Pharmacology Biochemistry & Behavior, Vol. 25, pp. 1237–1243, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Chlordiazepoxide and Tonic Immobility: A Paradoxical Enhancement

DAWN R. RAGER, GORDON G. GALLUP, JR.[1] AND JASON W. BECKSTEAD

Department of Psychology, State University of New York at Albany, Albany, NY 12222

Received 16 May 1986

RAGER, D. R., G. G. GALLUP, JR. AND J. W. BECKSTEAD. *Chlordiazepoxide and tonic immobility: A paradoxical enhancement.* PHARMACOL BIOCHEM BEHAV **25**(6) 1237–1243, 1986.—Four experiments were conducted with chickens to examine the effects of chlordiazepoxide on tonic immobility, which has been implicated as an innate fear response. Not only did chlordiazepoxide produce a paradoxical dose-dependent increase in the duration of tonic immobility, but birds treated with chlordiazepoxide showed significantly enhanced shock-termination thresholds. Using two separate tolerance paradigms, the enhancement due to chlordiazepoxide was shown to be independent of the sedative and/or muscle relaxant effects of the drug. These findings have interesting implications for the supposed anxiolytic effects of the benzodiazepines and the relationship between fear and serotonin in avian species.

Benzodiazepines Tonic immobility Fear Anxiety Chickens Drug tolerance
Chlordiazepoxide Serotonin

TONIC immobility (TI) is a peculiar response that has been observed in a wide variety of species. The response is characterized by a temporary loss of the righting reflex and profound motor inhibition, and is typically induced by a brief period of physical restraint. Initially, an organism will resist such restraint. However, once the struggling subsides and the organism is gently released, it may remain immobile for periods of time ranging anywhere from a few seconds up to several hours. One prevailing view of TI is that it functions as a predator defense ([38]; for a review see [11]). Related to this view of TI as an evolved predator defense is the idea that it may be related to fear. Specifically, manipulations designed to increase fear have been shown to potentiate tonic immobility. For example, pre-exposure to electric shock [12], loud noise [17], or a stimulus that has conditioned fear properties [14], or testing under conditions of a simulated predatory encounter [16] all prolong TI duration. Conversely, reducing fear through handling and familiarization prior to testing [18,33], through repeated testing [30], through the use of a tranquilizer [13], or by presentation of a stimulus that had previously been paired with termination of shock [29] attenuates TI duration.

Much of the recent work on TI has aimed at identifying the neurochemical changes underlying the response. Pharmacological manipulations affecting at least three neurotransmitter systems have been shown to affect tonic immobility (for a review see [15]). In particular, serotonin (5-HT) has been hypothesized to play a major role in mediating the response, and a serotonergic midbrain-raphe model of TI has been proposed, elaborated, and refined [2, 21, 30, 49]. The most recent revision of the model [2] theorized that decreased activity at post-synaptic 5-HT receptors results in potentiation of TI, while 5-HT receptor stimulation attenuates the response.

Thus both fear and 5-HT appear to play important roles in TI, but as yet there has been no formal attempt to integrate and relate these factors to the immobility response. The benzodiazepines, a class of drugs believed to exert effects on both of these factors, may provide a means of examining the relationship between fear and serotonin on tonic immobility. Several recent studies provide support for the fear-reducing effects of the benzodiazepines. For example, Davis [6] using the potentiated startle to measure conditioned fear in rats, found that diazepam and flurazepam attenuated this response. Similarly, Helmstetter and Fanselow [20] reported that midazolam blocked the freezing and analgesia usually elicited in rats placed in a context previously paired with shock. There is also evidence that the anxiolytic or fear-attenuating effects of the benzodiazepines may be due, at least in part, to their effects on the serotonergic system [19, 22, 25, 40, 44, 37, 50]. While the benzodiazepines are believed to exert their anxiolytic effects by facilitating the action of γ-aminobutyric acid (GABA) [7, 10, 40, 41], GABA is known to have an inhibitory influence on other neurotransmitters, including serotonin [8,34]. Thus, benzodiazepines may indirectly affect serotonergic function. Numerous studies have demonstrated changes in serotonergic function following both acute and chronic benzodiazepine administration. For example, Wise, Berger and Stein [50] reported a reduction in 5-HT turnover following single and repeated doses of oxazepam, and recently, McElroy, Feldman and Meyer [28] replicated these results using chlordiazepoxide.

[1]Requests for reprints should be addressed to Gordon G. Gallup, Jr., Psychology Department, S.U.N.Y. at Albany, 1400 Washington Avenue, Albany, NY 12222.

Consistent with these findings, diazepam increased retention of intracisternally injected (C14)5-HT and (C14)5-HIAA (a 5-HT metabolite) as compared to saline injected controls [4]. Also, various benzodiazepines have been reported to elevate brain tryptophan, 5-HT, and 5-HIAA levels [23, 34, 35, 48], which according to a recent study [35], results from decreased utilization of 5-HT rather than decreased synthesis. Electrophysiological studies have also provided evidence for benzodiazepine effects on the serotonergic system. For example, Trulson, Preussler, Howell and Frederickson [46] reported that chlordiazepoxide and diazepam both produced decreases in raphe unit activity in freely moving cats, although these decreases were only significant at doses that also produced ataxia. Similarly, several benzodiazepines were reported to reduce multiunit activity in the dorsal raphe of encephalé isolé rats [27]. These studies provide strong evidence for benzodiazepine-induced changes in serotonergic function, although whether these changes are directly related to the anxiolytic effects of these drugs remains open to question (e.g., [24, 42, 46]). Thus, the present series of studies were designed to examine the effects of a benzodiazepine on TI, to set the stage for a subsequent set of studies relating these effects to changes in fear and serotonergic activity.

EXPERIMENT 1

Since TI is most often measured in terms of response duration, the first experiment was conducted to examine the effects of various doses of chlordiazepoxide (CDP) on TI duration in domestic chickens.

METHOD

Subjects

The subjects were 48 straight-run Production Red chickens *(Gallus gallus)*, obtained from Welp, Inc. (Bancroft, IA) one day posthatch. The birds were group-reared in thermostatically controlled commercial brooders and received approximately 14 hours of artificial light per day. Chick feed and water were continuously available.

Apparatus

Four test boxes, each located in a separate sound-attenuated room were used for measuring TI duration. Each box was constructed of plywood with an open front, and measured 45 cm long × 29 cm wide × 35 cm high. A photocell (with adjustable height) was mounted on the left side wall of each box in order to detect response termination (i.e., recovery of an upright position). Each photocell was connected to a timer (Lafayette Instruments Model 54020) located outside the box, which was used to measure the duration of immobility. Each timer was wired to a light panel mounted outside the room, which allowed the experimenter to monitor the responses from outside the test rooms.

Procedure

When subjects were 32 days of age, they were removed from the brooders and randomly assigned to one of four groups. Each bird was weighed and received an intraperitoneal (IP) injection of either 0, 5, 10, or 20 mg/kg chlordiazepoxide HCl (Sigma) dissolved in 2.0 ml/kg distilled water vehicle. Subjects were then placed in individual cardboard boxes and transported to the test rooms, where they remained until ten minutes after injection. Immobility testing was conducted by an experimenter who was blind with regard to drug treatment. Each bird was removed from the cardboard box and placed in the TI box. Tonic immobility was induced by restraining the bird on its right side for 15 seconds, after which time the experimenter gently released the bird, activated the timer, and left the room. If a bird failed to remain immobile until the experimenter left the room, the induction procedure was repeated at 60 second intervals for up to five times. If TI could not be induced in five attempts, that subject received a duration score of zero seconds. For subjects that remained immobile, termination of TI was defined by a righting response, and a 3600 second ceiling was imposed on TI duration.

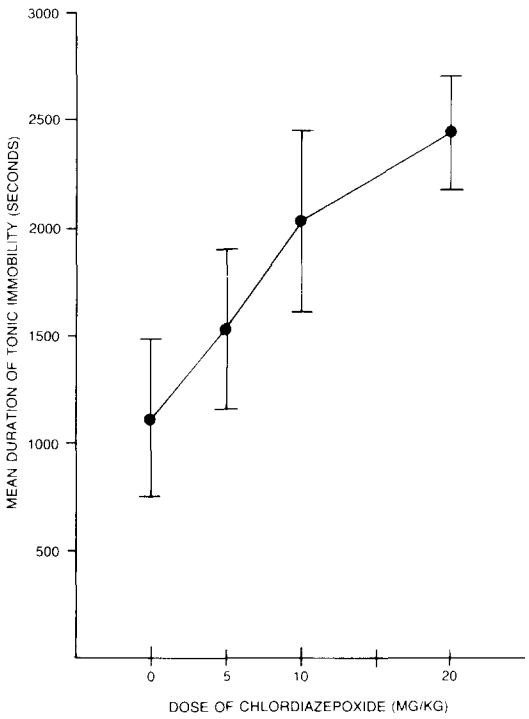

FIG. 1. Mean duration of tonic immobility in seconds (±S.E.M.) as a function of drug dosage.

RESULTS

In the three groups that received CDP, all of the subjects remained immobile following the first induction, while in the control group, five of the 12 subjects required more than one induction to elicit the response. Figure 1 depicts the mean TI durations for the 0, 5, 10, and 20 mg/kg doses of chlordiazepoxide.

To normalize the data, the duration scores were subjected to a square root transformation. An analysis of variance (ANOVA) of the transformed scores revealed a significant treatment effect, $F(3,44)=3.441$, $p=0.024$. Subsequent trend

analysis showed this treatment effect to be dose-dependent, with a significant linear component, $F(1,44)=9.82$, $p=0.003$.

EXPERIMENT 2

The duration of tonic immobility is typically used as a measure of response magnitude, and in some cases the number of inductions required to elicit the reaction is recorded as a measure of response susceptibility. In addition to these traditional methods for measuring TI, several investigators [26, 37, 45] have employed a third measure referred to as the arousal or shock-termination threshold (STT). This technique involves inducing TI and measuring the amount of electrical current that must be applied before the animal rights itself, thereby terminating the immobility episode. Since CDP increased TI duration in chickens in Experiment 1, and since Tompkins [45] reported that CDP increased arousal thresholds in rabbits, the second experiment was designed to examine the effect of CDP on shock-termination thresholds in chickens.

METHOD

Subjects and Apparatus

Twenty straight-run Production Red chickens, reared under conditions identical to those described in the first experiment, served as subjects. Shock was delivered through wires leading from the terminals of a Lafayette shock generator (Model 82400), and shock-termination thresholds were read directly from the meter of this apparatus.

Procedure

At 32 days of age, the subjects were randomly divided into two groups. One half of the birds were weighed, and received IP injections of 20 mg/kg of CDP dissolved in 2 ml/kg distilled water. The remaining birds were weighed, and received equivolume vehicle injections. Following injection each subject was transported to the test room in a cardboard box where it was left undisturbed for ten minutes. Next, the bird was removed from the box and placed on a table where a wire leading from each of the two terminals on the shock apparatus was wrapped around each leg. The experimenter, seated at arms length from the bird, induced TI by restraining the subject on its right side for 15 seconds and then slowly releasing the bird. If the subject failed to remain immobile for 15 seconds after release, the induction procedure was repeated every 60 seconds for up to five times. Birds that failed to remain immobile after five induction attempts were eliminated from the study. Fifteen seconds after TI onset, the experimenter gradually increased shock intensity from zero mA at the rate of approximately 0.1 mA per second. Shock intensity was increased until a righting response was observed or until the 1.0 mA ceiling was reached. Shock-termination threshold was thus defined as the intensity of shock required to elicit a righting response (see [13] for more detail on STT methodology).

RESULTS

With the exception of two birds (one CDP-treated and one control) that required two inductions to elicit TI, all subjects remained immobile following the first induction. The mean shock intensities required to disrupt TI were 0.253 and 0.161 mA for the CDP-treated and control groups, respectively. An ANOVA showed that birds that received CDP evidenced significantly higher shock-termination thresholds than control subjects, $F(1,18)=6.544$, $p=0.019$.

EXPERIMENT 3

The results of Experiments 1 and 2 seem counterintuitive in light of the relationship between fear and tonic immobility. One would expect the anxiolytic effects of the benzodiazepines to attenuate, not potentiate, the TI response. However, in addition to their anxiolytic effects, the benzodiazepines have muscle relaxant and sedative properties. Thus, the enhancement of TI observed in Experiments 1 and 2 may be a result of such muscle relaxant/sedative side effects. Indeed, Tompkins [45] suggested that the increased arousal thresholds observed in rabbits treated with CDP reflected the sedative properties of the drug. There is evidence, however, that tolerance develops differentially to the anxiolytic and sedative/muscle relaxant effects of the benzodiazepines [50]. While there is general agreement that tolerance to the sedative/muscle relaxant effects develops rapidly, there is some controversy as to whether tolerance develops to the anxiolytic effects. However, those studies reporting anxiolytic tolerance have also shown that it develops more slowly than does tolerance to the sedative/muscle relaxant effects (see [9,38] for reviews). Thus Experiment 3 was designed to assess the effects of chlordiazepoxide on TI duration, while controlling for possible sedative/muscle relaxant effects through the use of a tolerance paradigm.

METHOD

Subjects and Apparatus

The subjects were 40 straight-run Production Red chickens. All animals were obtained, housed, and fed as described in the previous experiments. Testing was conducted in the TI boxes described in Experiment 1.

Procedure

When the birds were 17 days of age, they were randomly divided into two groups and each subject was fitted with a plastic leg band denoting group membership. All birds were weighed and one half of the subjects received IP injections of 20 mg/kg of CDP dissolved in 2 ml/kg distilled water vehicle, while remaining birds received equivolume vehicle injections. Following injections all the subjects were returned to the brooders. This procedure was repeated for a total of five days at approximately the same time (between 0900 and 1100 hours) each day. Due to experimenter error in injection, one subject from the water pretreatment group was discarded. Testing for TI was conducted on the sixth day. On the test day, half of the birds in each pretreatment condition (CDP or water) were injected with 20 mg/kg of CDP (IP) while the remaining subjects in each pretreatment condition received IP injections of the distilled water vehicle. Thus there were four groups of birds: (1) H_2O pretreatment-H_2O test, (2) H_2O pretreatment-CDP test, (3) CDP pretreatment-H_2O test, and (4) CDP pretreatment-CDP test. Subjects were then transported individually to the test rooms in cardboard boxes, where they

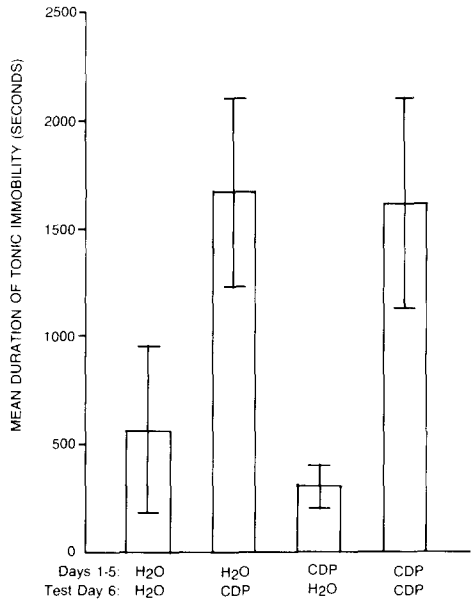

FIG. 2. Mean duration of tonic immobility (±S.E.M.) as a function of pretreatment with chlordiazepoxide or water, and testing with either chlordiazepoxide or water.

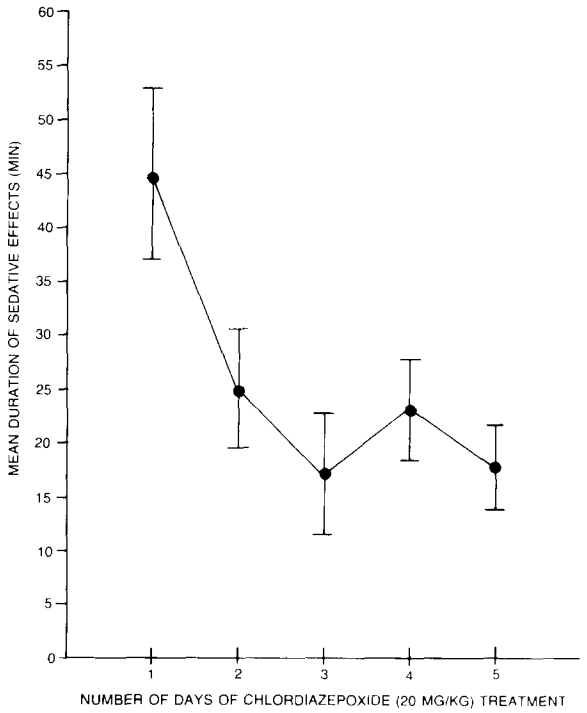

FIG. 3. Mean recovery time in minutes (±S.E.M.) as a function of repeated daily injections of chlordiazepoxide.

remained undisturbed until ten minutes after injection. Testing for TI was conducted in the manner described in the first experiment, with a 3600 second ceiling imposed on duration. Due to a malfunction in one of the test boxes, one subject from the H_2O-CDP group was eliminated from the study.

RESULTS

A 2×2 ANOVA revealed no significant effect of drug treatment on the number of inductions required to elicit tonic immobility, $p>0.05$. The mean number of inductions required were 1.1, 1.0, 1.5, and 1.1 for the H_2O-H_2O, H_2O-CDP, CDP-H_2O, and CDP-CDP groups, respectively. The mean TI durations for these four groups are shown in Fig. 2.

To correct for heterogeneity of variance, the raw duration data were subjected to square root transformations, and a 2×2 ANOVA was performed on the transformed scores. When administered ten minutes before TI testing, CDP significantly prolonged TI duration, regardless of whether subjects had prior exposure to the drug, $F(1,34)=13.886$, $p<0.001$.

EXPERIMENT 4

The results of Experiment 3 appear inconsistent with the interpretation that the enhancement of TI observed in the first two experiments resulted from the sedative/muscle relaxant effects of chlordiazepoxide, since the birds receiving repeated injections of CDP should have developed tolerance to these effects by the time they were tested. However, most of the data for tolerance to benzodiazepines has been derived from experiments with mammals [9, 39, 50], although there is at least one report of tolerance to the sedative/muscle relaxant effects of these drugs in pigeons [5]. Thus, there remains the possibility that chickens respond to chronic benzodiazepine treatment differently. Therefore, in the fourth experiment, we attempted to verify and more precisely document the development of tolerance to the sedative effects of CDP in chickens prior to TI testing.

METHOD

Subjects and Apparatus

The subjects were 20 straight-run Production Red chickens obtained and maintained in the manner described in the previous experiments. Tonic immobility testing was conducted in the boxes described in the first experiment.

Procedure

At 18 days of age, the birds were randomly divided into two groups and fitted with plastic leg bands which identified birds by group and subject number. Birds in one group received IP injections of 20 mg/kg of CDP dissolved in 2 ml/kg distilled water, and birds in the second group received IP injections of 2 ml/kg distilled water only. Pairs of birds were then placed in cardboard boxes, with each pair consisting of one CDP-treated bird and one control. At ten minute intervals following injection two experimenters observed each

TABLE 1

DURATION OF TONIC IMMOBILITY AND NUMBER OF INDUCTIONS NEEDED TO ELICIT THE RESPONSE AS A FUNCTION OF PRETREATMENT WITH CHLORDIAZEPOXIDE OR YOKED WATER CONTROLS (SEE TEXT)

	Number of inductions	Duration of TI (seconds)
CDP-treated		
Mean	1.20	745.5
S.E.M.	0.13	183.1
Control		
Mean	2.30	272.4
S.E.M.	0.37	119.8

pair of birds, and independently determined whether the animal that received CDP appeared sedated as compared to the control subject. A bird was judged to be sedated if it exhibited at least one of the following behaviors to a greater extent than the water-treated member of the pair: inactivity, ataxia, drooping head and/or wings, eyes closed, failure to stand upright. Each pair was observed every ten minutes until both experimenters agreed that the CDP-treated bird had recovered from the sedative effects of the drug. When the last CDP-treated subject had recovered, all birds were returned to a common brooder. This procedure was repeated at approximately the same time every day for a total of five consecutive days. On all of these days each CDP-treated subject was always placed with the same control subject. For each individual CDP-treated bird, the mean amount of time required to recover from the sedative effects of the drug on the fourth and fifth days was calculated. This amount of time served as the injection-test interval for TI testing which was conducted on day six. Pairs of subjects were weighed, injected with either vehicle or CDP and placed in cardboard boxes, as on the previous days. Following the injection-test interval (determined for each pair of subjects in the manner described above), TI was induced using the procedures described in the previous experiments, with a 3600 second ceiling imposed on duration.

RESULTS

A one-way repeated measures ANOVA revealed that the mean amount of time required to recover from the sedative/muscle relaxant effects of the drug in the CDP-treated group decreased significantly with repeated exposure, $F(4,36)=6.994$, $p<0.001$. A subsequent trend analysis showed this effect to have significant linear and quadratic components, $F(1,44)=17.38$, $p=0.0002$ and $F(1,44)=7.06$, $p=0.011$, respectively. Figure 3 depicts these data. Table 1 shows the means and standard errors of the CDP-treated and control groups for both induction and duration of tonic immobility. Analyses confirmed that CDP-treated birds required significantly fewer inductions, $F(1,18)=7.949$, $p=0.011$, and remained immobile significantly longer than control birds, $F(1,18)=4.676$, $p=0.042$.

GENERAL DISCUSSION

In the first experiment, administration of CDP ten minutes prior to testing resulted in a dose-dependent increase in TI duration. Similarly, in Experiment 2 pretreatment with CDP increased the amount of shock needed to disrupt the immobility response. One possible interpretation of these results is that they are due to the sedative and/or muscle relaxant properties of the drug employed. However, this account seems unlikely given the results of the third and fourth experiments. With repeated exposure, tolerance to the sedative/muscle relaxant effects of benzodiazepines develops rapidly [5, 9, 39, 50]. Yet in Experiment 3, CDP administered ten minutes prior to testing prolonged TI duration both in birds that received five days of CDP pretreatment, and in birds that received only vehicle pretreatment. However, since we were unaware of any studies demonstrating the development of tolerance to the sedative/muscle relaxant effects of chlordiazepoxide in chickens, there remained the possibility that this species responds differently to chronic CDP treatment. Thus, in Experiment 4 it was shown that birds did indeed develop tolerance to the sedative/muscle relaxant effects of chlordiazepoxide. Although subjects treated with CDP showed some sedation across all five days of treatment, they recovered from these effects more quickly with repeated exposure to the drug. This reduction in time-course of sedation resulting from repeated exposure to CDP has also been observed in pigeons [5]. Experiment 4 also showed that when tolerant birds were again injected with CDP and tested for TI using an injection-test interval that should have allowed for complete recovery from any residual sedative/muscle relaxant effects of the drug, they still exhibited longer immobility durations than control subjects.

In addition to the effects of CDP on the magnitude of TI, and its sensitivity to disruption by electric shock, CDP also appeared to enhance susceptibility to the response. For example, in the first experiment all subjects treated with CDP (even at the lowest dose) exhibited TI on the first induction, while five of the 12 control subjects required more than one induction. Again, this effect of CDP on susceptibility to TI does not appear to be due to the sedative/muscle relaxant effects of the drug, since in Experiment 4 tolerant birds that received CDP prior to TI testing required significantly fewer inductions to elicit the response than control birds. This is particularly interesting since repeated handling during the tolerance phase of the experiment would be expected to antagonize the TI response [33], as appeared to be the case for the control birds. Yet the effect of CDP was to produce a dramatic reinstatement of susceptibility, akin to what has been reported for aversive events [11,32].

In light of these findings it is important to comment on previous reports of tranquilization effects on tonic immobility. Most work has focused on a water-soluble tranquilizer, metoserpate HCl (Pacitran, CIBA), which was developed specifically to offset the emotional effects of handling and shipping on domestic fowl in commercial settings (see [1,3]). In direct contrast to the effects of CDP on TI, Gallup, Nash and Brown [13] found that chickens treated with metoserpate HCl were significantly less susceptible to TI (i.e., required more inductions) than controls, and in those subjects that showed the reaction the duration of TI was inversely proportional to drug dosage. Gallup, Rosen and Brown [14] not only replicated this effect with chickens, but found that a stimulus which had previously paired with shock would potentiate TI and this effect could be blocked by metoserpate HCl. More recently, using a different paradigm with chickens, Suarez and Gallup [43] found that metoserpate HCl significantly reduced distress-call, ambulation, and es-

cape latencies in an open-field. Using an antipsychotic drug, chlorpromazine, Maser, Gallup, Hicks and Edson [31] also found high doses antagonized the duration of TI in chickens. Again, the TI-attenuating effect of this major tranquilizer is opposite the TI-potentiating effects of CDP reported in the present experiments.

Although the results of these experiments appear contrary to what is known about the effects of fear on TI, this benzodiazepine-induced enhancement of the response may not be inconsistent with what is known about the neurochemistry of tonic immobility. As mentioned previously, Boren and colleagues [2] have theorized that reduced stimulation of post-synaptic 5-HT receptors enhances tonic immobility. There is also evidence to suggest that the benzodiazepines reduce serotonergic activity following both acute and chronic administration [4, 27, 28, 46, 50]. Thus, in the present experiments, administration of CDP may have reduced 5-HT neurotransmission, so that the benzodiazepine-induced enhancement of TI observed may, in fact, be consistent with the Boren *et al.* model. However, these results remain puzzling with regard to the issue of fear.

Although anxiety and fear are often considered synonymous, they may in fact be distinguishable from one another. Fear is usually considered to be specific to a particular situation or stimulus, while the source of anxiety is often more diffuse and cannot be readily identified (i.e., as conveyed by the term "free-floating" anxiety). In light of the results of the present experiments, the notion of separate neurochemical substrates of fear and anxiety might seem worthy of further investigation.

ACKNOWLEDGEMENTS

The authors thank Dave Radin, Jason Hurley, Denise Scarano, Jerry Scarnato and Susan Silver for help in data collection, and Don Warren for comments on an earlier draft of the manuscript.

REFERENCES

1. Belloff, G. B. and B. Hsu. Su-9064, a water soluble tranquilizer for handling broilers and replacement pullets. *Avian Dis* **7:** 50–55, 1963.
2. Boren, J. L., G. G. Gallup, Jr., S. D. Suarez, L. B. Wallnau and G. J. Gagliardi. Pargyline and tryptophan enhancement of tonic immobility: Paradoxical attenuation with combined administration. *Pharmacol Biochem Behav* **11:** 17–22, 1979.
3. Champion, L. R., H. C. Zindel, R. K. Ringer and J. H. Wolford. The performance of started pullets treated with Su-9064 (Pacitran) prior to transport. *Poult Sci* **45:** 1359–1368, 1966.
4. Chase, T. N., R. I. Katz and I. J. Kopin. Effect of diazepam on fate of intracisternally injected serotonin-C14. *Neuropharmacology* **9:** 103–108, 1970.
5. Cooper, S. J. and A. Posadas-Andrews. Food and water intake in the non-deprived pigeon after chlordiazepoxide administration. *Psychopharmacology (Berlin)* **65:** 99–101, 1979.
6. Davis, M. Diazepam and flurazepam: effects on conditioned fear as measured with the potentiated startle paradigm. *Psychopharmacology (Berlin)* **62:** 1–7, 1979.
7. Enna, S. J. Role of γ-aminobutyric acid in anxiety. *Psychopathology* **17:** 15–24, 1984.
8. Feldman, R. and L. F. Quenzer. *Fundamentals of Neuropsychopharmacology*. Sunderland, MA: Sindauer Associates, Inc., 1984.
9. File, S. E. Tolerance to the behavioral actions of benzodiazepines. *Neurosci Biobehav Rev* **9:** 113–121, 1985.
10. Gallagher, D. W., P. Mallorga, J. W. Thomas and J. F. Tallman. GABA-benzodiazepine interactions: physiological, pharmacological, and developmental aspects. *Fed Proc* **39:** 3043–3049, 1980.
11. Gallup, G. G., Jr. Animal hypnosis: Factual status of a fictional concept. *Psychol Bull* **81:** 836–853, 1974.
12. Gallup, G. G., Jr., H. S. Creekmore and W. E. Hill, III. Shock-enhanced immobility reactions in chickens: support for the fear hypothesis. *Psychol Rec* **20:** 243–245, 1970.
13. Gallup, G. G., Jr., R. F. Nash and C. W. Brown. The effect of a tranquilizer on the immobility reaction in chickens: additional support for the fear hypothesis. *Psychon Sci* **23:** 127–128, 1971.
14. Gallup, G. G., Jr., T. S. Rosen and C. W. Brown. Effect of conditioned fear on tonic immobility in domestic chickens. *J Comp Physiol Psychol* **78:** 22–25, 1972.
15. Gallup, G. G., Jr., J. L. Boren, S. D. Suarez and L. B. Wallnau. The psychopharmacology of tonic immobility in chickens. In: *The Brain and Behavior of the Fowl*, edited by T. Ookawa. Tokyo: Japan Scientific Societies Press, 1983.
16. Gallup, G. G., Jr., R. F. Nash, N. H. Donegan and M. K. McClure. The immobility response. A predator-induced reaction in chickens. *Psychol Rec* **21:** 513–519, 1971.
17. Gallup, G. G., Jr., R. F. Nash, R. J. Potter and N. H. Donegan. Effect of varying conditions of fear on immobility reactions in domestic chickens *(Gallus gallus)*. *J Comp Physiol Psychol* **73:** 442–445, 1970.
18. Gilman, T. T., F. L. Marcuse and A. U. Moore. Animal hypnosis: a study of the induction of tonic immobility in chickens. *J Comp Physiol Psychol* **43:** 99–111, 1950.
19. Gray, J. A. Anxiety as a paradigm case of emotion. *Br Med Bull* **37:** 193–197, 1981.
20. Helmstetter, F. J. and M. S. Fanselow. The effects of benzodiazepines on the freezing and analgesia produced by Pavlovian conditional fear stimuli. Presented at the 1986 annual meeting of the Eastern Psychological Association, New York, NY.
21. Hicks, L. E., J. D. Maser, G. G. Gallup, Jr. and P. H. Edson. Possible serotonergic mediation of tonic immobility: effects of morphine and serotonin blockade. *Psychopharmacologia* **42:** 41–56, 1975.
22. Iversen, S. D. 5-HT and anxiety. *Neuropharmacology* **23:** 1553–1560, 1984.
23. Jenner, P., C. D. Marsden and J. Pratt. Actions of benzodiazepines and other anticonvulsants on 5-HT turnover in mouse brain. *Br J Pharmacol* **74:** 812P–813P, 1981.
24. Johnston, A. L. and S. E. File. 5-HT and anxiety: promises and pitfalls. *Pharmacol Biochem Behav* **24:** 1467–1470, 1986.
25. Jones, B. J., I. A. Paterson and M. H. T. Roberts. Microinjections of methyl-β-carboline-3-carboxylate into the dorsal raphe nucleus: Behavioural consequences. *Pharmacol Biochem Behav* **24:** 1487–1489, 1986.
26. Klemm, W. R. Drug potentiation of hypnotic restraint of rabbits, as indicated by behavior and brain electrical activity. *Lab Anim Care* **15:** 163–167, 1965.
27. Laurent, J. P., M. Mangold, U. Humbel and W. Haefely. Reduction by two benzodiazepines and pentobarbitone of the multiunit activity in substantia nigra, hippocampus, nucleus locus coeruleus and nucleus raphe dorsalis of encephalé isolé rats. *Neuropharmacology* **22:** 501–511, 1983.
28. McElroy, J. F., R. S. Feldman and J. S. Meyer. A comparison between chlordiazepoxide and CL 218,872, a synthetic non-benzodiazepine ligand for benzodiazepine receptors, on serotonin and catecholamine turnover in brain. *Psychopharmacology (Berlin)* **88:** 105–108, 1986.

29. Maser, J. D., G. G. Gallup, Jr. and R. Barnhill. Conditioned inhibition and tonic immobility: Stimulus control of an innate fear response in the chicken. *J Comp Physiol Psychol* **83:** 128–133, 1973.
30. Maser, J. D., G. G. Gallup, Jr. and L. E. Hicks. Tonic immobility in chickens: Possible involvement of monoamines. *J Comp Physiol Psychol* **89:** 319–328, 1975.
31. Maser, J. D., G. G. Gallup, Jr., L. E. Hicks and P. H. Edson. Chlorpromazine dosage and duration of tonic immobility: Biphasic effects. *Pharmacol Biochem Behav* **2:** 119–121, 1974.
32. Nash, R. F. and G. G. Gallup, Jr. Effect of different parameters of shock on tonic immobility. *Behav Res Methods Instrum* **7:** 361–364, 1975.
33. Nash, R. F. and G. G. Gallup, Jr. Habituation and tonic immobility in domestic chickens. *J Comp Physiol Psychol* **90:** 870–876, 1976.
34. Nsihikawa, T. and B. Scatton. Evidence for GABAergic inhibitory influence on serotonergic neurons originating from the dorsal raphe. *Brain Res* **279:** 325–329, 1983.
35. Pratt, J. A., P. Jenner and C. D. Marsden. Comparison of the effects of benzodiazepines and other anticonvulsant drugs on synthesis and utilization of 5-HT in mouse brain. *Neuropharmacology* **24:** 59–68, 1985.
36. Pratt, J. A., P. Jenner, E. H. Reynolds and C. D. Marsden. Clonazepam induces decreased serotoninergic activity in the mouse brain. *Neuropharmacology* **18:** 791–799, 1979.
37. Rager, D. R. and G. G. Gallup, Jr. Tonic immobility in chickens *(Gallus gallus)*: shock-termination thresholds. *J Comp Psychol* **99:** 350–355, 1985.
38. Ratner, S. C. Comparative aspects of hypnosis. In: *Handbook of Clinical and Experimental Hypnosis*, edited by J. E. Gordon. New York: Macmillan, 1967.
39. Rosenberg, H. C. and T. H. Chiu. Time course for development of benzodiazepine tolerance and physical dependence. *Neurosci Biobehav Rev* **9:** 123–131, 1985.
40. Sepinwall, J. and L. Cook. Behavioral pharmacology of antianxiety drugs. In: *Handbook of Psychopharmacology, Vol 13*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1978.
41. Sepinwall, J. and L. Cook. Mechanism of action of the benzodiazepines: behavioral aspect. *Fed Proc* **39:** 3024–3031, 1980.
42. Shepard, R. A., D. A. Buxton and P. L. Broadhurst. Drug interactions do not support reduction in serotonin turnover as the mechanism of action of benzodiazepines. *Neuropharmacology* **21:** 1027–1032, 1982.
43. Suarez, S. D. and G. G. Gallup, Jr. Predatory overtones of open-field testing in chickens. *Anim Learn Behav* **9:** 153–163, 1981.
44. Thiebot, M.-H., M. Hamon and P. Soubrie. Attenuation of induced-anxiety in rats by chlordiazepoxide: role of raphe dorsalis benzodiazepine binding sites and serotoninergic neurons. *Neuroscience* **7:** 2287–2294, 1982.
45. Tompkins, E. C. The use of the immobility reflex (animal hypnosis) as a possible procedure for detecting sedative activity. *Life Sci* **15:** 671–684, 1974.
46. Trulson, M. E., D. W. Preussler, G. A. Howell and C. J. Frederickson. Raphe unit activity in freely moving cats: effects of benzodiazepines. *Neuropharmacology* **21:** 1045–1050, 1982.
47. Tye, N. C., S. D. Iversen and A. R. Green. The effects of benzodiazepines and serotonergic manipulations on punished responding. *Psychopharmacology (Berlin)* **18:** 689–695, 1979.
48. Valzelli, L., S. Bernasconi, E. Coen and V. V. Petkov. Effect of different psychoactive drugs on serum and brain tryptophan levels. *Neuropsychobiology* **6:** 224–229, 1980.
49. Wallnau, L. B. and G. G. Gallup, Jr. A serotonergic, midbrain-raphe model of tonic immobility. *Biobehav Rev* **1:** 35–43, 1977.
50. Wise, C. D., B. D. Berger and L. Stein. Benzodiazepines: anxiety-reducing activity by reduction of serotonin turnover in the brain. *Science* **177:** 180–183, 1972.