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Effects of Diazepam and Ethanol Alone and in Combination on Conditioned Suppression of Key-Pecking in the Pigeon[1]

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EDWARDS, J. D. AND D. A. ECKERMAN. *Effects of diazepam and ethanol alone and in combination on conditioned suppression of key-pecking in the pigeon.* PHARMAC. BIOCHEM. BEHAV. **10**(2) 217–221, 1979.—Pigeons were intermittently given grain reinforcement for key pecks. Occasional 30-sec keylight changes (warning stimulus) were followed by a brief electric shock, which suppressed responding during the warning stimuli. This suppression was reduced by diazepam and ethanol, yet combinations of the two drugs did not reduce suppression (antagonistic effect). Each drug reduced responding in the absence of the warning stimulus, and combinations of the drug produced still greater reductions in this safe-period responding (synergistic effect).

Diazepam Benzodiazepines Ethanol Drug interaction Anxiety reduction Conditioned suppression Pigeons Variable-interval reinforcement

DIAZEPAM and ethanol are both used to relieve anxiety [11, 12, 13], and frequently these drugs are simultaneously used [15]. Laboratory evidence documents that each drug reduces effects which are held analogous in non-humans to human anxiety [3, 4, 10, 15]. One laboratory model to certain human anxieties is the conditioned-suppression procedure in which a warning stimulus is presented for a time before an unavoidable aversive event such as an electric shock [7]. Ongoing appetitive behavior is typically disrupted during the warning stimulus, with the characteristics of the disruption depending on the type of behavior and the schedule of reinforcement maintaining the behavior [3,4]. For example, key-peck responding of pigeons that is reinforced according to a variable-interval three minute reinforcement schedule is suppressed during the warning stimulus (in a variable-interval three minute reinforcer schedule a key-peck produces food on the average once per three minutes [6]).

Conditioned suppression of this type is attenuated both by acute administration of benzodiazepines such as chlordiazepoxide or diazepam [4, 8, 9] and by ethanol ([15], but see [8]). That is, rate of responding during the preshock stimulus is increased. In the present study, these effects were confirmed and the interaction between the two drugs was observed. In evaluating these effects, care must be taken to separate effects representing altered suppression of responding from mere disruption of slow-rate responding (i.e., anxiety relief versus rate dependency [10,16], and some effort was made to evaluate this issue.

METHOD

Animals

Four experimentally experienced male white Carneaux pigeons (6 to 9 years old) were maintained at 75% of free-feeding weight. Experience included food reinforcement of key-pecking with various reinforcement schedules. Water and grit were continuously available in individual home cages. Diet consisted of sifted Payne's wild bird seed (reinforcer mix) supplemented as needed by Purina Pigeon Chow.

Apparatus

A two-key operant conditioning chamber was used. The chamber measured 28.6 (h)×34.6 (w)×29.4 cm (l). The walls were made of natural finish aluminum and the floor was wire mesh. Only one of the two pecking keys was used. It was mounted behind a 2.5 cm dia. hole located 3.8 cm to the left of the midline and 26.4 cm above the floor. Pecks activated the attached microswitch; otherwise there were no exteroceptive feedback stimuli provided. The key could be transilluminated with white or red light. An opening centered 15 cm below the response keys provided access to grain. During grain presentations, the opening was illuminated and the key was dark. No houselight was used.

Electric shock was administered to the birds through 0.05 mm dia. stainless steel strand wire electrodes implanted on either side of the breast bone. Electrode leads were secured

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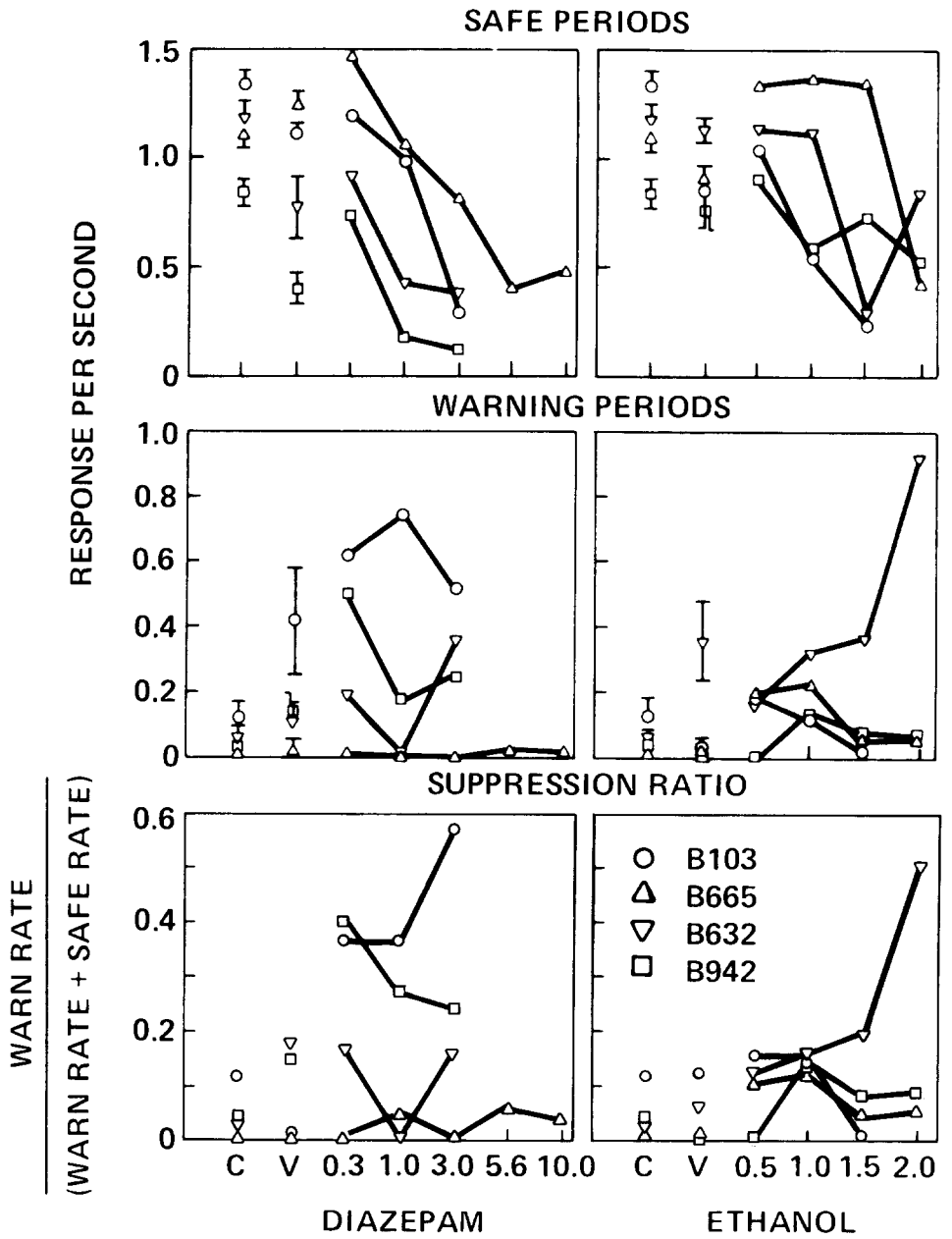

FIG. 1. Effects of each drug on responding for individual birds. Rate of responding is shown separately for safe-periods (top panels) and warning periods (middle panels). The bottom panels show the ratio between these rates in the typical suppression ratio. Standard error of the mean is identical for control (no-administration) and for vehicle control sessions where the mean is based on seven or more values. Means of the two or more administrations are shown for each drug dose.

by a leather harness on the pigeon's back and connected by a plug and ceiling commutator through switching circuits to a variable power transformer offering a range of AC (60-cycle) voltages.

Masking noise in the room prevented auditory detection of electromechanical recording and programming equipment housed several small rooms away.

Procedure

Baseline conditioned suppression and reinforcer schedule. Birds were given 4-sec access to mixed grain for key-pecks according to a variable-interval schedule [6] averaging one reinforcement per three min (VI 3-min). This schedule of reinforcement was continued throughout each daily 90-min session. Typically, the key was transilluminated with white light. At three or four times in a session (determined according to a variable-interval series), the key illumination changed from white to red for 30-sec. Shock was presented at the end of this red "warning" light. Shock voltage was increased and then adjusted for each pigeon until the minimum voltage was found which consistently eliminated

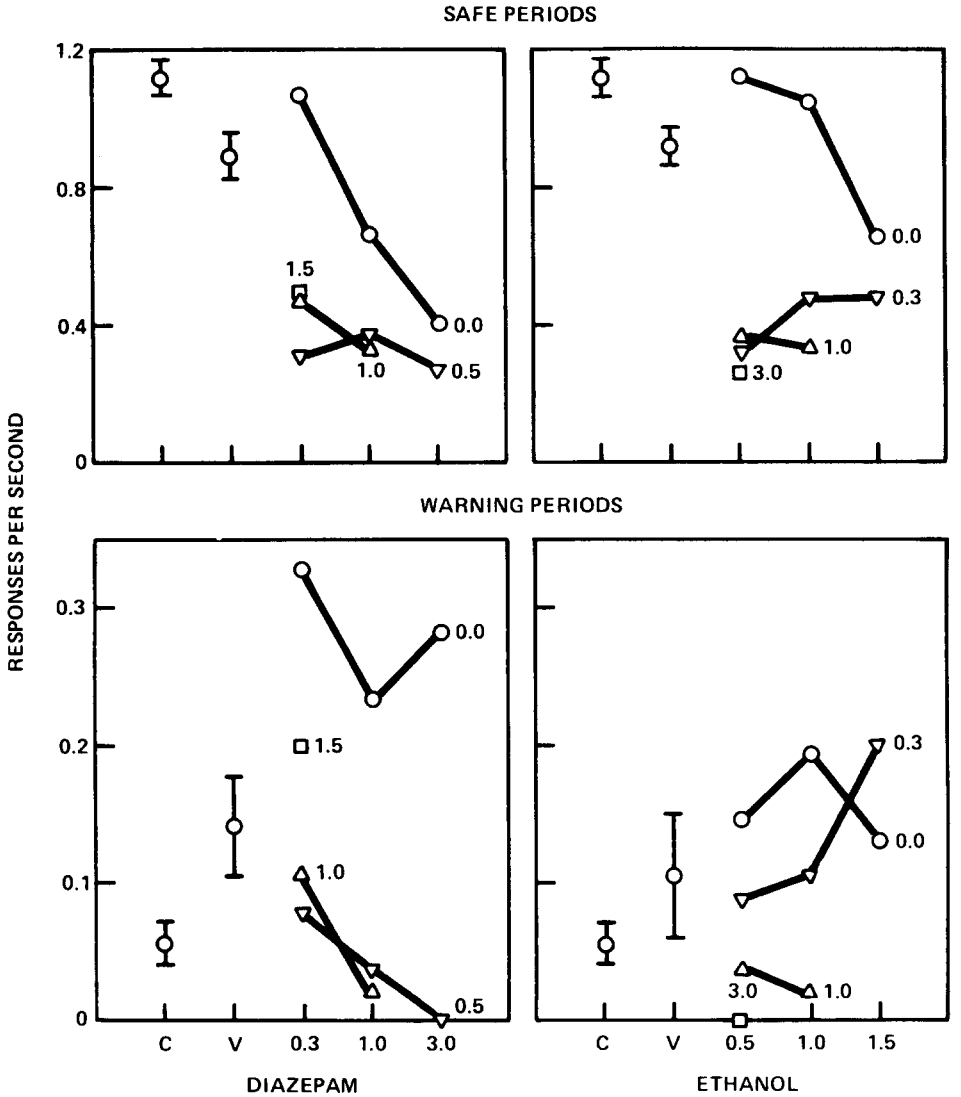

FIG. 2. Effects of drug combinations. Rate of responding is shown separately for safe-periods (top panels) and warning periods (bottom panels). Data are the mean rate over sessions and at least three birds. Standard error of the mean is shown for control (no-administration) and for vehicle control (both vehicles administered) sessions. The parameter is the dosage of the other drug.

responding during each red light period. Training with this schedule continued for 94 daily sessions. Prior training had (a) produced a stable performance on the VI 3-min schedule when no warning signals or shocks were presented, and (b) demonstrated that the red-light periods in themselves affected responding only briefly and to a small degree.

Drug procedures. Diazepam (in a commercially available solution, from Hoffman-LaRoche, Inc.) was injected IM in pectoral muscle in constant volume injections of 1 ml/kg. Ethanol (in 10% aqueous solution) was loaded by gavage into the proventriculus. All drugs and vehicles were given 10-min prior to a session.

The schedule of dosages was divided into two phases. Phase 1 consisted of single-drug administrations. The drug administered first was counterbalanced across birds. Doses were given in increasing and then decreasing series across the doses indicated in Fig. 1. Phase 2 consisted of combined administrations of the drugs. The combinations were chosen on the basis of Phase-1 results for each bird. Combinations given at least three birds are indicated in Fig. 2.

Throughout Phases 1 and 2, a 3-session cycle was maintained wherein daily sessions involved first no administration, then vehicle administration (during Phase 2, both vehicles), then administration of drug(s).

RESULTS

Safe-Period Responding

In the absence of the warning stimulus (safe period), diazepam and ethanol in large doses each reduced responding under the variable-interval schedule (see Fig. 1 and Fig. 2). The reliability of this effect is affirmed by a Friedman two-way analysis of variance by ranks [14], comparing safe period rate for vehicle control sessions to that for sessions

with each dose of a drug. Separate analyses compared diazepam doses to their control and ethanol doses to their control. In these two analyses, values were included for the first, second, and third 30-min periods for each drug administration for all four birds. For both diazepam and for ethanol, the analyses showed the effects to be reliable, with probability of chance effects in each case being less than 0.01.

The diazepam vehicle itself reduced safe-period responding in 3 of 4 birds (not B665), as is affirmed by a median test [14] comparing non-injection control to vehicle control sessions. Separate analyses were done for each bird, with the effects shown to be reliable for two with $p<0.01$ and for the third with $p<0.05$. The ethanol vehicle also reduced safe period responding, but did so reliably ($p<0.01$) only for Bird 103. No trend was seen in safe period rate within the session either for non-injection or for vehicle control sessions.

Judged against the vehicle control values, there was a bitonic effect of both drugs, wherein the lowest dose increased safe period responding while higher doses decreased responding. The increase for the lowest dose occurred reliably as judged by a sign test [14]. For diazepam the increase was reliable with $p<0.01$, and for ethanol with $p=0.01$. Since responding did not increase over that for non-injection control values, however, the bitonic effect can be seen as slight.

There was a time course of action of diazepam within a session, in that the reduction was statistically reliable in the first and second but not the third 30-min of sessions (judged from separate Friedman analyses for each 30-min). No time course of action was apparent for ethanol, as no single 30-min showed a statistically reliable effect while the entire session did as noted above.

Diazepam and ethanol had synergistic effects in reducing safe period rate of responding. Average values for the various combined doses are shown in Fig. 2. When combinations are compared to single-dose values, rate was consistently lowered by adding the other drug (sign test shows $p<0.001$ either when compared to the single dose of diazepam or of ethanol).

Warning-Period Responding

While safe-period responding was decreased, warning period responding was increased in three of four birds by diazepam. For each of these birds, two diazepam doses exceeded the standard error of the mean of vehicle control sessions (see Fig. 1). Ethanol also increased warning period responding. Three birds' responding increased beyond the standard error of the mean of vehicle control sessions at two or more doses, and the remaining bird showed an increase at the largest dose. When the warning period responding is expressed as a suppression ratio (bottom panels of Fig. 1), the decreased suppression of responding is seen for the same birds as noted above. The effect is most impressive for Birds 942 and 103 under diazepam, where warning period and safe period responding were close to equal under the drug.

When diazepam and ethanol were given together, however, warning period rate was lower than for either diazepam or ethanol given alone. A sign test confirms this reduction to be statistically reliable ($p<0.001$), judged against either the diazepam value or the ethanol value. The effect is clearly shown in the bottom panels of Fig. 2. Regarding warning stimulus responding then, diazepam and ethanol are antagonistic.

DISCUSSION

Since both ethanol and diazepam increased responding suppressed by the warning stimulus, the present study replicates and extends the apparent anxiety-relief of these two agents when acutely administered [3, 4, 8, 9, 15]. There is, as noted at the outset, a danger, however, with this interpretation. Perhaps these drugs merely increased a low-rate behavior. Data from the present study do not separate these alternatives, although Miczek [9] has shown that chlordiazepoxide selectively increased rat's responding suppressed by a stimulus preceding shock but not by a stimulus preceding food; though both stimuli suppressed responding.

Though the two drugs affected warning stimulus responding comparably when given alone, they were antagonistic when jointly given. Was this merely due to narcosis? To judge this, the effect of the drugs on safe-period responding may be used. Each drug reduced responding in safe-periods and they were synergistic in this regard when jointly given. If this reduction in safe-period responding is taken to measure narcosis, there were comparable single-drug and combined-drug values. That is, when two small doses were combined, the safe period responding was sometimes reduced no more than for larger single-drug administrations (see Fig. 2). Yet, these single-drug administrations elevated warning stimulus responding while the combined-drug administrations did not. There seems, then, to be a true antagonism between the drugs in their effect on warning-stimulus responding even while there is a synergism in their effect on safe-period responding. Again, the effect of combining drugs is found to be situation- and measure-specific [1].

Because the conditioned suppression procedure has at least logical and perhaps biological similarities to human anxiety, we may develop this last cautionary statement even one step farther. That responding during safe periods is reduced by the drugs is evidence of their disruptive effect. This disruptive effect is augmented when these drugs are given jointly. That responding during warning periods is increased is evidence of relief from suppression of responding. This relief is cancelled when these drugs are given jointly. The present study thus strongly suggests that ethanol and diazepam indeed do not mix as regards what is the predominate reason for giving either—relief from anxiety.

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