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RAPID COMMUNICATION

Cross-Generalization Between an Ecologically Relevant Stimulus and a Pentylenetetrazole-Discriminative Cue[1]

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GAUVIN, D. V. AND F. A. HOLLOWAY. *Cross-generalization between an ecologically relevant stimulus and a pentylenetetrazole-discriminative cue.* PHARMACOL BIOCHEM BEHAV **39**(2) 521–523, 1991.—Twelve male Sprague-Dawley rats were trained in a two-choice pentylenetetrazole vs. saline drug discrimination (DD) task under an FR10 schedule of food reinforcement. Two-minute reinforced test sessions with the two training stimuli engendered exclusive injection-appropriate responding. Rats were injected with saline and then exposed for 20 min to the presence of a domestic cat pretreated with catnip. Immediately following the predator exposure, rats were tested for stimulus generalization in the DD task. The predator/prey interaction engendered 92% PTZ-appropriate responding. These data suggest that the interoceptive state associated with species-specific defense reactions in rats is similar to the cues produced by a pharmacological agent within a behavioral assay which has been suggested as an animal model of human anxiety.

Pentylenetetrazole Drug discrimination Anxiety Predator-prey

THIS laboratory has previously reported data suggesting that the interoceptive (subjective) effects of pentylenetetrazole (PTZ) and chlordiazepoxide (CDP) reflect opponent dimensional domains of a single continuum, similar to the benzodiazepine/β-carboline spectrum described by Nutt (18). The discriminated operant [lever choice, (17)] of these drug-dependent cues has been found to covary with drug dose and to interact by mutual behavioral/pharmacological antagonism (10, 11, 17). Lal (12) and Lal and Emmett-Oglesby (13) have suggested that the interoceptive effects of PTZ may function as an animal analogue of human anxiety. Drugs and drug-withdrawal states characterized as "anxiety producing" in humans generalize to the PTZ cue in rats [cf. (7)]. Recently, Carey, Fry and White (6) have shown generalization to a PTZ-discriminative stimulus by both novel exteroceptive stimuli (i.e., floor, home intruder) and a classically conditioned "anxiogenic" (6) stimulus (tone associated with a CER task). Leidenheimer and Schechter (15) have also shown similar results using the inverse-agonist FG7142 to produce an interoceptive stimulus which demonstrated symmetrical cross-generalization to a PTZ cue (14) and to the interoceptive state induced by 20-min exposure to inescapable shock. This impressive data base suggests that the PTZ-discriminative cue, when trained in rats, produces generalization profiles to a range of interoceptive states which, on an intuitive basis, appears similar to "anxiety states" in humans. We have recently proposed an "affective state model" of drug discriminative control. Using a three-choice chlordiazepoxide, PTZ, saline drug discrimination task, we proposed a model which would predict that the affective basal conditions [adaptation level, AL; (11)] of the experimental subject would alter the response topography by shifting the operating range of the stimuli along a hypothesized psychological (affective) metric space. For example, if DD-trained subjects were tested with saline under "anxious" affective baseline conditions, PTZ-appropriate responding would be predicted.

One area that has not been adequately addressed in this animal model of human anxiety is those species-specific defense reactions (SSDR's) which elicit autonomic behavioral and physiological responses in rats and, indeed, in most mammals, which correspond to significant alterations in human physiological systems associated with 1) subjective reports of anxiety, 2) opiate-based analgesia, and 3) fight-or-flight responses (4, 5, 9). The perceptual-defensive-recuperative (PDR) model proposed by Lichtman and Fanselow (16) states that a defensive motivational state (i.e., an anxiety- or fear-like state as evidenced by height-

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ened emotional reactivity defined as increased vocalizations when handled, increased urination and defecation, or freezing) is sufficient to activate endogenous antinociceptive mechanisms as well as defensive behaviors. These defensive systems are activated by either an innate danger signal or learned danger stimuli and produce antipredator behaviors (SSDR's) and their associated internal subjective states. Blanchard et al. (4) have suggested that SSDR's can be used: 1) to understand the biology of defense, the neuroanatomic and neurochemical systems involved, and their relationships to both individual behaviors and to the process whereby one behavior gives way to another; 2) if important clinical problems such as depression and anxiety reflect changes, perhaps very complex changes, in the defense systems, then an understanding of these conditions can be facilitated by the creation of animal models which show important behavioral isomorphisms with the symptoms of the clinical condition; and 3) when creating tests for the evaluation of pharmacological agents which might alleviate the disorder, which can be done without understanding either the disorder or its biology, using a strategy which simply identifies compounds which produce the same pattern of results in laboratory tests (anxiety) and a criterion compound with known clinical efficacy to reduce it (anxiolytics).

The present study was undertaken to test the cross-generalization of a PTZ-discriminative cue to an ethologically relevant stimulus (domesticated cat) which has been well documented in the literature to produce significant SSDR's in the rat.

METHOD

The seminal work on SSDR's conducted at the Bekesy Laboratory of Neurobiology by Blanchard and colleagues (2–4) has focused on a number of critical factors required to induce significantly compromised behavior of rats (prey) in a predator/prey relationship. The predator/prey exposure utilized in the present study was based upon this body of research.

Drug Discrimination Task

Twelve male Sprague-Dawley rats were trained in a two-choice drug discrimination task using 15 mg/kg PTZ and saline. Rats were trained 6 days per week in 10-min lever-press, food-motivated, experimental sessions under an FR-10 schedule of reinforcement in standard operant chambers enclosed in sound-attenuating cubicles (Lafayette Inst., Lafayette, IN). The correct lever to earn food reinforcement was cued by the injection administered 15 min before the experimental session. Experimental contingencies were controlled and monitored by Commodore 64C microcomputer systems interfaced to the operant chambers (American Neuroscience Research Foundation, Yukon, OK). Training continued until each animal emitted less than 20 responses prior to the delivery of the first reinforcer and greater than 90% of the sessions' responses were emitted on the stimulus-appropriate lever. Each animal had to meet these training criteria for 6 consecutive days (i.e., SAL-PTZ-SAL-SAL-PTZ-PTZ) prior to testing. Two-minute reinforced test sessions were used for this protocol. Prior tests with predator exposure demonstrated that sessions of 5 min in length resulted in rats responding to the PTZ-appropriate lever for the first 3–4 min and then shifting to the saline lever for the rest of the session. This response topography was believed to be the result of learned environmental "safety cues" (i.e., the operant chamber, food deliveries, etc.) reducing the subjective experience elicited by the test situation. The two training cues were tested (SAL and 15 mg/kg PTZ) and compared to the internal state produced by a SAL injection administered immediately prior to a 20-min exposure of 12 rats to a predator/prey situation.

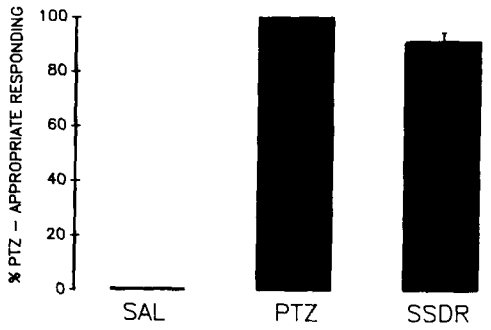

FIG. 1. Percentage of total session responses emitted on the PTZ-appropriate lever during 2-min reinforced test sessions. Rats were tested with saline, the training dose of PTZ, or saline prior to 20-min exposure to the presence of a domestic cat (SSDR).

Predator/Prey Exposure

Strict adherence to NIH guidelines for the care and use of animals in research governed the design of this protocol. First, this protocol did not allow any physical contact between predator and prey. Second, each rat received only one exposure to the predator/prey situation. Reviewing the literature revealed at least two critical factors associated with induction of SSDR's in rats by exposure to a domesticated cat: 1) the cat must be moving; the mere sight and/or smell of a cat is insufficient; and 2) olfactory cues from other rats help to elicit SSDR's. Based upon the above limitations, four rats were placed in a plastic (shoebox) housing cage with approximately 1.5 cm of hardwood chips in the bottom of the box to allow for defensive burying reactions. The top stainless steel closure device was installed and the entire plastic box was placed in a larger ($6 \times 10 \times 11$ cm) clear Plexiglas enclosure. A domesticated cat was borrowed for one day of laboratory exposure and then returned to its permanent place of residence. Since the cat's permanent residence was a local small farm, it had an extensive history of predator/prey relationships (this was not necessary to induce SSDR's in the rat). To increase the level of arousal and stimulate interaction with the sequestered rats, the cat was pretreated for 10 min with an ecologically relevant stimulant (catnip) prior to predator/prey exposure. Preliminary tests with other rats resulted in both the cat and rats sleeping through the entire session once the subjects learned of their sequestered environment (safety cues). Predator and prey were observed for the 20-min exposure time.

RESULTS

The 10-min exposure of the cat to the catnip significantly increased the amount of time the cat spent investigating, prowling, and pouncing in the presence of the rats. The rats were behaviorally excited, and the attacking cat elicited defensive burying into the wood chips. During the 20-min exposure, rats continually attempted to bury themselves under the other rats, producing a frenzied "push and shove" between rats. Figure 1 shows the results of all three tests in the drug discrimination task. Saline produced less than 1% PTZ-appropriate responding, whereas 15 mg/kg (the training dose) of PTZ engendered 100% PTZ-appropriate responding. More importantly, 20-min exposure to the predator/prey relationship engendered 92% PTZ-appropriate responding in ten rats. Two of the rats failed to respond during the discrimination test session, due to the expression of more

severe behavioral SSDR's (i.e., freezing, defecation, urination) which lasted for the entire two-min test session.

DISCUSSION

The results of the current investigation provide a clear demonstration of generalization between a pharmacologically induced interoceptive state (PTZ) and an endogenous interoceptive state produced by exposure to an ecologically relevant stimulus (predator/prey). We believe that the data further support our previous suggestions that the PTZ drug discrimination task provides a behavioral assay for a hypothesized psychological "affective" metric space best categorized as a single continuum bounded on one end by "anxiety," and "anxiolysis" on the opponent end. The "anxious" basal state of the rats induced by SSDR's to predator exposure appeared to shift the "neutral" saline state along the continuum such that the neutral stimulus now engendered significant PTZ-appropriate responding. The results from the present study may be the result of the endogenous release of a hypothesized (1) anxiogenic ligand for the benzodiazepine receptor during SSDR expression; however, to date, no clear evidence exists for this proposed ligand.

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