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Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice

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Abstract

The effects of treatment with anxiogenic or anxiolytic agents and exposure to acute restraint stress on emotional behavior in mice were examined using an automatic hole-board apparatus. Changes in the emotional state of mice were evaluated in terms of changes in exploratory activity, i.e., total locomotor activity, numbers and duration of rearing and head-dipping, and latency to the first head-dipping. The typical benzodiazepine anxiolytics diazepam (0.05–0.5 mg/kg, i.p.) and chlordiazepoxide (0.5–4 mg/kg, i.p.) dose-dependently increased the number and duration of head-dips at doses that did not produce sedation. In contrast with these anxiolytics, the typical anxiogenic drugs *N*-methyl-β-carboline-3-carboxamide (FG7142, 0.125–10 mg/kg, i.p.) and methyl-β-carboline-3-carboxylate (β-CCM, 0.1–2 mg/kg, i.p.) decreased both the number and duration of head-dips, and increased the latency to head-dipping. Moreover, decreases in the number and duration of head-dips, and an increase in the latency to head-dipping, were also observed in animals that were exposed to acute restraint stress. These effects of acute restraint stress were suppressed by treatment with diazepam at a dose that alone did not produce significant behavioral effects (0.1 mg/kg, i.p.). In addition, non-benzodiazepine anxiolytic flesinoxan (0.1 mg/kg, i.p.), a 5-HT_{1A} receptor agonist, also had an effect on the restraint stress-induced decrease in head-dipping behavior. The present study shows that the changes in several exploratory behaviors could be objectively measured using our automatic hole-board apparatus. Therefore, this system can serve as a useful tool for evaluating the changes in various emotional states of animals. Moreover, we also found that treatment with anxiolytics or anxiogenics and exposure to acute restraint stress affected head-dipping behavior. These results suggest that changes in head-dipping behavior in the hole-board test may reflect the anxiogenic and/or anxiolytic state of animals.

Keywords: Hole-board; Emotional behavior; Anxiolytic; Anxiogenic; Restraint stress; (Mouse)

1. Introduction

Recent evidence has shown that specific binding sites for benzodiazepine exist on a chlorideionophore-associated complex, with a receptor for γ-aminobutyric acid (GABA), and binding sites for convulsant and anticonvulsant compounds (Olsen, 1982). In the development of new anxiolytic drugs that offer a clear advantage over existing drugs, several new compounds that act at this receptor complex have been synthesized. It is important to investigate the behavioral actions produced by these compounds in several different test procedures.

Most of the procedures used to assess anxiety in rodents are particularly adapted to detect anxiolytic activities. In general, animals are exposed to anxiogenic conditions, i.e., either a novel environment (elevated plus-maze test, social interaction test, open field test, hole-board test) or a conflict situation (Vogel punishment drinking test or Geller–Seifter test) (Geller and Seifter, 1960; Vogel et al., 1971; File and Wardill, 1975; File and Hyde, 1978; Pellow et al., 1985), and these procedures have been used to study the anxiolytic properties of many compounds, especially benzodiazepine.

The hole-board test, which was first introduced by Boissier and Simon (1962, 1964), offers a simple method for measuring the response of an animal to an unfamiliar environment. Previously, the hole-board test has been used to assess emotionality, anxiety and/or responses to stress in animals (Rodriguez Echandia et al., 1987). Some advantages of this test are that several behaviors can be readily observed and quantified, which makes possible a compre-

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hensive description of the animals' behavior. However, this advantage is also a defect in that the behaviors affected by anxiety- and/or anxiogenic-relevant manipulations often vary between animals. Therefore, to overcome this problem, it is important to identify behavior(s) of animals that are affected by anxiety and/or an anxiolytic state. To establish a more detailed behavioral analysis, we recently modified this apparatus, and developed an automatic hole-board apparatus (Fig. 1). We expect that this system will make it possible to automatically measure changes in various exploratory activities of animals; therefore, this system may be a useful tool for objectively estimating various emotional states of animals.

Although several investigators have previously examined the effects of benzodiazepine anxiolytics such as diazepam on behavior in the hole-board test, the results were not consistent, i.e., exploratory behaviors were either increased (Nolan and Parkes, 1973; Crawley, 1985) or decreased (Weischer, 1976). Moreover, the effects of several anxiogenics such as benzodiazepine inverse agonists on the hole-board test have been studied very little (File et al., 1985). The aim of the present study was to establish the pharmacological value of our hole-board system for the evaluation of the anxiogenic and/or anxiolytic state of animals. The effects of reference anxiolytics and anxiogenics on various exploratory behaviors were examined using the automatic hole-board apparatus. The putative anxiolytic compounds selected were diazepam, chlordiazepoxide and flesinoxan, and the anxiogenic compounds were the β-carboline derivative *N*-methyl-β-carboline-3-carboxamide (FG7142) and methyl-β-carboline-3-carboxylate (β-CCM). These compounds have produced anxiolytic or anxiogenic effects with other methods, such as in the elevated plus maze (Pellow et al., 1985; Pellow and File, 1986; File and Zangrossi, 1993; Simon et al., 1993; Rodgers et al., 1994). In addition, it has been previously reported that changes in some spontaneous behaviors, i.e., locomotion, exploration and rearing, can occur as the results of exposure to various stressful stimuli (Stone et al., 1984). Therefore, the effect of acute restraint stress on the exploratory behavior of mice in the hole-board test was also investigated.

2. Materials and methods

2.1. Animals

Male ICR mice (Charles River, Japan) weighing 25–30 g were housed at a room temperature of 23 ± 1°C with a 12-h light–dark cycle (light on 7:00 AM to 7:00 PM). Food and water were available ad libitum.

2.2. Apparatus

The automatic hole-board apparatus (model ST-1, Muromachi Kikai, Japan) consisted of a gray wooden box ($50 \times 50 \times 50$ cm) with four equidistant holes 3 cm in diameter in the floor. An infrared beam sensor was installed on the wall to detect the number and duration of rearing and head-dipping behavior, and the latency to the first head-dipping. Other behavioral performance such as locus, distance and speed of movement of mice in the hole-board was recorded by the overhead color CCD camera. Namely, the head of mice are painted yellow and color CCD camera chase its center of gravity. Data from CCD camera was collected through a custom-designed interface (CAT-10, Muromachi Kikai, Japan) as a reflection signal. Head-dipping behaviors were double-checked via an infrared beam sensor and an overhead color CCD camera. Thus, only when both the head intercepted the infrared

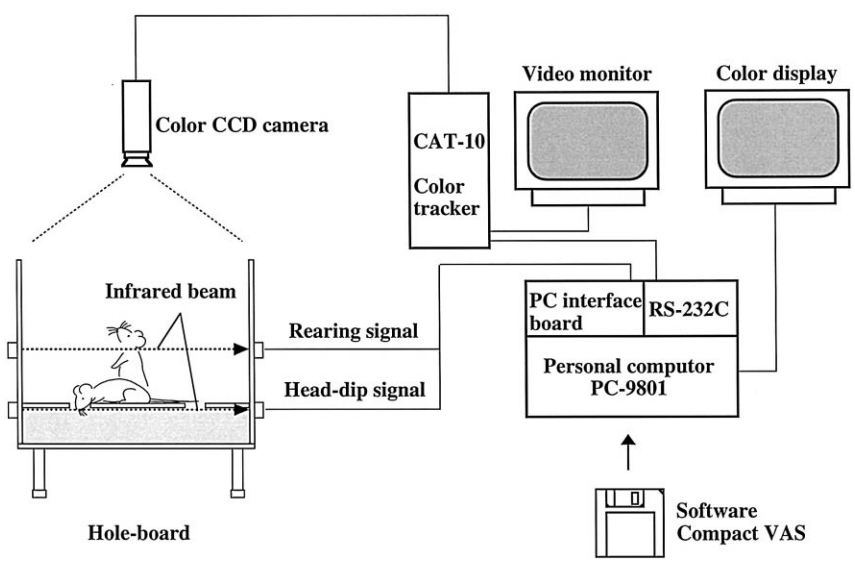

Fig. 1. The automatic hole-board apparatus (model-ST1).

beam, and the head was detected at the hole by the CCD camera was head-dipping behavior counted. All of the data were analyzed and stored in a personal computer using analytical software (Comp ACT HBS, Muromachi Kikai, Japan).

2.3. Behavioral procedure

2.3.1. Effects of anxiogenic or anxiolytic agents on the exploratory behavior of mice in the hole-board test

For the hole-board experiments, groups of animals were injected with drugs or vehicle. Thirty minutes later, each animal was placed in the center of the hole-board, and allowed to freely explore the apparatus for 5 min. Total locomotor activity, number and duration of rearing and head-dipping and latency to the first head-dip were automatically recorded.

2.3.2. Effects of acute restraint stress on the exploratory behavior of mice in the hole-board test

Animals were restrained in a snug-fit apparatus or left in their home cage for 30, 60 or 120 min. Each animal was injected with vehicle, and the behavioral experiments were performed 30 min later. Moreover, the effect of treatment with diazepam or flesinoxan on the changes in exploratory behavior produced by 60 min of acute restraint stress was also investigated. Animals were exposed to 60 min of restraint stress, and then diazepam (0.1 mg/kg, i.p.), flesinoxan (0.1 mg/kg, i.p.) or vehicle was injected. The behavioral experiments were performed 30 min later.

2.4. Drugs

The drugs used in the present study were diazepam (Wako, Japan), chlordiazepoxide hydrochloride (Sigma, USA), *N*-methyl-β-carboline-3-carboxamide (FG7142; Research Biochemicals, USA), methyl-β-carboline-3-carboxylate (β-CCM; Research Biochemicals, USA) and flesinoxan hydrochloride (provided by Solvay, Netherlands). β-CCM and flesinoxan was dissolved in saline, and all other drugs were suspended by ultrasound in saline with a drop of Tween 20.

2.5. Statistical analysis

The data are presented as the mean ± S.E.M. One-way repeated measures analysis of variance (ANOVA) followed by Dunnett's test was used for the statistical evaluation ($P < 0.05$ and 0.01).

3. Results

3.1. Effect of diazepam on exploratory behavior in mice

The effect of diazepam on exploratory behavior in mice is shown in Fig. 2. Diazepam (0.05–1 mg/kg, i.p.) produced a dose-dependent decrease in locomotor activity ($F(4,47) = 3.946$; $P < 0.01$) as well as in the number ($F(4,47) = 4.479$; $P < 0.01$) and duration ($F(4,47) = 5.168$; $P < 0.01$) of rearing behaviors, and this difference was statistically significant at 1 mg/kg ($P < 0.01$). In contrast, the number and duration of head-dips was significantly increased by treatment with diazepam at doses that did not produce sedation (0.5 mg/kg, $P < 0.05$). However, a sedative dose (1 mg/kg, i.p.) did not modify the head-dipping behavior. In addition, the latency to head-dipping tended to decrease following treatment with non-sedative doses of diazepam (0.05–0.5 mg/kg, i.p.), al-

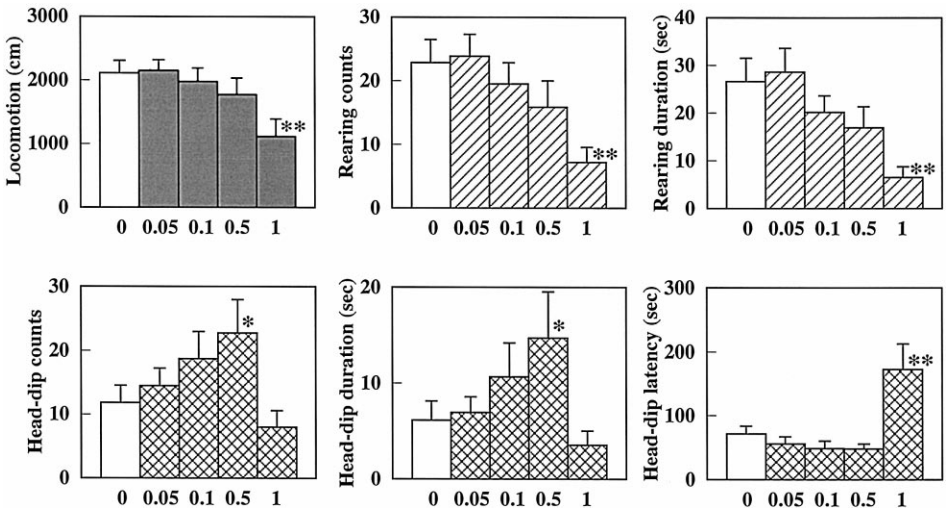

Fig. 2. Effect of diazepam on exploratory behavior in mice tested on the hole-board. Diazepam (0.05–1 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 8–12 mice. * $P < 0.05$, ** $P < 0.01$ vs. vehicle-treated group (open column).

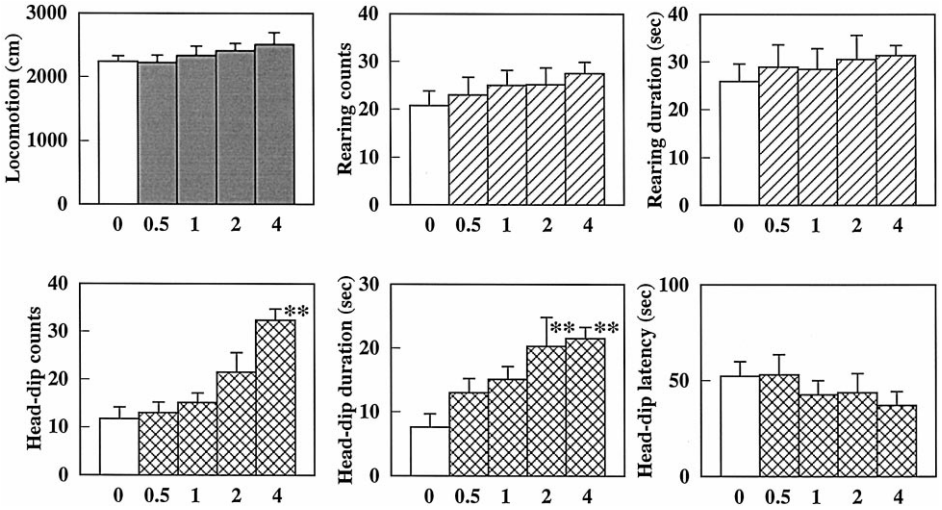

Fig. 3. Effect of chlordiazepoxide on the exploratory behavior in mice tested on the hole-board. Chlordiazepoxide (0.5–4 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 8–12 mice. $**P < 0.01$ vs. vehicle-treated group (open column).

though a significant increase was observed at a sedative dose (1 mg/kg, i.p., $P < 0.01$).

3.2. Effect of chlordiazepoxide on exploratory behavior in mice

The effect of chlordiazepoxide on exploratory behavior in mice is shown in Fig. 3. Chlordiazepoxide (0.5–4 mg/kg, i.p.) did not modify locomotor activity or the number or duration of rearing behaviors. As in the group treated with diazepam, the number ($F(4,43) = 11.752$; $P < 0.01$) and duration ($F(4,43) = 5.686$; $P < 0.01$) of head-dips was dose-dependently increased by treatment with chlordiazepoxide, and this increase was statistically significant at 2 and 4 mg/kg ($P < 0.01$). Moreover, the latency to head-dipping tended to decrease following treatment with chlordiazepoxide.

3.3. Effect of FG7142 on exploratory behavior in mice

The effect of FG7142 on exploratory behavior in mice is shown in Fig. 4. FG7142 (1.25–10 mg/kg, i.p.) did not significantly modify locomotor activity or the number or duration of rearing behaviors, although rearing did tend to decrease. In contrast to the groups that were treated with anxiolytics, the numbers ($F(3,39) = 3.078$; $P < 0.05$) and duration ($F(4,48) = 3.004$; $P < 0.05$) of head-dips dose-dependently decreased following treatment with FG7142, and this decrease was statistically significant at 5 and 10

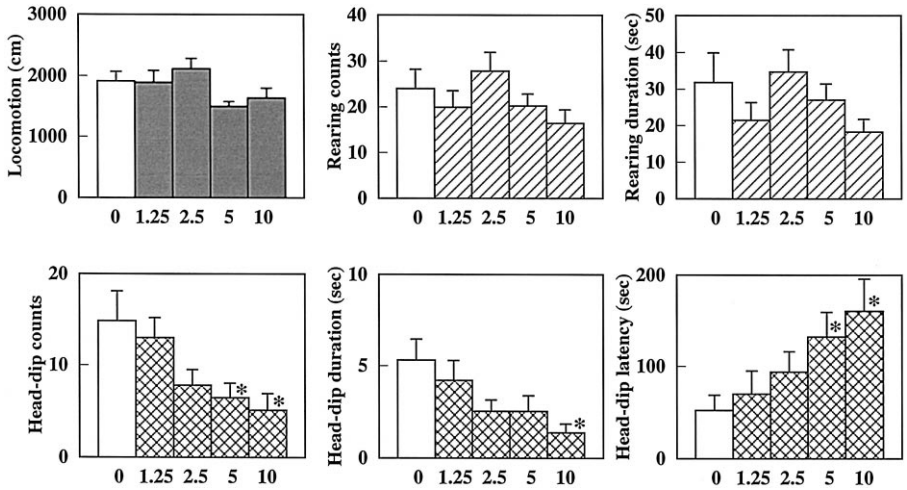

Fig. 4. Effect of FG7142 on exploratory behavior in mice tested on the hole-board. FG7142 (0.125–10 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 10–11 mice. $*P < 0.05$ vs. vehicle-treated group (open column).

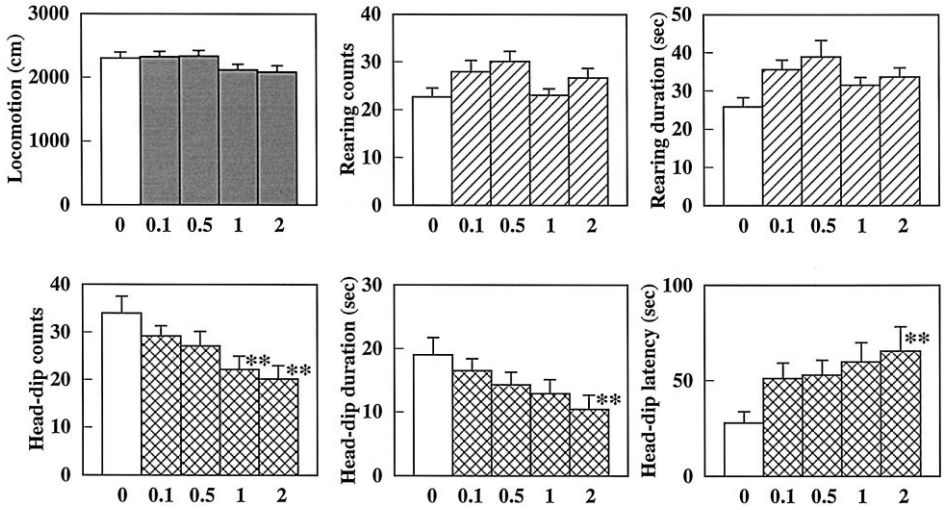

Fig. 5. Effect of β-CCM on exploratory behavior in mice tested on the hole-board. β-CCM (0.1–2 mg/kg, i.p.) or saline (10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 12 mice. ∗∗ $P < 0.01$ vs. vehicle-treated group (open column).

mg/kg ($P < 0.05$). Moreover, the latency to head-dipping was dose-dependently increased by treatment with by FG7142 ($F(4,50) = 2.774$; $P < 0.05$).

3.4. Effect of β-CCM on exploratory behavior in mice

The effect of β-CCM on the exploratory behavior in mice is shown in Fig. 5. β-CCM (0.1–2 mg/kg, i.p.) did not modify locomotor activity or the number or duration of rearing behaviors. As with FG7142, the number ($F(4,55) = 3.624$; $P < 0.05$) and duration ($F(4,55) = 2.6316$; $P < 0.05$) of head-dips dose-dependently decreased following treatment with β-CCM, and this decrease was statistically significant at 1 and 2 mg/kg ($P < 0.01$). Moreover, the latency to head-dipping dose-dependently increased following treatment with β-CCM ($F(4,55) = 2.590$; $P < 0.05$).

3.5. Effect of acute restraint stress on exploratory behavior in mice

The effect of acute restraint stress on exploratory behavior in mice is shown in Fig. 6. Acute restraint stress (30, 60 and 120 min) did not modify locomotor activity or the number or duration of rearing behaviors. As in the groups treated with anxiogenics, the number and duration of head-dips decreased with restraint stress, while the latency to head-dipping increased. The most drastic changes in

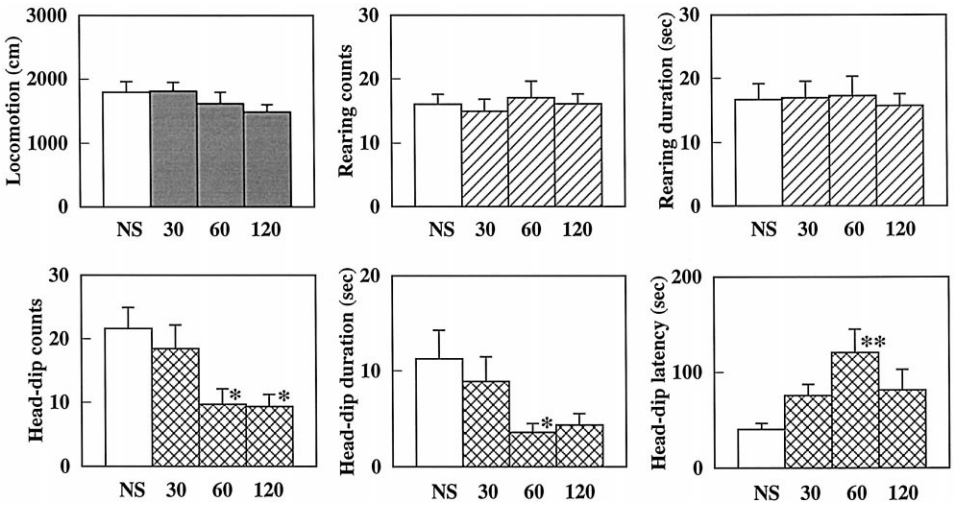

Fig. 6. Effect of acute restraint stress on exploratory behavior in mice tested on the hole-board. Mice were exposed to acute restraint stress for 30, 60 or 120 min, and vehicle (10 ml/kg, i.p.) was then injected. Thirty minutes later, exploratory behavior was measured. Each column represents the mean with S.E.M. of 11–12 mice. ∗ $P < 0.05$, ∗∗ $P < 0.01$ vs. non-stressed (NS) group (open column).

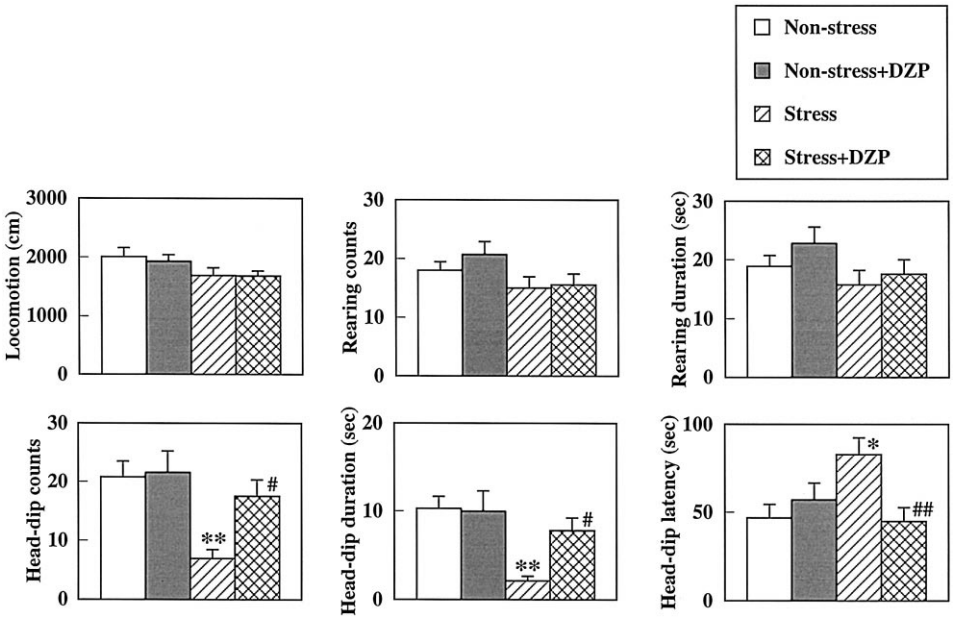

Fig. 7. Effect of diazepam (DZP) on the changes in head-dipping behaviors produced by acute restraint stress in mice tested on the hole-board. Mice were exposed to acute restraint stress (60 min), and diazepam (0.1 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.) was then injected. Thirty minutes later, exploratory behavior was measured. Each column represents the mean with S.E.M. of 15 mice. $*P < 0.05$, $**P < 0.01$ vs. non-stressed group (open column). $\#P < 0.05$, $\#\#P < 0.01$ vs. stress plus vehicle group (shaded column).

head-dipping behavior caused by restraint stress were observed in the 60-min restraint group.

3.6. Effect of diazepam on the changes in head-dipping behavior produced by restraint stress in mice

The effect of diazepam on the changes in head-dipping behavior in mice produced by restraint stress is shown in Fig. 7. Acute restraint stress (60 min) produced a significant decrease in both the number and duration of head-dips ($P < 0.01$), and an increase in the latency of head-dipping ($P < 0.05$). Both the decrease in head-dipping behavior and the increase in the latency to head-dipping produced by restraint stress were suppressed by treatment with diazepam (0.1 mg/kg, i.p.) at a dose that alone did not produce a significant behavioral effect.

3.7. Effect of flesinoxan on exploratory behavior in mice

The effect of flesinoxan on exploratory behavior in mice is shown in Fig. 8. Flesinoxan (0.03–3 mg/kg, i.p.)

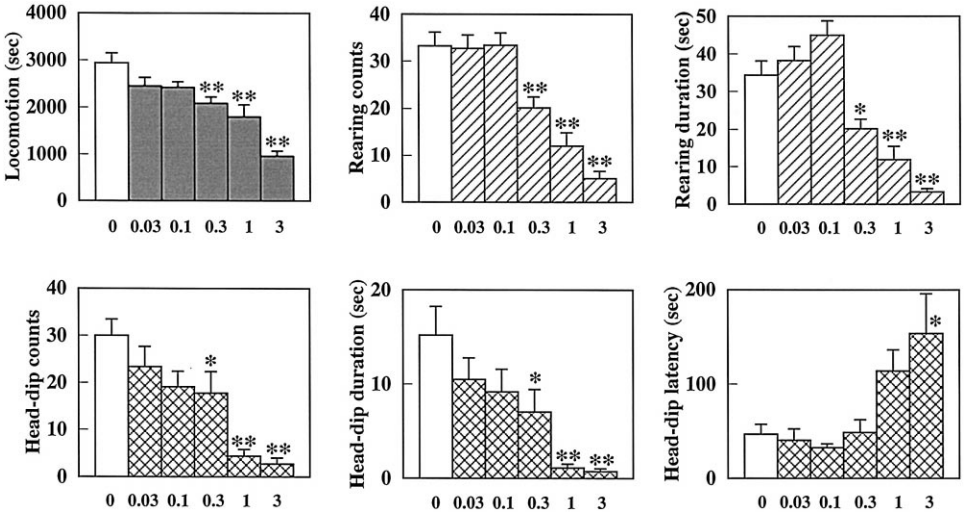

Fig. 8. Effect of flesinoxan on exploratory behavior in mice tested on the hole-board. Flesinoxan (0.03–3 mg/kg, i.p.) or saline (10 ml/kg, i.p.) was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E.M. of 7–8 mice. $*P < 0.05$, $**P < 0.01$ vs. vehicle-treated group (open column).

produced a dose-dependent decrease in locomotor activity ($F(4,47) = 3.946$; $P < 0.01$), as well as in the number ($F(4,47) = 4.479$; $P < 0.01$) and duration ($F(4,47) = 5.168$; $P < 0.01$) of rearing behaviors, and this difference was statistically significant at 0.3, 1 and 3 mg/kg ($P < 0.01$). The number ($F(4,47) = 4.479$; $P < 0.01$) and duration ($F(4,47) = 4.479$; $P < 0.01$) of head-dips was also dose-dependently decreased by treatment with flesinoxan, and statistical significance was observed at 0.3, 1 and 3 mg/kg ($P < 0.05$ and 0.01). Moreover, the latency to head-dipping dose-dependently increased following treatment with flesinoxan ($F(4,55) = 2.590$; $P < 0.05$).

3.8. Effect of flesinoxan on the changes in head-dipping behavior produced by restraint stress in mice

The effect of flesinoxan on the changes in head-dipping behavior in mice produced by restraint stress is shown in Fig. 9. Acute restraint stress (60 min) produced a significant decrease in both the number and duration of head-dips ($P < 0.01$), and an increase in the latency of head-dipping ($P < 0.05$). Treatment with flesinoxan (0.1 mg/kg, i.p.) at a dose that alone did not produce a significant behavioral effect (see Fig. 8) significantly suppressed the restraint stress-induced decrease in head-dip duration ($P < 0.05$). Moreover, the decrease in head-dip count and the increase in the latency to head-dipping produced by restraint stress also tended to reverse to control level by treatment with flesinoxan, although statistical significance was not observed.

4. Discussion

The present data demonstrate that typical benzodiazepine anxiolytics, diazepam and chlordiazepoxide, have clear and consistent effects on head-dipping behavior in the hole-board test. Both the number and duration of exploratory head-dips were dose-dependently increased by treatment with either compound at doses that did not produce sedation. This observation is consistent with previous reports of an increase in the frequency and duration of exploratory head-dips exhibited on a hole-board following the injection of non-sedative doses of diazepam. However, this effect was reversed at high doses of diazepam, which induced sedation (Suzuki et al., 1990). Similarly, Nolan and Parkes (1973) reported that not only diazepam, but also chlordiazepoxide increased head-dipping behavior at doses similar to those used in the present study. Interestingly, another procedure that is used to estimate the anxiolytic effect, i.e., the elevated 'zero-maze', gave results similar to those in the present study (Shepherd et al., 1994). In this procedure, both diazepam and chlordiazepoxide significantly increased the frequency of head-dipping. Moreover, these effects of diazepam and chlordiazepoxide on head-dipping behavior were generally evident at doses lower than those required to significantly

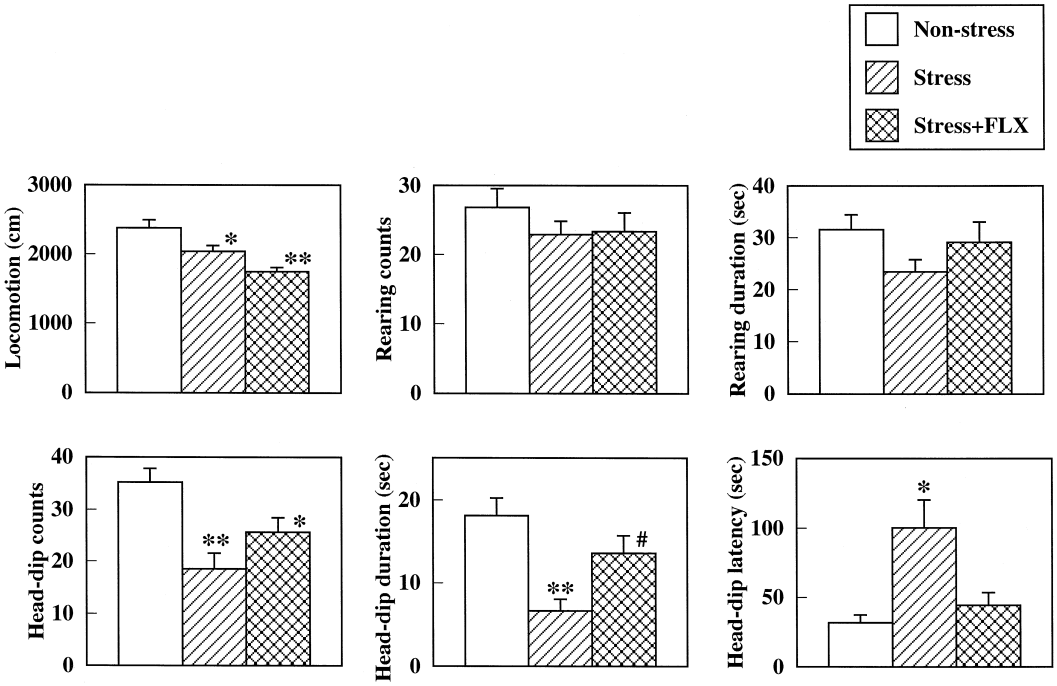

Fig. 9. Effect of flesinoxan (FLX) on the changes in head-dipping behaviors produced by acute restraint stress in mice tested on the hole-board. Mice were exposed to acute restraint stress (60 min), and flesinoxan (0.1 mg/kg, i.p.) or saline (10 ml/kg, i.p.) was then injected. Thirty minutes later, exploratory behavior was measured. Each column represents the mean with S.E.M. of 12 mice. $*P < 0.05$, $**P < 0.01$ vs. non-stressed group (open column). $\#P < 0.05$ vs. stress plus saline group (shaded column).

affect the time spent on the open quadrant in the elevated plus-maze test. These results indicate that the use of a more detailed behavioral analysis appears to increase the ability to measure anxiolytic activity. Based on these previous reports, the present results indicate that head-dipping behavior is sensitive to changes in the emotional state of the animal, and suggest that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behavior.

In contrast to the effects of anxiolytics such as diazepam and chlordiazepoxide, the effects of anxiogenics such as a benzodiazepine inverse agonist on the results of the hole-board test have received little attention (File et al., 1985). In the present study, we demonstrated that both FG7142 and β-CCM, a β-carboline derivative, produced effects on head-dipping behavior that were opposite those of anxiolytics such as diazepam and chlordiazepoxide. Both FG7142 and β-CCM dose-dependently decreased the number and duration of head-dips and increased the latency to the first head-dip. It is important to note that the doses of anxiogenics used in the present study previously produced the anxiety in another procedure (Pellow and File, 1986; File and Zangrossi, 1993; Simon et al., 1993). This suggests that a decrease in head-dipping behavior in the hole-board test may reflect the anxiogenic state of animals, and that both the anxiogenic and anxiolytic states of animals may be estimated using the hole-board test.

Similar to the effects of treatment with anxiogenics, exposure of mice to acute restraint stress also produced a decrease in head-dipping behavior and an increase in the latency to head-dipping. It has been previously reported that the exposure of animals to various stressful stimuli decreases some exploratory behaviors (Stone et al., 1984). In the hole-board test, a pronounced inhibition of head-dipping behavior was observed in animals that had been exposed to stressful stimuli (Rodriguez Echandia et al., 1987). This report is consistent with our present findings, and suggest that various stressful stimuli affect head-dipping behavior in animals. Moreover, we also found in the present study that the decrease in head-dipping behavior produced by acute restraint stress was reversed by treatment with diazepam at doses that alone did not produce significant behavioral effects. These results suggest that acute restraint stress may produce anxiety, and that the decrease in head-dipping behavior reflects this emotional state. It is important to note that the behavioral changes induced by acute stress were similar to those in the groups treated with anxiogenics, i.e., a decrease in head-dipping behavior was observed in both groups. These observations support our hypothesis that a decrease in head-dipping behavior in the hole-board test reflects the anxiogenic state of animals. Considering the results of the present study, and those given in several previous reports, we conclude that the change in head-dipping behavior in the hole-board test can provide information regarding the emotional state of animals.

The search for novel classes of anxiolytics that are free of the numerous problems associated with chronic benzodiazepine use is an important but hard task. It is generally recognized that animal models of anxiety must be validated. However, a problem associated with this approach is the lack of an appropriate behavioral profile that is sensitive to several anxiolytics, i.e., both benzodiazepine and non-benzodiazepine anxiolytics. In the present study, non-benzodiazepine anxiolytic flesinoxan, a 5-HT_{1A} receptor agonist, produced the decrease in locomotor activity, as well as rearing and head-dipping behaviors. Similar results were obtained in the previous study that used open-field test (Ahlenius et al., 1991), indicating that flesinoxan has sedative properties. Moreover, we found that not only diazepam but also flesinoxan suppress the acute restraint stress-induced decrease in head-dipping behavior at a dose that alone did not produce a significant behavioral effect. Recently, it has been reported that the anxiogenic activity of 1-(3-chlorophenyl) piperazine, a 5-HT_{2c} receptor agonist, modifies head-dipping behavior (Shepherd et al., 1994). Therefore, it is possible that changes in this behavior reflect changes in the emotional state, which involves not only benzodiazepine, but also non-benzodiazepine mechanisms.

5. Conclusion

The present study demonstrated that the changes in several exploratory behaviors that may be produced via various emotional states could be objectively measured using our automatic hole-board apparatus. Therefore, this system maybe a useful tool for evaluating various emotional states. We also found that treatment of mice with anxiolytics and anxiogenics affected head-dipping behavior, and that the effects of these compounds were opposite each other. Moreover, exposure of mice to acute restraint stress produced effects similar to those of anxiogenics, and these effects were reversed by treatment with anxiolytics. These results suggest that changes in head-dipping behavior may reflect the anxiogenic and/or anxiolytic state of animals, and that our automatic hole-board apparatus is a novel, sensitive and reliable model of anxiety that detects both anxiolytic and anxiogenic activity.

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