Pharmacology Biochemistry and Behavior, Vol. 58, No. 4, pp. 915–921, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00299-2

Effect of Diazepam and A β-Carboline on Open-Field and T-Maze Behaviors in 2-Day-Old Chicks

RAÚL H. MARÍN, IRENE D. MARTIJENA AND AUGUSTO ARCE[1]

Cátedra de Química Biológica, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299 (5000), Córdoba, Argentina

Received 27 August 1996; Revised 20 January 1997; Accepted 12 February 1997

MARÍN, R. H., I. D. MARTIJENA AND A. ARCE. *Effect of diazepam and a β-carboline on open-field and T-maze behaviors in 2-day-old chicks.* PHARMACOL BIOCHEM BEHAV **58**(4) 915–921, 1997.—The effects of diazepam and the β-carboline FG 7142 in chicks were examined on several behavioral measures in open-field and T-maze tasks. In the open field, only the higher doses of diazepam affected behavior, suggesting a sedative-like effect, while FG 7142 influenced behavior as would a fear-inducing manipulation. After a low dose of either drug was injected, testing in a T-maze showed that diazepam improved and FG 7142 impaired the escape performance from the isolation chamber, without affecting the time spent in the T-corridor. In three groups of chicks selected on the basis of their first escape performance, only lower performance chicks were affected by an anxiolytic dose of diazepam. T-maze results suggest that: (a) T-maze is more sensitive than open-field test to behavior changes induced by anxiolytic doses of diazepam; (b) isolation chamber behavior could be an index of general emotionality in young chicks; (c) diazepam and FG 7142 do not modify the social motivation to escape the maze; (d) higher performance chicks present an escape behavior of a less anxious type than lower performance chicks. The results suggest that the GABAergic system is involved in the behavioral expression of fear and anxiety in young chicks. © 1997 Elsevier Science Inc.

Open-field T-Maze task Diazepam β-Carboline Chick behavior

THE subtype A of the gamma-aminobutyric acid (GABA) receptors ($GABA_A$ receptor) is a pentameric ligand-gated chloride channel protein, and the site of action of a variety of pharmacologically important drugs including benzodiazepines (BZDs) (26). The central-type benzodiazepine receptor (CBR) is an allosteric modulatory site of the $GABA_A$ receptor (2).

Diazepam (DZ) is a classical BZD, a CBR agonist (15, 27,28) that increases the effects of GABA on the $GABA_A$ receptor (8,13), with an anxioltytic effect mediated by an enhancement of chloride conductance. On the other hand, CBR inverse agonists such as β-carbolines exert a negative modulatory effect on the function of the $GABA_A$ receptor. Administration of β-carbolines elicits behavioral effects in the opposite direction to the BZDs, for instance, the β-carboline FG 7142 has been reported to induce anxiety in laboratory animals and in humans (4,7,24). Anxiolytic drugs might be expected to reduce fear associated with the new environment, and hence, to release supressed behavior. On the other hand, anxiogenic drugs enhance fearfulness in a variety of behavioral tasks (8,29), then the effects of anxiogenic drugs should be the inhibition of reinstatement behavior by increasing the fear response.

The open-field test has been extensively used to assess emotionality in animals (1,3,9,10,14,20). In domestic fowls, open-field behavior has been defined as an interaction or compromise between tendencies to reinstate contact with conspecifics and evade predation (10,19). The one-trial T-maze has been developed as a learning paradigm for use in young chicks (11).

In the present work, we study the effects of DZ and FG 7142 on several behaviors of young chicks in an open-field test and in a T-Maze task. The objetive was to determine the roles of fear of isolation and need for social interaction and reinstatement in the expression of behavioral measures in these tasks.

EXPERIMENT 1

A dose–response curve of DZ and the response to two doses of FG 7142 on open-field behaviors were assessed.

[1]Address correspondence to A. Arce: Fax: (54–51)332097.

METHOD

Subjects

Chicks (Cobb Harding, of both sexes) were obtained from a commercial hatchery, INDACOR (Argentina). A total of 80 birds in groups of 20 each, were housed in brooders in a room with constant temperature (31°C) and humidity at a 12 D: 12 L cycle (lights on at 0700 h) with food and water freely available and maintained in these conditions until they reached 2 days old.

Drugs

DZ and FG 7142 (Sigma Chemical Co.) were suspended in distilled water added with Tween 80 (1 drop every 2 ml) and dispersed by ultrasound. Injections were given intraperitoneally (IP) 20 min before each test, at a volume of 0.2 ml/100 g of body weight. Control chicks received injections of vehicle (water/Tween 80 mixture).

Open-Field Apparatus

A 60 × 60 cm open-field activity monitor, with 30-cm high sides was used. It was made of wood and painted white. The floor of the activity monitor was marked off into 25 equal-sized squares, and was illuminated by a 100 W overhead bulb.

Procedure

Chicks were removed from the brooder, weighed, and randomly assigned to one of eight groups that received either DZ (0.05, 0.1, 0.2, 0.5, or 1 mg/kg), FG 7142 (0.1 or 1 mg/kg), or vehicle alone. After the appropriate injection was given, the chick was placed in a cardboard box and carried to the experimental room. Twenty minutes after the injection the chick was removed from the box and placed in the center square of the open-field apparatus. The ambulation latency (time elapsed before the bird left the center square), locomotor activity (the number of squares crossed), the number of escape attempts from the apparatus (jumps at the walls), and the number of defecations was registered during 5 min by an observer who was blind to the drug treatment. The floor of the open-field was wiped clean after each chick was tested.

RESULTS AND DISCUSSION

Figure 1a shows the dose–response curve of DZ and response to two doses of FG 7142 on the ambulation latency. A one-way analysis of variance (ANOVA) revealed significant differences between groups, $F(7, 72) = 5.69$, $p = 0.0001$. LSD pairwise comparison of means test showed significant differences in DZ group (1 mg/kg) and FG 7142 group (1 mg/kg) compared to the control vehicle group ($p < 0.05$).

Figure 1b shows the dose–response curve of DZ and response to two doses of FG 7142 on the number of squares crossed. A one-way ANOVA revealed significant differences between groups, $F(7, 72) = 3.54$, $p = 0.0027$. LSD pairwise comparison of means test showed significant differences in DZ groups (0.5 and 1 mg/kg) and FG 7142 group (1 mg/kg) compared to the control vehicle group ($p < 0.05$).

Figure 1c shows the dose–response curve of DZ and response to two doses of FG 7142 on the number of escape attempts. A one-way ANOVA revealed significant differences between groups, $F(7, 72) = 2.15$, $p = 0.0493$). LSD pairwise comparison of means test showed significant differences in DZ group (1 mg/kg) and FG 7142 group (1 mg/kg) compared to the control vehicle group ($p < 0.05$).

Figure 1d shows the dose–response curve of DZ and response to two doses of FG 7142 on the number of defecations. A one-way ANOVA revealed significant differences between groups, $F(7, 72) = 2.98$, $p = 0.0087$. LSD pairwise comparison of means test showed significant differences in DZ group (1 mg/kg) and FG 7142 group (0.1 mg/kg) compared to the control vehicle group ($p < 0.05$).

The results show that at lower doses (0.05, 0.1, 0.2 mg/kg) DZ had no effect on the open-field parameters, while a decrease in the number of squares crossed was induced by the higher doses of DZ (0.5 and 1 mg/kg). Increase on the ambulation latency and decrease in both the number of escape attempts and defecations were induced by the highest dose of DZ (1 mg/kg). Taken together, the results indicate that the sedative effect of DZ on open-field behaviors was evidenced at a 0.5 mg/kg dose and clearly observed at a 1 mg/kg dose. It is relevant that these and even higher doses of DZ were considered anxiolytic by several reports, i.e., 2 mg/kg in mice (31); 1.5 in rats (29); 1.75 mg/kg in pigeons (25).

On the other hand, FG 7142 at a dose of 1 mg/kg significantly increased the ambulation latency, decreased both the number of squares crossed and escape attempts, and decreased but not significantly, the number of defecations. FG 7142 at a dose of 0.1 mg/kg induced similar tendencies than at a dose of 1 mg/kg on these behavioral parameters, except on the number of defecations, which was significantly increased. These results (Fig. 1d) are consistent with the hypothesis from Gallup et al. (9), who suggested that defecation in chicks is suppressed during intense fear and represents a reaction to more moderate fear. Taken together, the results suggest that FG 7142 at either doses displayed an anxiogenic-like behavior. The anxiogenic effect of FG 7142 influenced open-field behavior as would a fear-inducing manipulation, which is consistent with a previous report in chicks, but at a dose of 2.5 mg/kg (23).

It has also been reported that in open-field experiments the number of defecations is positively correlated with the ambulation scores (9). Our results in the FG 7142 treated chicks also showed a positive correlation between number of defecations and squares crossed (Fig. 1b and d).

EXPERIMENT 2

The T-maze test has been employed to select either chicks with different susceptibility to swim stress (22) or with different growth capacity (unpublished data). Because the lower dose of DZ (0.05 mg/kg) and FG 7142 (0.1 mg/kg) had no effect on the locomotor activity and other parameters in the open-field test, these doses were chosen to be assayed on the T-maze test. The present experiment was designed to further support the hypothesis that the T-maze is a more sensitive test to assess fear/anxiety in chicks than the open-field test.

METHOD

Subjects

A total of 220 2-day-old chicks were obtained and maintained as indicated in Experiment 1.

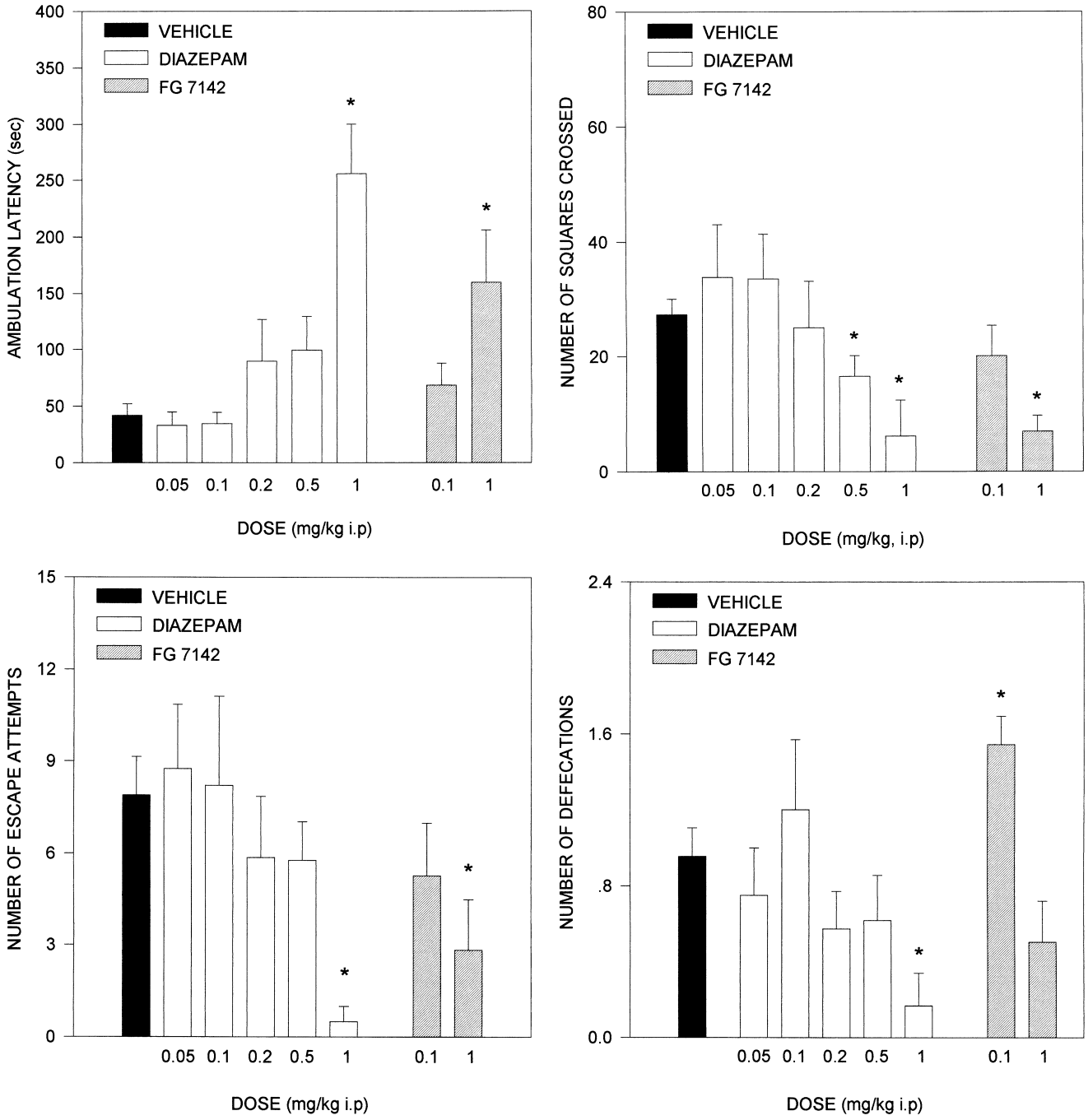

FIG. 1. Effects of DZ and FG 7142 on open-field behaviors: (a) ambulation latency; (b) number of squares crossed; (c) number of escape attempts, and (d) number of defecations. Bars represent the mean, and vertical lines the SEM. Chicks were injected with vehicle, diazepam (0.05, 0.1, 0.2, 0.5, or 1 mg/kg, IP) or Fg7142 (0.1 or 1 mg/kg, IP) 20 min before the experimental session. *$p < 0.05$, compared to the vehicle control group (LSD test); $n = 24$ for the vehicle control group and $n = 8$ for the remaining drug-treated groups.

Drugs

DZ (0.05 mg/kg), FG 7142 (0.1 mg/kg) and vehicle were prepared and injected as indicated in Experiment 1.

T-Maze Apparatus

The apparatus, as described by Gilbert et al. (11) consisted of a T-maze joined to an isolation chamber, placed inside a communal brooder, but separated from the brood area by chicken wire. A small 10 × 10 cm mirror was located at the T-junction of the maze, just above the floor of the T-maze, to facilitate the arrival of the chick to the T-junction. Then, the chick could choose to enter any of the maze arms. The brood area was illuminated with a bright lamp (100 W) suspended immediately above it, and food and water were freely available. The apparatus and brooder were contained in a 95 × 60

cm box that was kept in a small room (2 × 3 m) held at constant temperature and humidity during testing.

Procedure

The procedure was essentially as described in previous reports (11,22). Groups of 20 birds each, were individually marked with spray dye 1 day before the test. Each group was placed in the communal brooder area before training to interact with each other freely for at least 1 h. Training always commenced at 1000 h. Chicks were removed from the communal brooder, weighted, and randomly assigned to one of three groups that received either DZ (0.05 mg/kg), FG 7142 (0.1 mg/kg), or vehicle. Twenty minutes after the injection, each chick was placed individually in the center of the isolation chamber facing away from the T-corridor. The time taken for the chick to arrive to the exit of the apparatus (total first escape time) was recorded. Time taken for the chick to leave the isolation chamber because the begining of the test (latency to escape from isolation chamber) and the time spent in the T-corridor were also registered. Then the chick was immediately returned to its broodmates. After 3 h the chick was placed again in the isolation chamber and the second trial began, without been injected again. The same behavioral measures as indicated for the first trial were recorded. After the completion of each test the maze was wiped clean.

RESULTS AND DISCUSSION

Figure 2 shows the effects of DZ and FG 7142 on the first escape time in the T-maze test. A repeated measures ANOVA revealed significant differences between groups, $F(2, 217) = 6.40$, $p < 0.01$, significant differences within groups, $F(1, 217) = 50.40$, $p < 0.01$, as well as a significant interaction, $F(2, 217) = 3.41$, $p < 0.01$. LSD pairwise comparison of means test showed significant differences in the total first escape time between DZ (38.56 s) compared to the vehicle control group (53.63 s) ($p < 0.015$), and FG 7142 (69.67 s) compared to the vehicle control group (53.63 s) ($p < 0.016$). Moreover, LSD test showed a significant difference between the first and second escape time in vehicle group ($p < 0.001$), DZ group ($p < 0.036$), and FG 7142 group ($p < 0.001$). No differences were observed between groups in the total second escape time. The escape performance was inversely correlated with the escape time.

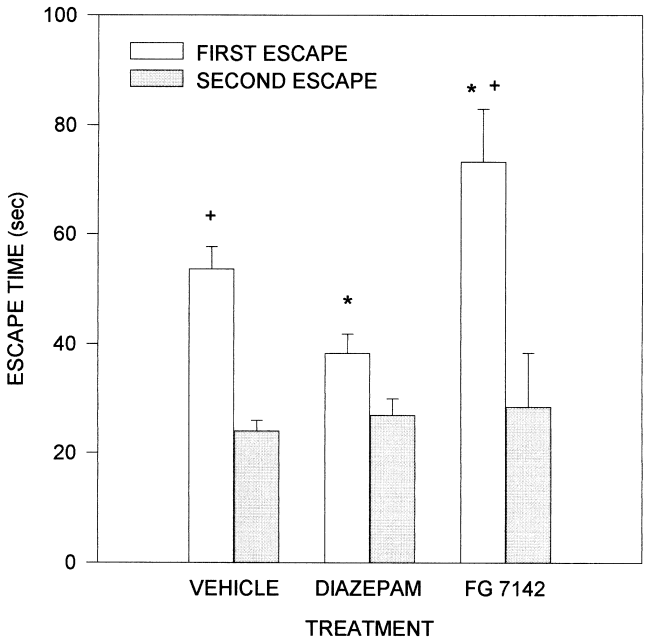

FIG. 2. Effects of DZ and FG 7142 over the escape behavior in the T-Maze test. Bars represent the mean, and vertical lines the SEM. Chicks were injected with vehicle, DZ (0.05 mg/kg, IP) or FG 7142 (0.1 mg/kg, IP) 20 min before the first escape. ANOVA shows significant differences between groups. $^{*}p < 0.016$, compared with vehicle control group. $^{+}p < 0.001$, compared with the respective second escape time (LSD test); $n = 110$ for the vehicle control group, $n = 68$ for the DZ group and $n = 42$ for the FG 7142 group.

To analyze the behavioral effects of DZ and FG 7142 on the two maze sections, time taken for the chicks to escape the isolation chamber (latency to escape), and the time spent in the T-corridor were compared between the three experimental groups (Table 1). One-way ANOVA on the latency to escape, revealed significant differences between groups, $F(2, 217) = 5.71$, $p < 0.005$. LSD pairwise comparison of means test showed significant differences on the latency to escape between DZ (18.01 s), FG 7142 (45.31 s) and vehicle-treated (33.96 s) groups ($p < 0.05$). No differences were observed between these three groups in the time spent in the T-corridor.

The decrease and increase in the total first escape time induced by the DZ and FG 7142, respectively, suggest that the first escape behavior was sensitive to low doses of an anxiolytic or anxiogenic drug. A similar result (midazolam at 0.1 mg/kg tended to decrease the latency to escape the T-maze) was reported (11). Hence, it is probable that chicks with a higher escape performance display an escape behavior of a less anxious type than chicks with a lower escape performance.

The escape performance depended on the latency to escape from the isolation chamber, suggesting that the behavior in this section of the T-maze could be useful as an index of general emotionality. It was observed that the time spent in the isolation chamber was associated with immobility; this motor inhibition of short duration might be a fear indicator. After this initial phase of inhibited response, an investigation phase with calling and locomotor activity was progressively developed. The decrease of latency in the DZ group is in agreement with the fact that anxiolytic drugs reduce fear associated with the new environment, and hence, release suppressed behavior. The increase of latency in the FG 7142 group was principally associated with immobility behavior, which is consistent with increased anxiety.

TABLE 1

EFFECTS OF DZ AND FG 7142 ON THE FIRST ESCAPE PERFORMANCE IN THE TWO SECTIONS OF THE T-MAZE APPARATUS

	Latency to Escape From Isolation Chamber	Time Spent in the T-Corridor
Vehicle	34 ± 3.1 (110)	19 ± 2.6 (110)
Diazepam	18 ± 3.2* (68)	20 ± 3.0 (68)
FG 7142	45 ± 5.6* (42)	24 ± 4.2 (42)

Each value is the mean ± SEM of the scored time (s) in the isolation chamber and T-corridor of the T-maze apparatus, in 2-day-old chicks. DZ (0.05 mg/kg, IP) and FG 7142 (0.1 mg/kg, IP) were injected 20 min before the first escape. Number of chicks is indicated in parentheses.

$^{*}p = 0.05$ compared to vehicle control chicks (LSD test).

At the beginning of the test, the chick did not see the mirror because it was placed in the center of the chamber facing away from the T-corridor. Only when the chick began the exploration phase in the chamber it could see its reflection and immediately walk into the T-corridor. The time spent in the T-corridor might be an index of social reinstatement. No differences were observed between groups on the time taken into the T-corridor, suggesting that the drugs, at the administered doses, did not modify the social motivation to escape the maze.

It was reasoned that chicks who remembered the way out from the first escape would show an improved performance, leaving the isolation chamber more quickly on the second escape. The lack of differences between groups in the second escape time suggests that DZ and FG 7142 do not affect retention on this task at the doses used in the present work.

EXPERIMENT 3

Preinjection of an anxiolytic dose of DZ or an anxiogenic dose of FG 7142 respectively increased or decreased the first escape performance in the T-maze test (Experiment 2). Furthermore, it was reported that chicks selected on the basis of their second escape performance in the T-maze test exhibited different susceptibility to acute stress associated to increases of both central and peripheral-type BZD receptors (22). In the present experiment we study if chicks with different first escape performance present different susceptibility to an anxiolytic DZ dose on the second escape performance.

METHOD

Subjects, Drugs, and Apparatus

Subjects were 129 2-day-old chicks, obtained and mantained as indicated in Experiment 1. The T-maze was described in Experiment 2. An anxiolytic dose of DZ (0.05 mg/kg) and vehicle were prepared and injected as described in Experiment 1.

Procedure

The procedure was essentially as described in Experiment 2. Three chick groups were selected on the basis of their first escape time. Chicks that escaped in less than 25 s were termed the high-performance (H-P) group, the ones that escaped in 25–75 s were termed the moderate-performance (M-P) group, and the ones that escaped in 75–300 s were termed the low-performance (L-P) group. Chicks that took longer than 300 s to escape were discarded (less than 3% of total of the chicks). Chicks selected for each condition (H-P, M-P, and L-P) were removed from the communal brooder, weighed, and randomly assigned to one of two groups, and then injected with vehicle or DZ 20 min before the second escape.

RESULTS AND DISCUSSION

Figure 3 shows the effect of DZ on the second escape performance in chicks previously selected according to the first escape performance. Two-way ANOVA on the second escape time (selection × treatment) revealed significant differences of selection, $F(2, 123) = 11.14$, $p < 0.0001$, a significant effect of DZ treatment, $F(1, 123) = 17.73$, $p < 0.0001$, as well as a significant interaction, $F(2, 123) = 5.40$, $p < 0.006$. LSD pairwise comparison of means test showed significant differences ($p < 0.05$) between the H-P, M-P, and L-P control groups (16.30, 27.00, and 53.64 s, respectively). The DZ significantly increased the second escape performance in L-P group (25.62 s) compared with the control (53.64 s) ($p < 0.05$). Significant differences in the second escape performance were not observed between M-P and H-P groups, suggesting that this drug selectively affects the L-P more anxious chick group. This results can be compared with previous results (22) that showed that L-P chicks displayed lower increase than H-P chicks in the forebrain BZD receptors associated to acute swimming stress. Taken together, the results suggest that the anxiety level is inversely correlated with the degree of BZD receptor increase associated to stress in chicks.

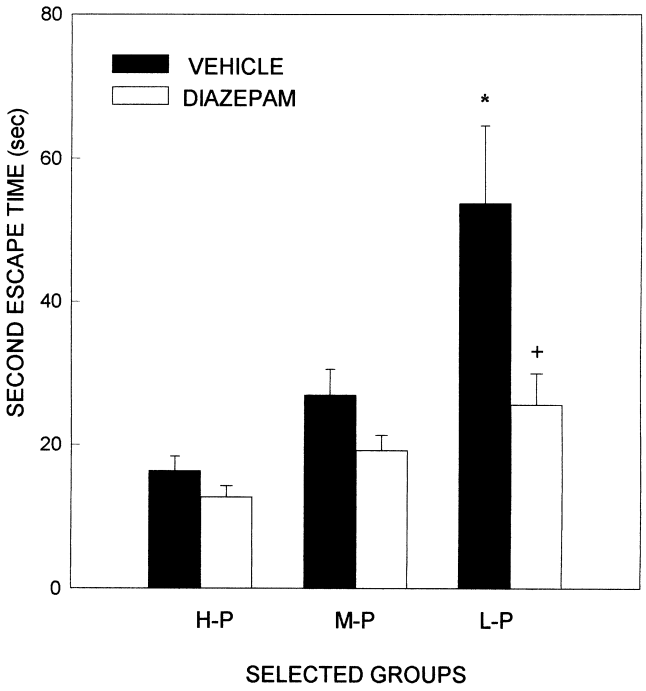

FIG. 3. Effect of DZ (0.05 mg/kg, IP) over the second escape behavior of H-P, M-P, and L-P chick groups previously selected by their first escape time in the T-Maze test. Bars represent the mean and vertical lines the SEM. Chicks were injected with vehicle or DZ, 20 min before the second escape. $^{*}p < 0.05$, compared to the M-P and H-P control groups. $^{\#}p < 0.05$, compared to H-P control group. $^{+}p < 0.05$, compared to the vehicle control L-P group; $n = 40$ for the H-P group, $n = 64$ for the M-P group and $n = 25$ for the L-P group.

On the other hand, the significant differences observed between the three control selected groups in the second escape time (Fig. 3) suggest a direct relationship between the first and second escape performance. That is, chicks with a lower first escape time (H-P) showed a lower second escape time, and chicks with higher first escape time (L-P) showed a higher second escape time.

GENERAL DISCUSSION

Several behavioral constructs, such as general activity, exploration, social motivation, and predator avoidance have been evoked to explain open-field behaviors (6,10,20). It was proposed that the response to open field primarily represent a compromise between opposing tendencies to reinstate contact with its social companions and to avoid detection by potential predators (10). Thus, the lower ambulation latency, the higher number of squares crossed and the higher number of escape

attempts can be regarded as socially motivated behavior patterns that increase the likelihood of the isolated chick reinstating social contact in the open-field test (6,30).

In the maze-learning task, just as in the open-field test, chicks may present the same two motivations that control their behavior: fear and social reinstatement. In both tests, chicks are separated from their imprinted companions and placed in an unfamiliar environment, where they usually show signs of emotional stress (distress calling, freezing behavior, and in some cases, defecating). Chicks work to escape the isolation-induced stress and get back with the conspecifics (10,11,22). It was reported (22) that in T-maze selected chicks on their second escape performance, H-P chicks were more susceptible to acute stress associated to the increase of BZD receptors than L-P chicks, probably due to differences in the degree of endogenous emotionality. However, we do not know other reports in the literature on the factors that may control behavior in the T-maze test.

The behavioral effects of FG 7142 (Experiments 1 and 2) should be the inhibition of reinstatement behavior. These effects are consistent with the results suggesting that anxiogenic drugs enhance fearfulness in a variety of behavioral tasks (7,24,29). A low DZ dose improved the escape performance of the T-maze (Experiment 2). This result is consistent with the fact that anxiolytic drugs reduce fear associated to a new environment and, hence, release supressed behavior. It is not clear why the same anxiolytic dose of DZ (0.05 mg/kg) did not affect the open-field behavior (Experiment 1); however, several differences between tasks should be considered: in the T-maze task, after translation to the testing room, animals spent 1 h in habituation to the novelty to the environment in the presence of their cage mates; chicks in the isolation chamber can find their way out of the box having heard the call of their companions, and can display an escape-oriented behavior. Moreover, their own reflection in a mirror simulates the presence of a companion. On the other hand, in the open-field test, chicks are captured and placed into a big lighted arena without prior habituation, without sound stimulus, and they have not the posibility to escape. Thus, both tasks seem to involve a different bidirectional approach/avoidance conflict that may not model the same state of fear/anxiety. The behaviors modulated by anxiety/fear appear to be more sensitive to anxiolytic BZDs in the T-maze task than in the open-field task. It has also been reported that the nature and degree of conflict between the tendencies that influence the task behaviors may determine the efficacy of CBR ligands (17).

The behavioral change in the T-maze test induced by an anxiolytic dose of DZ, mainly occurs in the isolation chamber sector (Experiment 2) and in the L-P chick group (Experiment 3). The escape latency from the isolation chamber could be a useful index of general emotionality. The results of Experiment 2 also suggest that H-P chicks present an escape behavior of a less anxious type than L-P chicks.

Genetic variation in behavioral characteristics exist between and within population of Galliforms (21), and selection programs for divergence in behavioral traits have been undertaken in chickens (12). It was proposed that fear exerts a progressively inhibitory effect on activity of chicks (16,18), so domestic chicks genetically selected for high activity in a novel environment (5) were considered less fearful in a variety of situations than the corresponding "inactive" line. The existence of strain differences in fear/anxiety behavior suggest that this character may be open to genetic manipulation. Thus, the higher sensitivity of the T-maze test to pharmacological manipulations suggest that the escape performance can represent a useful selection criterion in future breeding programs.

The CBR plays a crucial role in the responses asociated with anxiety and stress and it was suggested that the $GABA_A$ system is involved in the behavioral expression of fear and anxiety in chickens (23). Taken together, our results in very young chicks suggest that the GABAergic system is involved in the behavioral expression of fear and anxiety in the T-maze and open-field task.

ACKNOWLEDGEMENTS

This article was supported by grants from CONICET, CONICOR, and SECyT-U.N.C., Argentina. R. H. M holds a research fellowship from the SECyT-U.N.C. The authors thank Dr. R. M. Gleiser for reading the manuscript.

REFERENCES

1. Archer, J.: Tests for emotionality in rats and mice: A review. Anim. Behav. 21:205–235; 1973.
2. Burt, D. R.; Kamatchi, G. L.: $GABA_A$ receptor subtypes: From pharmacology to molecular biology. FASEB J. 5:2916–2923; 1991.
3. Candland, D. K.; Nagy, Z. M.: The open-field: Some comparative data. Ann. NY Acad. Sci. 159:831–851; 1969.
4. Dorow, R.; Horowski, R.; Paschelke, G.; Amin, M.; Braestrup, C.: Severe anxiety induced by FG 7142, a β-carboline for benzodiazepine receptors. Lancet 9:98–99; 1983.
5. Faure, J. M.; Folmer, J. C.: Etude genetique de l'acticite precoce en open-field de jeune poussin. Ann. Genet. Sel. Anim. 7:123–132; 1975.
6. Faure, J. M.; Jones, R. B.; Bessel, W.: Fear and social motivation as factors in open-field behavior of the domestic chick: A theoretical consideration. Biol. Behav. 8:103–116; 1983.
7. File, S. E.; Pellow, S.; Braestrup, C.: Effects of the beta-carboline, FG 7142, in the social interaction test of anxiety and the hole-board: Correlations between behavior and plasma concentrations. Pharmacol. Biochem. Behav. 22:941–944; 1985.
8. Gallager, D. W.; Mallorga, P.; Thomas, J. W.; Tallman, J. F.: GABA-benzodiazepine interactions: Physiological, pharmacological and developmental aspects. Fed. Proc. 39:3043–3049; 1980.
9. Gallup, G. G., Jr.; Ledbetter, D. H.; Maser, J. D.: Strain differences among chickens in tonic immobility: Evidence for an emotionality component. J. Comp. Physiol. Psychol. 90:1075–1081; 1976.
10. Gallup, G. G., Jr.; Suarez, S. D.: An ethological analysis of open-field behaviour in chickens. Anim. Behav. 28:368–378; 1980.
11. Gilbert, D. B.; Patterson, T. A.; Rose, S. P. R.: Midazolam induces amnesia in a simple, one-trial, maze-learning task in young chicks. Pharmacol. Biochem. Behav. 34:439–442; 1989.
12. Graves, H. B.; Siegel, P. B.: Bidirectional selection for fearfulness of *Gallus gallus* chicks to an imprinting situation. Anim. Behav. 17:683–691; 1969.
13. Haefely, W.; Kulcsar, A.; Mohler, H.; Pieri, L.; Schaffner, R.: Mechanisms of action of benzodiazepines. New York: Raven Press; 1975.
14. Hall, C. S.: Emotional behavior in the rat: Defecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. 18:385–403; 1934.
15. Harvey, S. C.: Hypnotics and sedatives. In: Gilman, A. C. G.; Goodman, L. S.; Rall, T. W.; Murad, F. The pharmacological basis of therapeutics. New York: Macmillan; 1985.
16. Hogan, J. A.: An experimental study of conflict and fear; An analysis of the behavior of young chicks towards a mealworm. Part 1; The behavior of chicks which do not eat the mealworm. Behavior 26:45–95; 1965.

17. Holmes, P. V.; Drugan, R. C.: Differential effects of anxiogenic central and peripheral benzodiazepine receptor ligands in tests of learning and memory in rats. Psychopharmacology (Berlin) 704:249–254; 1991.
18. Jones, R. B.: The assessment of fear in the domestic fowl. In: Zayan, R.; Duncan I. J. H., eds. Cognitive Aspects of social behavior in the domestic fowl. Amsterdam: Elsevier; 1987.
19. Jones, R. B.: Social and environmental aspects of fear in the domestic fowl. In: Zayan, R.; Duncan, I. J. H., eds. Cognitive aspects of social behavior in the domestic fowl. Amsterdam: Elsevier; 1987.
20. Jones, R. B.; Faure, J. M.: Open-field behavior of male and female domestic chicks as a function of housing conditions, test situation and novelty. Biol. Behav. 7:17–25; 1982.
21. Jones, R. B.; Mills, A. D.; Faure, J. M.: Genetic and experiential manipulation of fear-related behavior in Japanese quail chicks (*Coturnix coturnix japonica*). J. Comp. Psychol. 105:15–24; 1991.
22. Marín, R. H.; Arce, A.: Benzodiazepine receptors increase induced by stress and maze learning performance, in chicks forebrain. Pharmacol. Biochem. Behav. 3:581–584; 1996.
23. Moriarty, D. D.: Anxiogenic effects of a β-carboline on tonic immobility and open field behavior in chickens (*Gallus gallus*). Pharmacol. Biochem. Behav. 51:795–798; 1995.
24. Pellow, S.; File, S. E.: Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525–529; 1986.
25. Savage, L. M.; Stanchfield, M. A.; Overmier, J. B.: The effects of scopolamine, diazepam, and lorazepam on working memory in pigeons: An analysis of reinforcement procedures and sample problem type. Pharmacol. Biochem. Behav. 48:183–191; 1994.
26. Sieghart, W.: $GABA_A$ receptors: Ligand-gated Cl^- ion channel modulated by multiple drug-binding sites. Trends Pharmacol. Sci. 13:446–450; 1992.
27. Söderpalm, B.: Pharmacology of the benzodiazepine: With special emphasis on alprazolam. Acta Psychiatr. Scand. 76(Suppl. 355):39–46; 1987.
28. Sternbach, L. H.: The benzodiazepine story. J. Med. Chem. 22:1–7; 1979.
29. Stout, J. C.; Weiss, J. M.: An animal model for measuring behavioral responses to anxiogenic and anxiolytic manipulations. Pharmacol. Biochem. Behav. 47:459–465; 1994.
30. Suárez, S. D.; Gallup, G. G. J.: Emotionality and fear in birds: A selected review and reinterpretation. Bird Behav. 5:22–30; 1983.
31. Vinader-Caerols, C.; Brain, P. F.; Parra, A.; Simón, V. M.: An ethological analysis of the effects of diazepam and nitrazepam on the responses of female mice to anosmic males encountered in a novel arena. Behav. Pharmacol. 3:427–433; 1992.