs. 1 and 2. For carbaryl and physostigmine, and also for the other three pesticides, the percentage of hits decreased abruptly with increasing dosage, declining over two successive doublings of the dose from near baseline levels to virtual response cessation. The bottom left and right sides of Figs. 1 and 2 show the effects of intertrial interval and dosage on response accuracy. The decrease in accuracy (A') with increasing dosage reflects the proportionally greater decline in p(Hit) than in p(FA).

Figures 3, 4 and 5 present the dose-response curves obtained in the CNM and in the Small Difference discrimination

procedure for all the pesticides and reference drugs. The dose-response curves obtained for the effects of the four pesticides and physostigmine on CNM performance were qualitatively similar to each other. Moreover, each compound's effect on CNM performance was similar to its effect on the Small Difference discrimination performance. (See bottom portions of Figs. 3, 4 and 5.) Substantial discrepancies between CNM and discrimination performance were sometimes observed at higher dosages, but only when the over-all level of responding was quite low.

Effects of carbaryl, physostigmine, and chlordiazepoxide on performance of the Large Difference discrimination are shown in Figs. 3 and 5. Although non-treatment (control) performance was substantially more accurate in the Large Difference discrimination than in the Small Difference discrimination, carbaryl and physostigmine had similar effects on performance of both discriminations. Chlordiazepoxide, on the other hand, affected Small Difference discrimination performance more than either Large Difference or CNM performance (see Fig. 5). Chlordiazepoxide was remarkable for the broad range of doses over which CNM performance was affected but not totally disrupted.

DISCUSSION

The four pesticides and physostigmine had similar effects on CNM performance: the percentage of hits and false alarms declined abruptly with dose, falling from control levels to near total response failure within two doublings of the dose. The magnitude of effects did not vary consistently with intertrial (retention) interval. Effects of these same compounds on performance of the Small Difference discrimination (for which the baseline accuracy was equivalent to that of the CNM) were qualitatively and quantitatively similar to their effects on CNM performance (cf. Table 1). In addition, carbaryl and physostigmine disrupted performance of a Large Difference discrimination (for which the trial stimuli were the same as for the CNM) to approximately the same extent that they disrupted performance on the CNM and on the Small Difference discrimination.

Thus, the pesticides and physostigmine did not selectively impair working memory: rather, they indiscriminately suppressed all behavior. If they had specifically affected working memory, there should have been a range of doses at which the animals continued to perform (although inaccurately) in the CNM, and carbaryl and physostigmine should have affected memory for the trial stimuli, as measured in the CNM, more than it affected discrimination of these same stimuli, as measured in the Large Difference discrimination. Neither of these effects was obtained. However, scopolamine, an alleged amnesic agent, produced precisely these effects on CNM performance in experiments carried out under similar conditions by Spencer, Pontecorvo, and Heise [22].

Estimates of the effective doses of the pesticides and physostigmine in the CNM were obtained by determining from the dose-response curves the doses that reduced the proportion of hits to 50% of baseline control levels. These doses are presented in Table 1, along with effective doses for these same compounds similarly estimated from the dose-response curves obtained from two delayed response working memory procedures: subject-initiated go-no go alternation and spatial reversals [11].

In general, similar effective doses were obtained for each compound in the various types of memory and discrimination

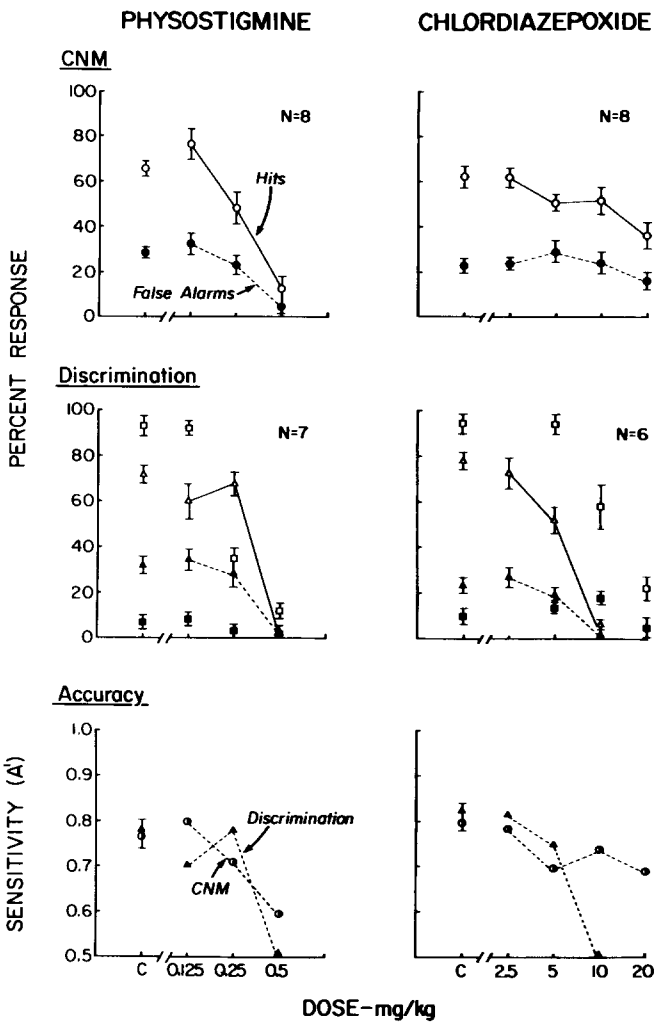

FIG. 5. Effects of physostigmine and chlordiazepoxide on CNM and discrimination control performance, for the three intertrial intervals combined. Same notation as Fig. 3.

procedures—a result consistent with the non-selective action of these compounds. A possible exception was deltamethrin, which was more effective in the CNM and its associated discrimination procedure than in the other procedures. Approximately the same effective doses as those listed in Table 1 for CNM performance have also been reported for carbaryl [1, 7, 21]; for propoxur [20,21]; for chlordimeform [16]; for deltamethrin [4,15], and for physostigmine [3] in other behavioral studies.

In the present experiments, successive injections were separated by at least 72 hours for any particular animal and the animals received different orders of different drugs. Nevertheless, the possibility of tolerance or carryover of effects of the pesticides must be considered, since each animal used in the CNM or the discrimination control procedures received twice-weekly dosings with each of a number of different compounds. However, there was no evidence of either tolerance or carryover in the present results. Treatment effects did not decrease with repeated injections, nor did effects of treatments depend upon the animal's recent treatment history. The absence of carryover effects with

TABLE 1

EFFECTS OF PESTICIDES AND PHYSOSTIGMINE ON RESPONDING IN WORKING MEMORY AND DISCRIMINATION PROCEDURES

Procedure	Measure	Dose (mg/kg) Carbaryl	Propoxur	Chlordimeform	Deltamethrin	Physostigmine
Continuous Non-Match:						
Working Memory	50% Reduction in Hits	8	7	4	3.5	0.3
Discrimination	50% Reduction in Hits	6.5	4	7	3.5	0.35
Go-no alternation [11]						
Working Memory	50% Reduction in Trial Initiations	7.5	4	6	17	
Discrimination	50% Reduction in Trial Initiations	7	8	4	>10	0.25
Spatial Reversals [11]						
Working Memory	50% Response Failure	7	9	8	10	0.4

carbaryl is in accord with the behavioral observations of others (e.g. [7]). The results are also consistent with the reported short durations of reduced cholinesterase activity following acute injections of carbaryl or porpoxur. For example, 16 mg/kg carbaryl reduced brain cholinesterase levels for 240 min, and 2 mg/kg propoxur reduced brain cholinesterase levels for 120 min [21].

In conclusion, the results obtained with the CNM procedure further substantiate the conclusions of our delayed response experiments [11]: carbaryl, propoxur, chlordimeform and deltamethrin do not specifically affect working memory, but rather have non-specific behavioral suppressant effects.

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