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BRIEF COMMUNICATION

# Acute Effects of Oral Low Doses of Pyridostigmine on Simple Visual Discrimination and Unconditioned Consummatory Acts in Rats[1]

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LIU, W.-F. *Acute effects of oral low doses of pyridostigmine on simple visual discrimination and unconditioned consummatory acts in rats.* PHARMACOL BIOCHEM BEHAV **41**(1) 251–254, 1992.—Pyridostigmine bromide (Pyr), a reversible cholinesterase inhibitor, is currently suggested to be the most effective pretreatment drug against intoxication with potent organophosphates (OP). This investigation was conducted to determine if oral low doses of Pyr would affect performance of a simple visual discrimination task, and further to assess the alterations of motor or motivational function that might underlie the performance deficits in the water-deprived rats. Rats were trained extensively on a successive light-intensity discrimination implemented with the use of a multiple schedule. The multiple schedule consisted of one fixed-ratio (FR-10) and one differential reinforcement of low rates (DRL-10 s) component, which were signalled by the 10-s discriminative stimuli of bright ($S^+$) and dim ($S^-$) houselights, respectively, in simple alternation. The light intensity difference ($S^-/S^+$) was about 0.6. Pyr, at doses (3–12 mg/kg), which did not cause overt symptoms, moderately decreased $S^+$ respondings but did not affect $S^-$ respondings. The ratio of $S^+/S^-$ respondings, an index of discrimination performance, was moderately decreased. Over the range of doses evaluated, Pyr also attenuated the corresponding water intake in a dose-dependent manner, but it did not significantly affect locomotor activity. The lowest effective doses of the above affected behaviors were virtually identical (6 mg/kg). These results suggest that the disruptive effects of a single oral low dose of Pyr on the rat operant performance involve motivational dysfunction rather than motor impairment.

Pyridostigmine Visual discrimination Operant behavior Consummatory acts Water intake Locomotor activity Cholinesterase inhibitor FR/DRL schedule

PYRIDOSTIGMINE bromide (Pyr), a reversible inhibitor of acetycholinesterase (AChE), used for the chronic treatment of myasthenia gravis (2,10), has been suggested for use in prophylaxis against intoxication with irreversible cholinesterase inhibitors (3, 4, 6, 11). In a recent study, we have demonstrated that acute oral ingestion of low doses of Pyr (≤12 mg/kg, i.e., ≤15% $LD_{50}$) to rats, which did not cause any signs of cholinergic toxicity, resulted in significant, mild to moderate decrement in controlled operant performance for water reward (13). Wolthuis and Vanwersch (15) reported a similar finding in rats, i.e., low doses of Pyr (≤10% $LD_{50}$) when administered intraperitoneally interfered with certain behavioral paradigms that involve higher CNS structures and motor function. Based on their findings, they suggested that more Pyr may cross the blood-brain barrier than is commonly believed. However, it is possible that the inhibition of AChE in the gut after both oral (PO) or intraperitoneal (IP) administration may affect behavioral performance in a nonspecific manner, since Pyr is relatively poorly absorbed from the gastrointestinal (GI) tract (2,14).

In the current experiments, our concern was to test whether ingestion of low doses of Pyr had any significant effects on visual function as assessed by measurement of operant performance on a simple light-intensity discrimination task in rats. This paradigm was an alternative fixed-ratio (FR), differential reinforcement of low rate (DRL) of water-reinforced schedule under the discriminative stimulus control of a bright or dim houselight, respectively. The results of the above experiment revealed that Pyr had an effect on this visual discrimination task, which was concomitantly accompanied by a decrease in reinforced response rate. This suggested to the author that more than specific effects on the visual discrimination might be involved. Therefore, a separate experiment on the measurement of unconditioned drinking behavior and locomotor activity in water-deprived rats was incorporated into the present investigation. This

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study was to determine whether or not the disruption of the discrimination performance might have resulted from the general effects of Pyr on motivational and/or motoric functions. The results of this ancillary experiment demonstrated that Pyr significantly decreased the unconditioned water intake, but less significantly decreased locomotor activity, in water-deprived rats. On the basis of the above findings, it is tempting to suggest that the disruptive effects of Pyr on this visual discrimination task involved motivational dysfunction rather than motor impairment. In other words, the reduced attention to the visual stimulus or the reduced performance on the discrimination resulted from a motivational reduction and not a visual discrimination decrement.

## METHOD

### *Subjects*

Male Sprague-Dawley rats weighing between 300–400 grams were used in this study. They were singly housed in polycarbonate cages in a temperature-controlled room on a 12-hour light/dark cycle beginning with lights on at 6:00 a.m. A 22-h schedule of water deprivation was used in the experiment of visual discrimination, while a 23.5-h schedule of water deprivation was used in the home cage drinking behavior and the locomotor activity measurements; food was available ad lib. These rats were run daily, beginning at 9:00 a.m., 5 days a week.

### *Drugs Employed*

Pyridostigmine bromide (Pyr) (mol.wt. = 261.4) was synthesized by the Organic Chemistry Unit of our division. Elemental analysis was satisfactory. The sample used was over 99% pure as shown by high-pressure liquid chromatography (HPLC) analysis. Pyr was dissolved in distilled water ($dH_2O$) and given as its salt. Pyr was administered by oral gavage in a volume of 5 ml/kg; the times and doses of the drug administrations are noted in the procedures for the following experiments.

### *Experiment 1: Effect of Pyr on Visual Discrimination Performance*

Four identical transparent operant chambers, each equipped with a response lever and water-dipper mechanism which delivered 0.01 ml of tap water, were used. Each chamber was housed in a larger sound-attenuated enclosure equipped with an exhaust fan which provided ventilation and a masking noise. A 10-W houselight bulb (110 V A.C.), the visual stimulus, was located above the ceiling of each enclosure. The intensity of the houselight in each chamber was controlled by a rheostat that was in series with the houselight. The intensity of the bright stimulus ($S^+$) was approximately 20 μA. A light intensity difference (dim/bright; $S^-/S^+$) of 0.6 was used in the present experiment. Events were scheduled by an Omron C-20 controller (Japan) and recorded by an Acer 1100 computer (Taiwan, R.O.C.)

Following water deprivation the rats (N = 16) were trained, by shaping procedures, to lever press for water reward. The rats were given a 1-h training session with a schedule of 30 s of bright light ($S^+$) followed by the dim light ($S^-$) for an interval of 10 s. During each $S^+$ period each response was reinforced (i.e., FR-1), while responses during the 10 s $S^-$ period postponed the onset of the next $S^+$ period by a further 10 s (i.e., DRL-10″). In the second hourly training session the $S^+$ period was reduced to 10 s. Thereafter, in successive sessions, the number of responses required for reinforcement was increased to 10 (FR-10). At this stage of the study the program, namely the multiple, alternative FR-10/DRL-10″ operant behavioral paradigm, was in use, and each session was then extended to 2 h. The rats were trained on this schedule until they reached a steady level of performance. Test sessions were conducted on Mondays through Fridays. Each animal was tested at the same time of day and in the same chamber. These rats were habituated to handling and to oral gavage before the Pyr dosing. However, one rat was eliminated due to excessive variability and low rates of responding, leaving 15 rats. These 15 rats were randomly assigned into five groups of 3 (N = 3/group) and treated with 0 ($dH_2O$), 3, 6, 9, and 12 mg/kg Pyr, respectively, orally by gavage immediately prior to the experimental sessions. The rats were normally dosed with Pyr on Fridays, and the performance on Thursdays served as control data. To maintain a more normal water/food balance, an additional 10 min of free access to drinking water was given in the home cage following the operant sessions.

Three parameters of operant responding were monitored: 1) the total number of lever presses during the $S^+$ periods (i.e., reinforced responses); 2) the total number of lever presses in the $S^-$ periods (i.e., nonreinforced responses); and 3) the ratio of $S^+/S^-$ responses (i.e., an index of the animal's visual discrimination performance). Performance on the day prior to the dosing was used to determine the nongavage control values; data were then expressed relative to performance during these sessions (percent of control).

### *Experiment 2: Effect of Pyr on Water Intake and Locomotor Activity*

This study was undertaken to determine whether the disruption of visual discrimination might be associated with an impairment of drinking motivation and/or locomotor function. The design of this study was a repeated measures for water intake and locomotor activity, in chronic subjects.

Nine rats were exposed to a 23.5-h water deprivation schedule such that they received 30 min of access to tap water each day, and every Friday they were also monitored for 5 min of locomotor activity, immediately after the 30-min drinking session. Total daily intakes were calculated (to the nearest 0.1 g) from the drinking bottle weights determined before and after a 30-min access period. After both the daily water consumption and the locomotor activity on Fridays had stabilized (i.e., both were stabilized after 5 weeks in the present experiment), Pyr treatment was initiated. In order to habituate the rats to the gastric intubation procedure, 5 ml/kg $dH_2O$ was administered orally 1 h prior to the drinking session on the test days (Fridays).

The locomotor activity of each individual rat was determined using a Stoelting Electronic Activity Monitor (Model 31409, Stoelting Company, Chicago, IL). This system essentially measures general motor activity by providing a digital output proportional to the animal's movements within a radio frequency field. The apparatus was set at 0.7 mA normal sensitivity and threshold reset time, and an activity level of 15. Immediately after the 30-min drinking session of Fridays, each rat was placed into a Plexiglas box (32 × 25 × 35 cm) centered on the sensing platform. Activity was recorded for 5 min.

Each rat was tested with each of the four doses of Pyr (3, 6, 9, and 12 mg/kg) or $dH_2O$ in a randomized sequence. The experiment was conducted once a week (on Fridays) in order to insure a stable baseline of locomotor activity. The day one week prior to the initiation of Pyr administration was used as the control level of locomotor activity for each rat.

### *Data Analysis*

Overall analysis of the behavioral data, transformed into arc-

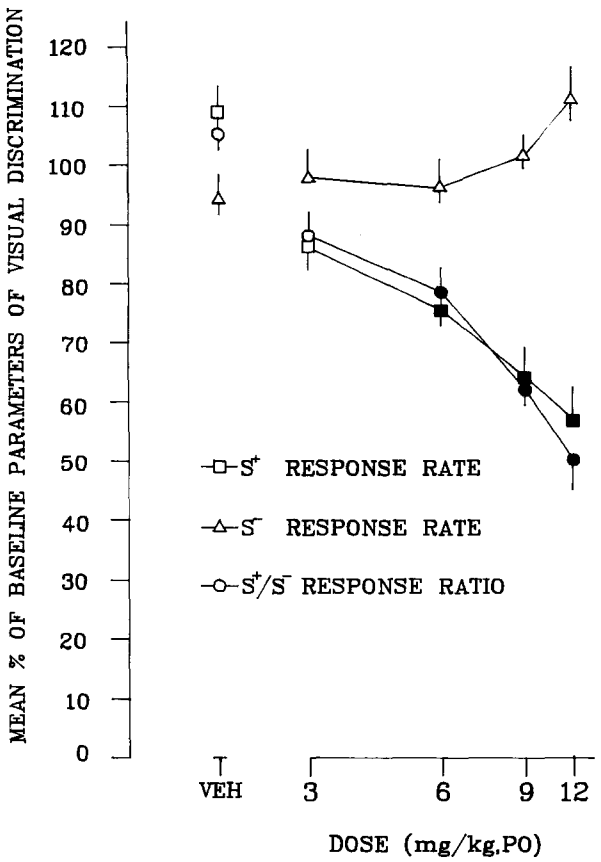


FIG. 1. Effects of oral pyridostigmine bromide on visual discrimination in water-rewarded rats. Each point represents the mean (±SEM) percentage change from the pregavage baseline response rates of the 120-min session. The solid symbols indicate significant differences from control values ($p$'s<0.05) determined by the Newman-Keuls test. N=3/dose group.

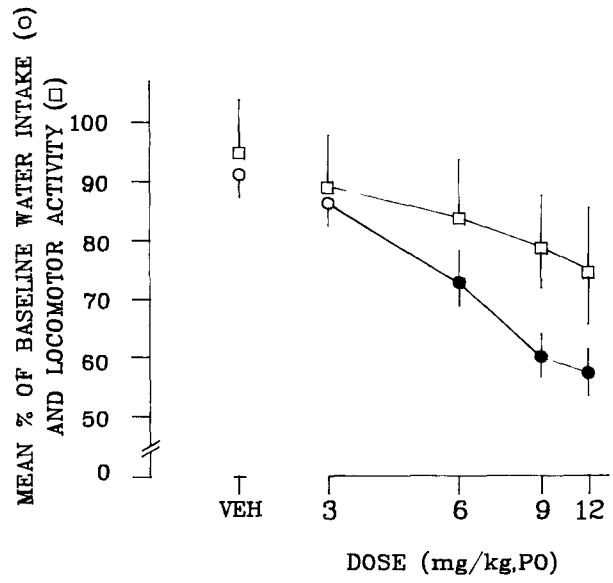


FIG. 2. Effects of oral pyridostigmine bromide on unconditional water intake and locomotor activity in water-deprived rats (N=9). Pyr was administered 1 h prior to a 30-min drinking session. The baseline water intake was averaged as 21.5±1.9 g/30-min. The solid circles represent significant differences from $dH_2O$ control ($p$'s<0.05) determined by the Newman-Keuls test. Pyr was administered 1½ h prior to a 5-min locomotor activity measurement. The baseline activity level was averaged as 289±25 counts/5 min. Repeated measures ANOVA showed no significant Pyr effect on locomotor activity.

sines, for treatment effects was initially carried out using the appropriate ANOVAs. Significant ($p$<0.05) effects were subsequently analyzed using the Newman-Keuls test to compare the control mean with the multiple treatment means. Significant treatment effects were accepted only at $p$<0.05. Regression analysis was also performed on all data sets for each measure to determine whether dose-related linear trends were present. Doses of the drug that decreased behavior to 50% control levels ($ED_{50}$) were determined by extrapolation from the descending, linear portion of the dose-effect functions. Confidence limits (95%) were calculated as $ED_{50} \pm t \times SE$.

## RESULTS

The overall baseline response performance for the $S^+$ period and the $S^-$ period, and the $S^+/S^-$ response ratio for the total 2-h session for the 15 rats were averaged to be 1916±167 (range 1125–2798), 41.7±3.8 (range 29–69) and 46.0±5.4 (range 23.7–64.4), respectively.

The effects of Pyr on these three parameters, expressed as the percent of baseline control on the preceding nongavage control day, is shown in Fig. 1. Pyr dose-dependently decreased both the $S^+$ response rate, $F(4,10)=19.8$, $p$<0.001, and the $S^+/S^-$ response ratio, $F(4,10)=17.3$, $p$<0.001, but did not affect $S^-$ response rate, $F(4,10)=1.5$, $p$<0.05. Both the $S^+$ response rate and $S^+/S^-$ response ratio were significantly less than the $dH_2O$ control at doses of 6–12 mg/kg. No overt signs of cholinergic toxicity were observed following these doses of Pyr treatments. $ED_{50}$ values for suppression of $S^+$ response rate and $S^+/S^-$ response ratio were calculated as 17.1 (9.3–14.9) mg/kg and 12.1 (6.9–17.2) mg/kg, respectively. Obviously, the $ED_{50}$ of $S^+$ response suppression just falls outside of the 95% confidence limits of the $S^+/S^-$ response ratio.

The effects of low doses of Pyr (3–12 mg/kg) on water intake and general locomotor activity of the water-deprived rats are shown in Fig. 2. Pyr decreased water intake in a dose-dependent manner, $F(4,32)=20.5$, $p$<0.001, but did not significantly decrease locomotor activity, $F(4,32)=1.9$, $p$>0.05. The water intake was significantly less than the vehicle control at doses of 6–12 mg/kg. $ED_{50}$ value for suppression of water intake was calculated as 18.9 (12.6–25.3) mg/kg.

Qualitatively similar patterns of dose-response curves for suppression of $S^+$ response rate, $S^+/S^-$ response ratio and water intake were produced by Pyr (Figs. 1 and 2). As noted above, the confidence limits for the $ED_{50}$ of Pyr on the suppression of water intake overlapped those on the $S^+$ response rate and $S^+/S^-$ response ratio; as a result the three behavioral measures were equally sensitive to Pyr even though the $ED_{50}$ for $S^+/S^-$ response ratio appeared to be lower than for suppression of water intake.

## DISCUSSION

The results of the present study demonstrate that orally administered low doses of Pyr (3–12 mg/kg), which did not cause

any overt toxic symptoms, produced dose-dependent decreases in the rate of FR responding for water reinforcement under a simple light intensity discrimination task. The effects are consistent with our previous report on rat operant responding under a multiple fixed-ratio, time-out schedule (13). Over the range of doses assessed, Pyr also reduced the corresponding unconditioned water consumption in a dose-dependent manner in water-deprived rats, but it did not significantly affect locomotor activity. These data suggest that the reduction of drinking motivation plays the major role for the disruption of this simple light intensity discrimination task, whereas the Pyr's motoric effect is of little functional consequence on the operant performance. This assumption is based mainly upon the previous findings that the doses of Pyr needed to produce a suppression of motor activity, such as open-field behavior and ambulatory activity, are about one-half lower than those produce an impairment in the motor performance of locomotor tasks that involve more skilled movements, such as the performance of accelerating rotarod, hurdle stepping and shuttle-box avoidance (7,15). In fact, a small increase in the $S^-$ response rate, although statistically nonsignificant, with the highest dose of Pyr (see Fig. 1) may reflect an absence of motor response impairment after low doses of Pyr administration. Therefore, the debilitating effects of a single oral low dose of Pyr on the operant performance are not likely to result from alterations in visual perception and/or motor function, but are most likely attributed to the motivational dysfunction by unknown physiological mechanism(s).

Several studies of clinical safety evaluation have reported that a single oral dose of 60 mg has no deleterious effect on stationary visual function (9), but an oral dose of 30 mg Pyr at 8-hour intervals, i.e., the recommended effective pretreatment regimen against organophosphate poisoning (4), has a minimal effect on performance of a visual-motor coordination task (1) in normal human subjects. In a recent study, Glikson et al. (5) reported that Pyr does not produce any significant neuromuscular effect in healthy subjects when taken in a dosage of 30 mg three times daily for 8 days, causing 20–30% inhibition of cholinesterase. However, both the single and the repeated therapeutic dosage regimens can also cause a mild to moderate gastrointestinal (GI) disturbance in a percentage of normal human subjects. In addition, a preclinical safety assessment has also reported that chronic administration of oral Pyr at doses as low as 0.05 mg/kg to dogs does have local effects on GI tract (10). Taken together, these data suggest Pyr may affect motivated psychomotor performance through its anticholinesterase actions on the GI tract related to enhanced gut motility, since Pyr has poor systemic bioavailability by this route (2,14). However, the locus of the site of action to the GI tract remains a matter of speculation.

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