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RAPID COMMUNICATION

Pentylenetetrazole Can Induce a Conditioned Place Preference

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GAUVIN, D. V., K. N. DORMER AND F. A. HOLLOWAY. *Pentylenetetrazole can induce a conditioned place preference.* PHARMACOL BIOCHEM BEHAV **40**(4) 987–990, 1991.—Thirty-two male Sprague-Dawley rats were randomly selected into 4 groups (n = 8/group) and conditioned in a standard place preference task. The groups differed in the dose of pentylenetetrazole (PTZ) administered prior to conditioning trials. With respect to the three treatment groups, placement into the, initially, nonpreferred side of the CPP apparatus was preceded by injections of 5.6, 10, or 17.8 mg/kg PTZ. The control group was injected with hypertonic saline (1.8% w/v) on the rats', initially, nonpreferred sides and isotonic saline (0.9% w/v) on their preferred sides, to control for any irritative effects of PTZ injections in the treatment groups. Six pairs of drug-saline conditioning trials were conducted with each subject. PTZ produced a dose-dependent increase in the amount of time spent in the drug-associated environment. Saline control subjects' preference scores did not change over the course of the study. These data suggest that PTZ is not aversive in the place learning task; more importantly, the data suggest that a dose-dependent shift in the hedonic valences associated with environmental stimuli can occur when these stimuli are repeatedly paired with PTZ administration. The data are discussed in terms of the stimulus properties of PTZ and the hypothetical "anxiety" state the drug may produce.

Pentylenetetrazole Rats Conditioned place preference Classical conditioning

PENTYLENETETRAZOLE (PTZ) is a synthetic CNS stimulant which has been widely used as a laboratory tool for the screening of anticonvulsant drugs (14,31). The discriminative stimulus properties of PTZ have been used as an animal analogue of human anxiety [cf. (21, 22, 30)].

Using the PTZ vs. saline drug discrimination task as a preclinical screening tool, a number of laboratories have reported that drug-withdrawal states characterized as "anxiety producing" in humans generalize (i.e., produce PTZ-appropriate responding) to the PTZ cue in rats. These include diazepam withdrawal (7, 8, 23), morphine withdrawal (9), nicotine withdrawal (19), chronic (1) and acute (15) ethanol withdrawal, and cocaine withdrawal (40). Recently, Carey et al. (4) have shown generalization of a PTZ discriminative stimulus both to novel exteroceptive stimuli (i.e., floor, home intruder) and to a classically conditioned anxiogenic stimulus (tone associated with a CER task). We have recently reported cross-generalization of a PTZ discriminative stimulus to exposure to a natural predator (17). Leidenheimer and Schechter (25) have also shown similar results using the benzodiazepine inverse-agonist FG7142, and cross-generalization to the interoceptive state induced by both PTZ and 20-min exposure to inescapable shock. This impressive data base strongly supports the view that PTZ discrimination in rats parallels the subjective reports of anxiety in humans.

We have recently reported the results of a three-choice drug discrimination task using PTZ, saline, and chlordiazepoxide as training stimuli (18). We have suggested that the discrimination is based on an opponent-process between competing stimuli along a single continuum, similar to the benzodiazepine/β-carboline spectrum described by Nutt (29). We believe the data, described above, supports our view that the PTZ drug discrimination task provides a behavioral assay for the measurement of the physical domain of pharmacological stimuli which corresponds to a hypothesized psychological "affective" metric space best categorized as a single continuum bounded on one end by "anxiety," and "anxiolysis" on the opponent end.

One argument against this proposed model of affective states is based on the notion that the PTZ discrimination is based on another hypothetical continuum, for example, euphoria/dysphoria. Since all of the drug withdrawal states that have generalized to the PTZ discriminative cue in rats (discussed above) are generally believed to be "dysphoric" in human drug abusers experiencing the withdrawal, it is possible that the PTZ cue reflects a generic "dysphoric" state in rats.

One preclinical behavioral assay which might address the proposed euphoric/dysphoric dimensionality of the PTZ discrim-

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inative stimulus is the Place Learning paradigm. This specific assay is sensitive to both negatively and positively hedonic valences of stimuli. It is based on the assumption that animals will approach stimuli which are, or have been associated with, hedonically positive stimuli. Conversely, animals will avoid stimuli which are, or have been associated with, hedonically negative stimuli [see (2) or (39) for details].

The purpose of the present study was to examine the hedonic valence associated with PTZ administration in rats. A range of subconvulsant doses of PTZ (5.6, 10, and 17.8 mg/kg) was selected which encompassed the normal range of training stimuli in the more typical PTZ vs. SAL drug discrimination task. If PTZ induces a "dysphoric" interoceptive stimuli, a conditioned place aversion should result; if, on the other hand, PTZ induces a positively hedonic state, then a conditioned place preference should result. Finally, if PTZ administration is associated with a hedonically neutral state, no shift in the rats' initial preference scores should occur. Therefore, the Place Learning paradigm appears to be a sensitive assay for all three hypothesized drug-induced affective states.

METHOD

Subjects

Thirty-two male Sprague-Dawley rats were purchased from Sasco, Inc. (Omaha, NE) and grouped housed in clear Plexiglas "shoe-box" cages in a colony room maintained on a 12-h light/dark cycle (lights on at 0630 h). Food and water were continuously available in the home cages. The colony room and animal care were maintained by an AAALAC-accredited team of technicians from the Department of Animal Resources (University of Oklahoma). Animals were randomly assigned to groups upon arrival. Eight rats were assigned to each of four groups which differed in the dose of PTZ administered during conditioning trials [0 (saline), 5.6, 10, and 17.8 mg/kg].

Place Conditioning

The place preference apparatus consisted of two main "conditioning compartments" ($40 \times 16 \times 24$ cm), separated by a small 10-cm alleyway partition and connected to each other by a third compartment ($10 \times 16 \times 24$ cm) in a straight alleyway configuration. the apparatus was constructed of Plexiglas (Cope Plastics, Oklahoma City, OK); the hinged top was clear Plexiglas, the walls were double-walled clear Plexiglas with sliding black Plexiglas inserts. The central chamber (Compartment 2) had a clear Plexiglas floor with gray walls. The two conditioning compartments had the following distinguishing stimulus characteristics. In Compartment 1 the walls were decorated with white and black vertical stripes with stainless steel bars for flooring (1 cm bar-to-bar). In Compartment 3 walls were decorated with white and black horizontal stripes with a wire-mesh floor. The apparatus was cleaned between rats by removing feces and wiping the walls, ceiling, and flooring with warm soapy water. Sets of removable guillotine doors could be inserted between individual compartments at the beginning of each session. The small partial-walls in the alleyways, separating each of the three compartments, remained in place during the conditioning sessions to allow for a clear, detectable, quantitative change in location of the rat from one compartment to the other. The number of compartment entries, time in each compartment, and general activity of the subjects were assessed and recorded by sets of infrared photobeams located near the floors of each compartment and linked by a photobeam controller (DIG-723, Med Associates, Inc., East Fairfield, VT) to a Commodore-64C microcomputer system. The microcomputer system controlled the experimental contingencies and recorded all measures from four sets of conditioning chambers simultaneously (American Neuroscience Research Foundation, Yukon, OK).

The first four days of the procedure consisted of a preexposure period with the guillotine doors removed, to allow for free access to all three compartments. Each rat was placed separately into the apparatus for 20 minutes. On the fifth day, a 20-minute "habituation" test session was conducted. The central compartment was sealed off from the two distal compartments by the placement of the guillotine doors. Initially, each rat was sequestered into this small central compartment. Once the computer program was initiated the guillotine doors were removed which completed a photobeam sensor circuit and started recording time, activity, and chamber data. The rats had free access to all three compartments. The relative time spent in each of the two conditioning compartments were calculated for each rat (total time spent in Compartments 1 and 3). The "preference" score was calculated (the total time spent in the least preferred compartment ÷ total time spent in Compartments 1 plus 3) and expressed as a percentage. This initial "habituation" test session data was used to compare changes in individual preferences/biases for one end of the compartment over the other. The most nonpreferred compartment was designated as the conditioned stimulus (CS+) side, which would be paired with PTZ during the following conditioning trials. The alternate compartment was paired with saline.

The PTZ/SAL pairing trials began on the day following the "habituation" test session. On alternate days, each rat in the treatment groups were administered an intraperitoneal dose of 5.6, 10, or 17.8 mg/kg PTZ (dependent on group assignment) on one day, and saline on the next day. The control group subjects received hypertonic saline on the nonpreferred side on one day, and isotonic saline in the preferred side on the alternate day. Hypertonic saline was treated as a "drug" condition in the this group to control for any abdominal irritation PTZ may produce in the drug treatment groups. Immediately after drug or saline injections, each rat was placed into the conditioning chambers for 20 minutes. Black Plexiglas partitions were inserted into the guillotine door guides to sequester each rat within one of the two conditioning compartments. PTZ or hypertonic saline was paired with the placement of the rat in the initially nonpreferred compartment (CS−). Immediately after each session, rats were returned to their home cages. Each rat received a total of 12 stimulus/environment pairing sessions (6: PTZ, 6: saline) and was then tested, undrugged, for the "final" side preference as described above.

Drugs

Pentylenetetrazole and sodium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Hypertonic saline solution (1.8% w/v) was prepared by adding 900 mg of sodium chloride to 100 ml of 0.9% normal sterile saline. All solutions were injected intraperitoneally on alternating sides of the abdomen in volumes of 1 ml/kg of body weight.

Data Analysis

The total time of the 20-min undrugged free-access test session spent in the nonpreferred compartment ÷ the total time spent in both conditioning compartments was expressed as a percentage, and is used as a measure of "side preference" and infers a hedonic valence for the environmental cues of the compartment. Data were analyzed using a two-factor (group × time)

independent group analysis of variance with Duncan's New Multiple Range test for a posteriori individual group comparisons. Additionally a paired t-test compared the change in the final (conditioned) test preference scores from the initial (habituation) preference scores. Statistical significance was set at $p<0.05$

RESULTS

Figure 1 shows the group mean (S.E.) of the initial (habituated) side preference scores (top panel) and the final (conditioned) preference scores (bottom panel) for all four groups of rats. The initial side preference scores did not significantly differ across groups, $F(3,56)=0.13$, all Duncan's p's>0.05. However, conditioning with PTZ resulted in a dose-dependent increase in the preference scores [bottom panel, Group factor: $F(3,56)=3.42$; Time factor: $F(3,56)=20.6$]. The groups conditioned with either hypertonic saline or 5.6 mg/kg PTZ did not result in a significant shift in preference scores [saline group: $F(1,56)=0.28$; $t(7)=0.06$; 5.6 PTZ: $F(1,56)=2.2$; $t(7)=0.45$]. The group conditioned with 10 mg/kg PTZ and 17.8 mg/kg PTZ significantly increased the amount of time spent on the CS+ (initially nonpreferred) side of the apparatus [Duncan's p's<0.01; 10 PTZ: $F(1,56)=11.9$; $t(7)=3.0$; 17.8 PTZ: $F(1,56)=21.7$; $t(7)=5.9$].

DISCUSSION

The results of the present study clearly demonstrate that PTZ is not aversive in the Place Learning paradigm; in fact, the results suggest a dose-dependent increase in the positively hedonic subjective effects produced by PTZ administration in rats. It is interesting to note that the dose engendering the largest shift in positive preference scores, in the present study, is the same dose usually used in PTZ-SAL drug discrimination studies, which have been characterized as an animal analogue of human anxiety (see Introduction). A review of the literature found supporting evidence that the subjective/physiological effects of PTZ are not inherently negative.

Irwin and Benuazizi (20) have reported that PTZ facilitated memory consolidation and learning in mice. These results are not surprising if one accepts on an intuitive level that the doses of PTZ used in this study induced low to moderate levels of interoceptive stimuli which are similar to human symptoms of "anxiety." Yerkes and Dodson (41) were probably the first researchers to report a positive relationship between anxiety and performance. Since that time, a number of psychologists have found supporting evidence for the positive effects of low to moderate levels of anxiety and task performance (5, 6, 13, 24, 28, 32, 33, 38) and cognitive functioning (3,11).

Prior studies in our lab and others have found that doses lower than 15 mg/kg (Sprague-Dawley rats) or 17.8 mg/kg (Long-Evans rats) do not effectively function as discriminative stimuli (personal observation and Emmett-Oglesby, personal communication). These observations suggest that the doses usually used as discriminative stimuli are just above threshold doses and therefore, would only induce a low to moderate level of a hypothesized "anxiety" state. Moderate levels of anxiety have facilitated learning in simple conditioning experiments (38). This facilitation may be due to the enhanced processing of simple sensory stimuli [cf. (10,11)]. The Place Conditioning task utilized in the present study is theoretically based on learning simple associations between subjective interoceptive stimuli and the environment in which they are perceived. It may be that: 1) PTZ enhances the physiological transmission of sensory stimuli (12,26); and/or 2) PTZ induces an interoceptive state, similar to human

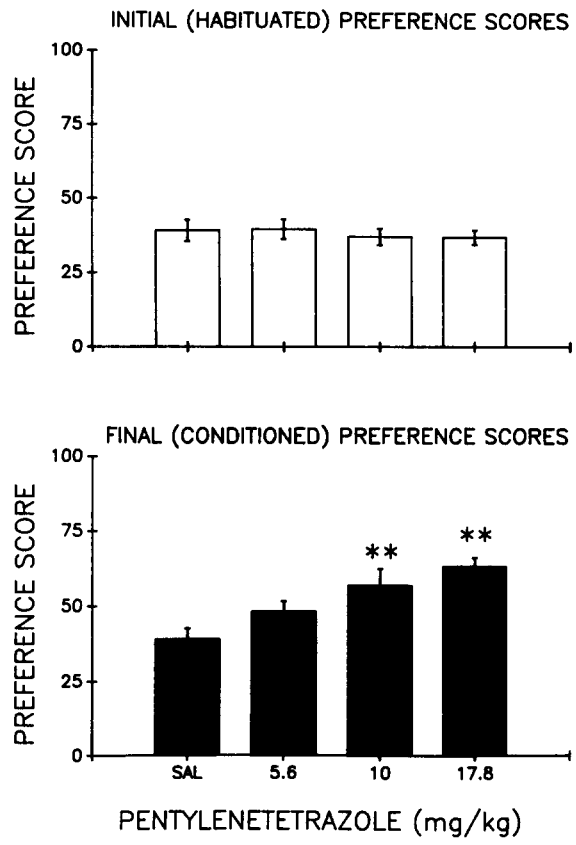

FIG. 1. Mean (S.E.) preference scores for four groups of rats prior (top panel) and after (bottom panel) place conditioning in which unique environmental stimuli were paired with various doses of pentylenetetrazole or saline. The total time of a 20-min session spent in the nonpreferred compartment ÷ total time spent in both conditioning compartments is expressed as a percentage, and is used as a measure of "side preference." Side preference infers a hedonic valence for the environmental cues of the compartment. Initial side preferences/biases (top panel) did not differ between groups and were used to determine which of two compartments would be paired with PTZ. After PTZ-SAL pairings, groups were retested for side preference (bottom panel). A significant, dose-dependent increase in preference scores resulted from the place conditioning task (**$p<0.01$).

"anxiety" which, in turn, acts as a motivational "drive" state (34,35) to enhance the conditioning of paired associations.

We believe the data from the present study and those previously conducted in our lab (16, 18, 27) strengthen the framework of the hypothetical animal model of human "anxiety" using the discriminative stimulus properties of PTZ. Further, these data support the view that the interoceptive stimuli produced by PTZ administration is not inherently dysphoric. Spyraki and colleagues (36,37) have reported that the benzodiazepine, diazepam, can also induce a conditioned place preference. Therefore, it seems that both an agonist (diazepam) and antagonist (PTZ) at the GABA-benzodiazepine-Cl^- ionophore complex can produce similar hedonically positive affective states in rats as measured by the conditioned place preference assay. These data suggest that both of these compounds can lie within similar domains of a hypothesized "euphoria/dysphoria" continuum. We believe these data weaken the argument that PTZ-induced stimuli inherently lie at the extreme "dysphoric" end of this affective continuum.

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