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Effects of Phencyclidine, Atropine and Physostigmine, Alone and in Combination, on Variable-Interval Performance in the Squirrel Monkey[1]

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CHAIT, L. D. AND R. L. BALSTER. *Effects of phencyclidine, atropine and physostigmine, alone and in combination, on variable-interval performance in the squirrel monkey.* PHARMAC. BIOCHEM. BEHAV. 11(1) 37–42, 1979.—The role played by cholinergic activity in the effects of phencyclidine (PCP) on schedule-controlled responding was studied in three squirrel monkeys trained to respond on a variable-interval (VI) 100 sec schedule of food presentation. A low dose of PCP (0.08 mg/kg IM) produced small increases in rates of responding. Higher doses (0.16–0.64 mg/kg) produced dose-dependent decreases in rates of responding. Atropine (0.05–3.2 mg/kg IM) and physostigmine (0.025–0.20 mg/kg IM) caused only decreases in response rates, the dose-response curve for atropine being particularly flat over a wide range of doses. When atropine was combined with PCP, no significant interaction was obtained. When physostigmine was combined with PCP, a complex interaction was observed. Evidence for partial antagonism of PCP by physostigmine was obtained only at the highest PCP dose tested. Atropine-physostigmine combinations resulted in response rates suggestive of antagonism.

Phencyclidine Physostigmine Atropine Variable-interval Squirrel monkey Drug interactions ACh

PHENCYCLIDINE (1-(l-phenylcyclohexyl) piperidine; PCP) is a drug with an unusual spectrum of central nervous system activity [6]. Originally introduced as a general anesthetic, it possesses some characteristics of analgesics, sedative-hypnotics, psychomotor stimulants and hallucinogens [4, 5, 11]. In the past several years it has emerged as a major drug of abuse in this country [7,27].

Several studies have attempted to elucidate the neurochemical mechanisms responsible for the complex behavioral effects of phencyclidine. The drug has been shown to affect levels or function of several central neurotransmitters—norepinephrine [15, 19, 22, 30, 32, 33], dopamine [14, 19, 22], serotonin [20, 31, 32, 33] and acetylcholine [12, 21, 24].

The effects of phencyclidine on cholinergic function are of special interest, since several reports have provided evidence that pharmacological manipulation of cholinergic activity can reverse or block the behavioral effects of phencyclidine or ketamine, a drug of similar structure and activity [25]. Maayani *et al.* [24] reported that treatment with tetrahydroaminoacridine (tacrine; THA), a cholinesterase inhibitor, prevented or reversed both the hyperactivity in mice and the anesthesia in guinea pigs induced by phencyclidine. Similarly, Winters and Kott [36] found that physostigmine pretreatment was effective in antagonizing the EEG hypersynchrony and catalepsy produced by ketamine in cats. Albin *et al.* [3] demonstrated in dogs that ketamine-induced anesthesia and emergence reactions could be antagonized by THA. These same researchers later extended these results to phencyclidine in the dog [2] and ketamine in humans [1].

Our laboratory has recently been studying the effects of phencyclidine on schedule-controlled behavior in the squirrel monkey. The present study represents an attempt to assess the role of the cholinergic system in the behavioral effects of phencyclidine in this species. Specifically, we wanted to determine whether physostigmine, a cholinesterase inhibitor, would antagonize, and whether atropine, an anticholinergic agent, would potentiate the behavioral disruption caused by phencyclidine. As a positive control we also studied the interaction of physostigmine and atropine. Essentially the same design and methodology was used in the present study as was used to study the interaction be-

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tween phencyclidine and pentobarbital in the squirrel monkey [9].

METHOD

Animals

Three male squirrel monkeys (*Saimiri sciureus*, Santa Cruz, Bolivia: Primate Imports, Port Washington, NY) with no previous exposure to drugs or experimentation served in the experiment. Initial free-feeding weights ranged from 874 to 1090 g. Weights were maintained throughout the study at approximately 85% of free-feeding weights. Animals' diet consisted of Purina Monkey Chow and supplementary vitamin C. The animals were housed in individual stainless steel cages in an isolated room with a controlled light-dark cycle (12 hr on–12 hr off). Water was continuously available in their home cages, but not during experimental sessions.

Apparatus

During experimental sessions animals were restrained about the waist in a Plexiglas primate chair, described in detail in a previous publication [8]. The chair was equipped with stimulus lights, a response lever and an automatic feeder which delivered 97 mg Noyes banana-flavored food pellets into a brass food trough. For experimental sessions the chair was placed in a light- and sound-attenuating isolation cubicle. A fan provided both adequate ventilation and masking noise. Solid state programming equipment, counters and a cumulative recorder were located in an adjacent room.

Procedure

Training. Animals were initially trained to lever press on a schedule of continuous food presentation. The schedule was then changed to a variable-interval (VI) 15 sec. Over the next month the average interval was gradually lengthened to a VI 100 sec. The monkeys were maintained under this schedule for an additional month in order to allow baseline rates to stabilize and habituation to saline injections.

The duration of intervals was determined by a probability gate (BRS/LVE PP-201). Because the output of this gate varied from day to day, the average interval during a particular session varied from a minimum of 80 to a maximum of 140 sec, with a mean of approximately 100 sec. An upper limit of 4 min was imposed to prevent cessation of responding during unusually long intervals.

Experimental sessions. Sessions began immediately after placing the animal into the isolation cubicle (immediately after injection on days when drug or saline was given). A white stimulus light was illuminated for the duration of the session, which was normally 2 hr. This light dimmed momentarily each time the animal made a response on the lever. Sessions were held on a 4-day cycle. On the first day data were collected but not analyzed. Data from the second day comprised the no-injection baseline. On the third day drug or saline was given before the session. No session was held on the fourth day of the cycle. This sequence continued for the duration of the study (about 32 weeks). Animals were run in the same order and at approximately the same time each day a session was held. They received from 51 to 90 food pellets during an experimental session (unless their responding was disrupted by drug). The balance of their daily food intake (and vitamin) was given in their home cages one hour after the end of each session.

Treatment regimens. After baseline responding had adequately stabilized, each animal received the following treatment regimens, in sequence: phencyclidine alone, atropine alone, atropine-phencyclidine combinations, physostigmine alone, physostigmine-phencyclidine combinations and atropine-physostigmine combinations. Doses were given in mixed order within each treatment regimen, and each animal received each drug or combination one time. During the combination regimens each drug was administered in combination with saline as a control for the injection procedure.

Drugs

Phencyclidine hydrochloride (Sernylan), atropine sulfate and physostigmine salicylate (Antilirium) were diluted with physiological saline to yield injection volumes of less than 1.0 ml/kg. All doses are expressed as the salt. Injections were given IM into the thigh immediately before the start of the session (unless noted otherwise). During the combination regimens one injection was given into one leg followed immediately by the other into the other leg.

Data Analysis

Because baseline rates of responding tended to gradually decrease, as well as become more variable, as the 2 hr session progressed, only data from the first hour of each session were used in calculating response rates. Response rates (responses/min) were determined from counters, and are expressed as percent of baseline: that is (response rate during the first hour of a drug session/response rate during the first hour of the preceding day's baseline session)$\times$100.

Because all three drugs given alone affect VI responding (at certain doses), the effects of giving doses of these drugs in combination are compared to the algebraic sum of the effects of the same doses of each drug when given in combination with saline. This represents a simple descriptive means of evaluating the effects of drug combinations. The expected effects were calculated for each animal and averaged. Response rates for an individual animal summating to less than 0 were averaged as 0.

RESULTS

Baseline Performance

Table 1 shows the mean response rates ($\pm$ SD) for all the baseline sessions during each of the six treatment regimens for each subject. No consistent trends occurred in baseline rates of responding in any animal over the entire course of the study. Animal P.L. always had much higher baseline rates of responding than the other two animals, but did not appear to respond to drug treatments in a manner systematically different from them.

Effects of Phencyclidine, Atropine and Physostigmine, Given Alone

The dose-response curves for each drug given alone are shown in Fig. 1. All three drugs produced dose-dependent decreases in rates of responding. Phencyclidine was the only drug which produced consistent response rate increases (0.08 mg/kg). The dose-response curve for atropine was flat over more than a ten-fold dose range (0.10–1.6 mg/kg). At

TABLE 1

MEAN BASELINE RESPONSE RATES IN INDIVIDUAL ANIMALS

Treatment	Animal B.K.	B.W.	P.L.
		resp/min ± SD	
PCP	6.9 ± 3.5	8.1 ± 2.0	30.9 ± 1.3
ATR	8.5 ± 1.9	15.4 ± 2.1	46.0 ± 2.8
ATR + PCP	9.2 ± 2.6	16.5 ± 2.4	46.0 ± 1.8
PHY	7.5 ± 1.5	10.3 ± 1.0	39.1 ± 2.7
PHY + PCP	11.4 ± 3.3	9.8 ± 1.2	35.1 ± 2.6
ATR + PHY	13.2 ± 4.5	11.4 ± 1.6	38.7 ± 4.3

these doses atropine produced a progressive disruption of rates of responding throughout the two hour session, with no clearly defined onset or offset—it never caused a total suppression of responding (Fig. 2). At doses greater than 0.4 mg/kg animals manifested considerable screaming and biting directed toward the experimenter after the experimental sessions ended.

The effect of physostigmine on responding was different from that of atropine. Figure 2 shows that the effect of physostigmine was relatively all-or-none; there was little effect on responding except during periods when responding was totally, or nearly totally suppressed. Onset of disruption was rapid and abrupt, with recovery to baseline response rates also occurring abruptly. Decreasing response rates with increasing dose of physostigmine actually represent prolongation of this period of total suppression of responding. When observed during this period, animals were found crouched over motionless in a corner of the chair. Doses of physostigmine greater than 0.2 mg/kg caused tremors and excessive salivation.

Effects of Phencyclidine, Atropine and Physostigmine, Given in Combination

Atropine-phencyclidine combinations (Fig. 3) resulted in no systematic deviations from response rates that are obtained from simple algebraic addition of the effects of atropine and phencyclidine when each was combined with saline (solid horizontal bars in Fig. 3). Thus, there is little evidence for an enhancement of the rate-decreasing effects of phencyclidine by atropine.

Figure 4 suggests that the effect of physostigmine on phencyclidine-induced changes in VI responding is dependent upon the dose of phencyclidine. When both doses of physostigmine were combined with either 0.08 mg/kg (a rate-increasing dose) or 0.16 mg/kg (a threshold rate-decreasing dose) of phencyclidine, response rates obtained were lower than those derived from algebraic summation. The opposite effect was observed, however, when both doses of physostigmine were combined with 0.32 mg/kg phencyclidine, a dose which decreased rates of responding by about 70%. Neither dose of physostigmine completely antagonized the rate-suppressant effect of phencyclidine.

The effects of the atropine-physostigmine combinations (Fig. 5) are in agreement with the known antagonistic interaction between these two drugs [34]. All combinations examined resulted in rates of responding greater than those obtained from algebraic summation of the effects of the same

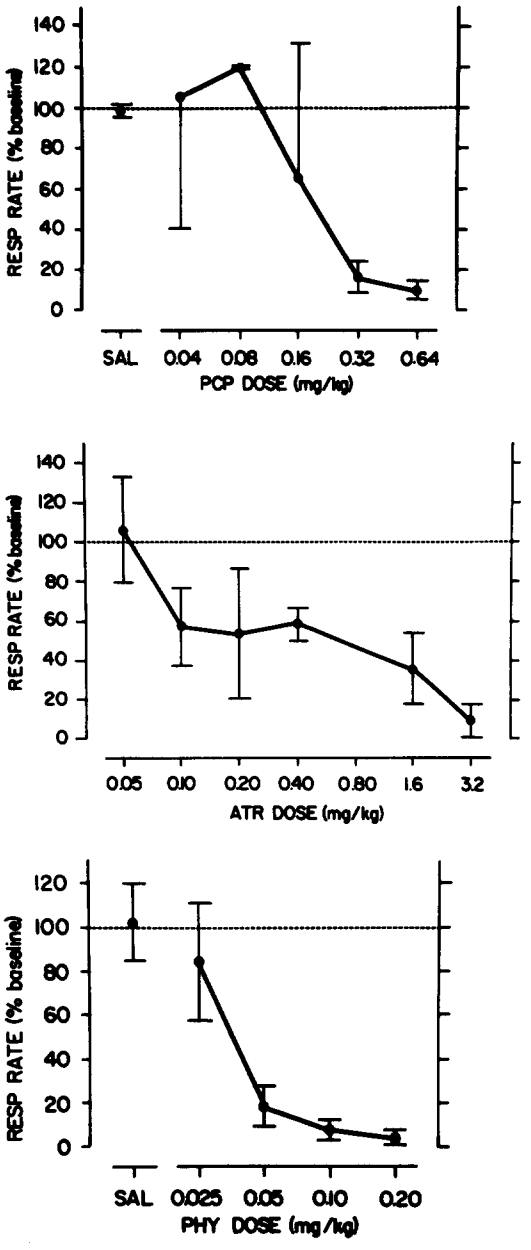

FIG. 1. Dose-response curves for phencyclidine (PCP), atropine (ATR) and physostigmine (PHY) on variable-interval responding. Each point represents the mean of one determination in each of three animals, except 0.08 mg/kg PCP, which is the mean for two animals. SAL refers to saline injection. Bars represent standard deviations.

doses of atropine and physostigmine when each was combined with saline.

DISCUSSION

The effects of phencyclidine on variable-interval responding in the present study are very similar to those reported previously in squirrel monkeys responding on the same schedule of reinforcement [9] and in rats responding on a variable-interval schedule of water presentation [26]. Low

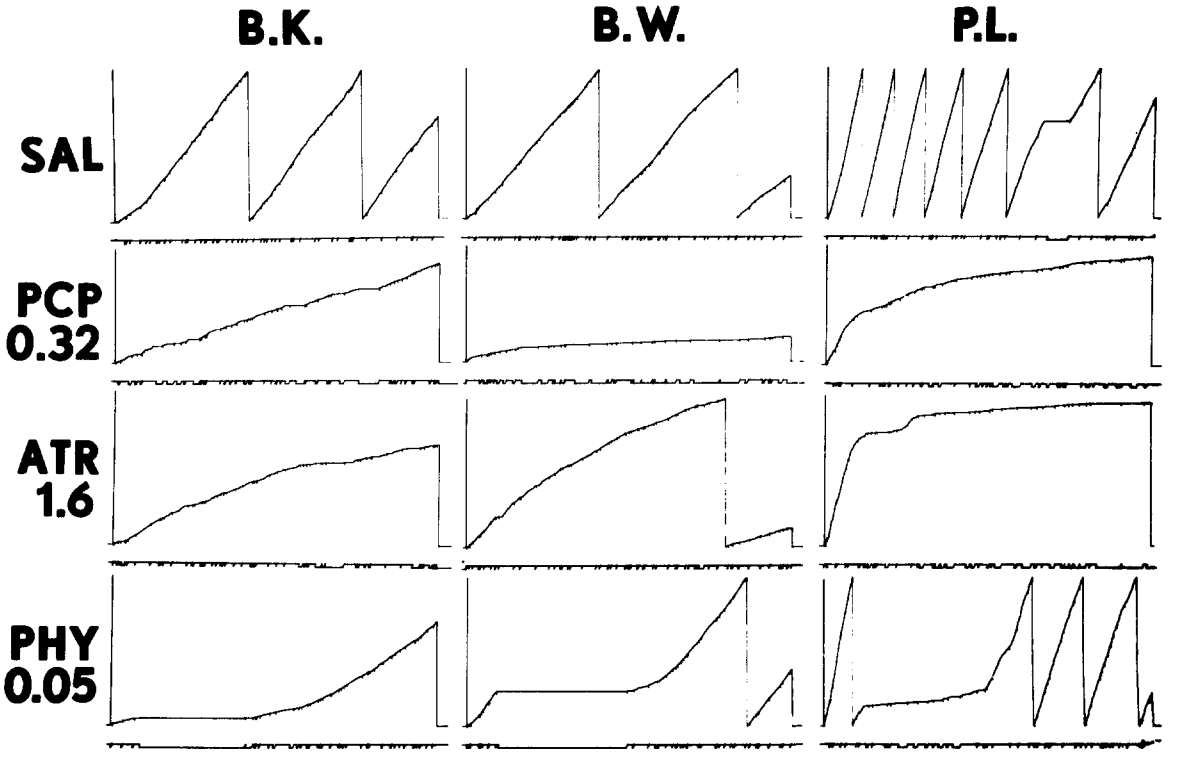

FIG. 2. Cumulative records showing responding of each animal after injection of saline, 0.32 mg/kg phencyclidine, 1.6 mg/kg atropine or 0.05 mg/kg physostigmine. Time is shown along the horizontal axis; responding along the vertical. Downward deflections along the horizontal line beneath each record indicate availability of a food pellet upon the next lever press. Diagonal deflections of the response pen indicate delivery of a food pellet. The response pen reset after every 500 responses and at the end of the session (2 hours).

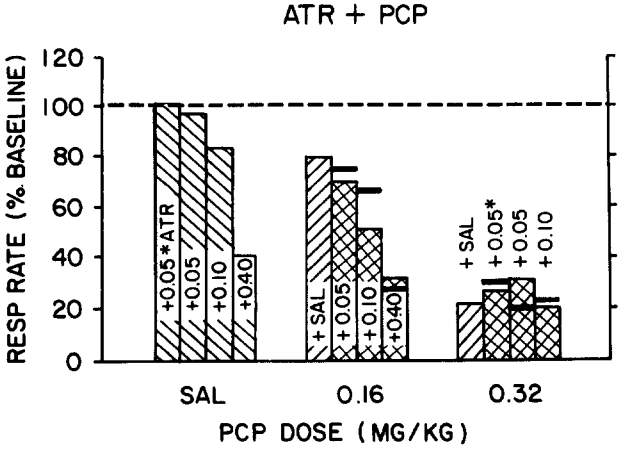

FIG. 3. Effects of atropine-phencyclidine combinations on variable-interval responding. Each vertical bar represents the mean of one determination of the indicated dose combination in each of three animals. The lefthand set of bars shows the effects of atropine given in combination with saline. Cross-hatched bars show the effects of atropine-phencyclidine combinations. Solid horizontal bars at each atropine-phencyclidine combination represent the mean rates of responding obtained by calculating the algebraic sum of the effects (as % change from baseline) of that dose of atropine combined with saline and that dose of phencyclidine combined with saline for each animal. Asterisks indicate that 0.05 mg/kg atropine was injected 90 min before the start of the session.

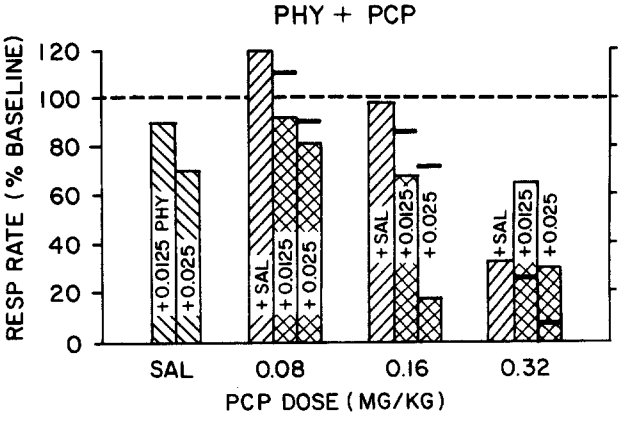

FIG. 4. Effects of physostigmine-phencyclidine combinations on variable-interval responding. See Fig. 3 for details.

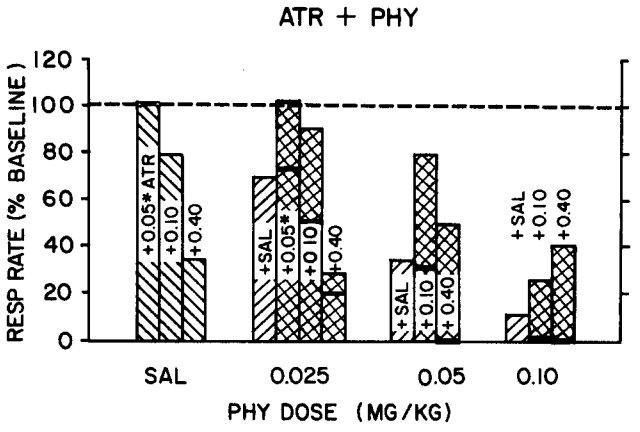

FIG. 5. Effects of atropine-physostigmine combinations on variable-interval responding. See Fig. 3 for details.

doses produce small to moderate (10–40%) increases in rates of responding, with higher doses producing decreases in responding.

Atropine produced only response rate decreases in the present study, in agreement with previously reported effects of anticholinergic agents on variable-interval responding in rats [17,18] and squirrel monkeys [16]. The failure of atropine to totally abolish responding, and the flat dose-response curve obtained in the present experiment have been noted previously [34,35], and have been attributed to the fact that the full anticholinergic effect of atropine may be exerted at low doses [23].

The relatively all-or-none disruption of operant responding and the abrupt return of baseline rates and patterns of responding after physostigmine have also been reported by Vaillant in pigeons [34,35] and in squirrel monkeys [35].

The results of the present study demonstrate that the methods employed were sensitive enough to detect an antagonistic interaction between atropine and physostigmine. No significant interaction between atropine and phencyclidine was found. Although physostigmine did show a tendency toward antagonism at the high dose of phencyclidine, the enhancement of the rate-decreasing effect of a lower dose of phencyclidine (0.16 mg/kg) by physostigmine suggests that physostigmine is not a specific antagonist of the rate-suppressant effect of phencyclidine on VI performance in this species.

Previous work in our laboratory has demonstrated that squirrel monkeys show a qualitatively different response to phencyclidine-pentobarbital combinations than do rhesus monkeys [10]. If species differences in the effects of other drugs on the response to phencyclidine can be demonstrated between rhesus and squirrel monkeys, it would not be unreasonable to assume that similar differences could exist between squirrel monkeys and other, less phylogenetically related, species. None of the studies cited in the introduction [1, 2, 3, 24, 36] in which a cholinesterase inhibitor was shown to block or reverse the behavioral effects of phencyclidine utilized squirrel monkeys. Thus, the failure of the present study to show a similar antagonism of phencyclidine by physostigmine may represent a species-specific effect.

Another likely explanation involves the differences in methodology and dependent variables measured between the present study and the others. The other studies measured such effects of phencyclidine as catalepsy, general anesthesia, motor activity, EEG hypersynchrony and emergence reactions (side effects after recovery from anesthesia). It is possible that physostigmine could attenuate or reverse these effects of phencyclidine while not antagonizing its effects on food-reinforced schedule-controlled behavior.

The effects of phencyclidine on cholinergic function are complex [24, 28, 29]. It can exert both anticholinergic and cholinesterase inhibitory activity. These two effects are antagonistic in nature. Thus, the effects of pharmacological manipulation of cholinergic activity on the effects of phencyclidine would depend upon which of these two actions is the more dominant upon the specific response being measured. In view of the substantial effects of phencyclidine on the other central neurotransmitters, it is unlikely that any type of manipulation of cholinergic function could reverse all of the CNS effects of phencyclidine. However, some of the adverse side effects of phencyclidine overdosage (for example, those which are due to central anticholinergic crisis) could possibly be successfully treated with cholinesterase inhibitors [13].

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