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Effects of Bifemelane on Discrimination Learning of Serotonergic-Dysfunction Rats

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NOMURA, M. *Effects of bifemelane on discrimination learning of serotonergic-dysfunction rats.* PHARMACOL BIOCHEM BEHAV 42(4) 721-731, 1992.—To investigate the effect of bifemelane hydrochloride on learning achievements of serotonin-deficient rats, animals were fed with tryptophan-deficient diets and operant type discrimination learning tests were performed. In general, serotonin-deficient rats show hyperactivity. In this study, total number of responses in reverse learning experiments was lower in rats that received 50 mg/kg bifemelane compared to the other serotonin-deficient groups. The ratio of correct responses to the total number of responses revealed low learning achievements in the control and low-dose groups, whereas the ratio in the high-dose group was nearly the same as in normal rats in the final few sessions of both the primary and reverse learning experiments. Throughout this study, the high-dose group showed a better improvement in learning achievement than the low-dose group. Therefore, bifemelane has certain effects on learning achievement from a) the functional activation of the serotonergic nervous system and b) changes in neurotransmitter levels in the brain (e.g., acetylcholine, noradrenaline) and overall energy metabolism.

Tryptophan-deficient rats Bifemelane hydrochloride Brightness discrimination learning test
Multiple-variable interval 15-s extinction schedule

CEREBRAL metabolic dysfunction has various causes. One mechanism causing this dysfunction is due to changes in the neurotransmitter levels in the brain. Tryptophan, an essential amino acid, is necessary for the growth and psychological development of animals. It is metabolized into serotonin, which is known to be involved in a variety of physiological functions, for example, sleep (18), eating behaviors (4,9,23), physical behaviors (8,26), the vascular system (17), and regulation of hormonal secretion (7). In addition, tryptophan derivatives are directly involved in the functional regulation of neurotransmitter activity in the serotonergic system. The relationship between serotonin and neuropsychiatric disorders, particularly depression, has also been discussed (7,24, 36).

The cerebral serotonergic system changes when animals are fed a tryptophan-deficient diet. These animals are quite useful in investigations on biological functions of serotonin (11, 12,31). It is also reported that rats fed with tryptophan-deficient diets showed marked impairment in their operant learning behavior (31,33).

Bifemelane hydrochloride (bifemelane) is reported to have an effect on the serotonergic system, as well as on the activation of cerebral acetylcholine and noradrenaline metabolisms (10,34). In this study, to investigate the effect of bifemelane on serotonin-deficient rats animals were given tryptophan-deficient diets and the effects of bifemelane on animals' behavior were examined by monitoring the operant learning achievement as an index.

METHOD

Animals

Forty male Wister rats (body weight 30–35 g, Charles River Japan, Inc., Atsugi, Japan) were divided into four groups (n = 10), that is, the normal diet group (normal group); the control group, which received the tryptophan-deficient diet (control group); an experimental group that received 10 mg/kg bifemelane in addition to the tryptophan-deficient diet (10-mg group); and another experimental group that received 50 mg/kg bifemelane in addition to the tryptophan-deficient diet (50-mg group). The tryptophan-deficient diet, which consisted mainly of maize, was given to rats from the weaning period (3 weeks after birth) to the completion of this study to create a serotonin-deficient condition in the brain (12). Bifemelane hydrochloride (Celeport®) was provided by Eisai Co., Ltd., Tokyo, Japan

At the age of 11 weeks, rats were transferred to individual cages and the quantity of food was reduced for 1 week to decrease the body weight to 85%. This weight was maintained throughout the learning experiments (30,43). Rats were allowed free access to water except during the learning experiments, which were conducted every morning for the same time

period in a Skinner box. The rearing temperature was maintained at 22 ± 2°C. Lighting was provided from 0700–1900 h (12 L : 12 D light/dark cycle).

Apparatus

The Skinner box (Ralph Gerbrands, Arlington, MA) dimensions were 30 × 28 × 26 cm. The front wall was made of an aluminum plate, while the walls of the other sides were acryl resin plates. The floor was a grid of bars (3 mm diameter) with 1-cm intervals. There was a round window (4 cm in diameter) 10 cm from the floor on the front wall. A light source was set outside this window to provide bright illumination (5×10^4 ft-L) and dark illumination (1/1,000 of the bright illumination) in the box during experiments. Brightness was changed by inserting a Kodak filter (ND 4.0) between the light source and the window. A food box was located outside the box and immediately below the window. The lever (3 × 2 cm) was located below the window in the box, 2 cm from the floor level on the right side of the front wall (2 cm from the right corner). When the rat pressed this lever, a feed pellet (45 mg) was supplied from the food box to the floor inside the Skinner box through a dispenser. The number of supplied feed pellet within certain time periods was controlled by this dispenser.

Controlling of the application of photo stimuli and supply of feed pellets and recording of response numbers were handled by a microcomputer (30). The operant Skinner box was located in a dark room and the activity of rats was observed on a TV monitor set in an adjoining control room via a dark-field video camera set on the ceiling of the box. White noise (60 dB) was used to mask extraneous noises in the laboratory.

Procedure

Preparation. Before starting the discrimination learning experiments, the rat was placed in a Skinner box and given five feed pellets when it came close to the lever. The rat at first recognized the location of lever. Then, the investigator gave feed pellets when the rat pressed the lever. In these ways, the rat learned how to get the pellets. This practice was continued for a maximum of 7 days or until the rat was able to get 40 pellets within 4 min by pressing the lever. After that, continuous reinforcement (CRF) of learning practices to get 40 pellets was conducted by changing the rate of pellet supply from one pellet in 5-s variable intervals (VI 5 s) on the first day to one pellet in VI 10 s on the next day. This experiment applied VI 15 s.

Primary learning. Brighter illumination (S+) and darker illumination (S−), both for 20 s, were each provided 20 times in accordance with the Gellermann series (5,15) with a 5-s interval without lighting. A pellet was supplied at the rate of VI 15 s when the rat pressed the lever during the S+ period, and no pellet was supplied when the rat pressed the lever during the S− period. This experiment was conducted once daily in the morning for 30 consecutive days. The number of lever-pressing responses during the S+ period was counted as the number of positive response (R+), and that during the S− period was counted as the negative response (R−) (43). Discrimination learning performance was evaluated in terms of the ratio of R+ to the total number of responses (R+ plus R−), that is,

$$\frac{R+}{(R+) + (R-)} \times 100.$$

Reversal learning. Following the 30-day primary learning period, the learning task was reversed: No pellet was supplied in the S+ period (R−), whereas pellets were supplied in the S− period (R+). This learning process was also continued for 30 consecutive days.

ANALYSES

Learning achievements were expressed by the mean ± SE for each group. Statistical analyses of data using analysis of variance (ANOVA) were first conducted to determine whether or not there were significant differences in data obtained for all four groups and for the performance of rats during the 30-day experiment period. Details of this method are not explained here but, briefly, it is a function of the N-1 group and the total number of rats minus number of groups. ANOVA was used to observe how widely the data varied. For example, we set up four groups for this study with each group consisting of 10 rats. Therefore, the function can be expressed as $F(3, 36)$ for analysis of group differences, $F(29, 1044)$ for analysis of session effect, and $F(87, 1044)$ to monitor interaction between group and day factors. After performing ANOVA, the obtained figure was compared to the analytical table to determine its confidence interval, p value. When the value was $p < 0.05$ or resulted in a significant difference, we determined whether or not there were any statistical differences between any two groups using the Games-Howell posthoc test (G-H tests) (14). Significant differences in specific session dates between any two groups were also analyzed using Student's t-test.

RESULTS

Primary Learning Experiments

Normal group. Results are summarized in Fig. 1A. Increasing R+ and decreasing R− were already obvious by day 6. The average number of R+ was 81 on day 1, which increased to 262 on the final day of the primary learning experiment (day 30). R− on day 1 was 74, almost the same number as R+, and it decreased progressively to 48 on day 30. As a result, the total number of response (R+ plus R−) per session did not change much after day 5, that is, it was between 280–310. The ratio of R+ to the total number of responses reached 80% on day 16 (Fig. 1A, lower).

Control group. The average number of R+ increased significantly from 100 on day 1 to 345 on day 30. On the other hand, the average number of R− increased until day 10, returned to the original level on day 20, and then slightly decreased. Even though the total response number in a session increased from 200 to about 450 (Fig. 1B), the ratio of R+ to the total number never reached 80% during the study period.

10-mg group. Results are shown in Fig. 2A. The R+ increased as experiments were repeated, that is, from 95 on day 1 to 355 on day 30. These numbers were nearly the same as those in the control group. On the other hand, the R− (109 on day 1) increased up to day 11, returned to the initial level on day 22, and remained around this level up to day 30. The ratios of the R+ to the total number of responses are shown in the lower part of Fig. 2A: The slope decreased after day 23, and the ratio reached 80% on day 28.

50-mg group. Results are shown in Fig. 2B. The R+ increased from 98 on day 1 to 390 on day 30. The R− was 110 on day 1 and increased up to day 10. It remained higher than the initial level up to day 20. The R− was lower than the initial level on day 24 and then decreased to 70 on day 30.

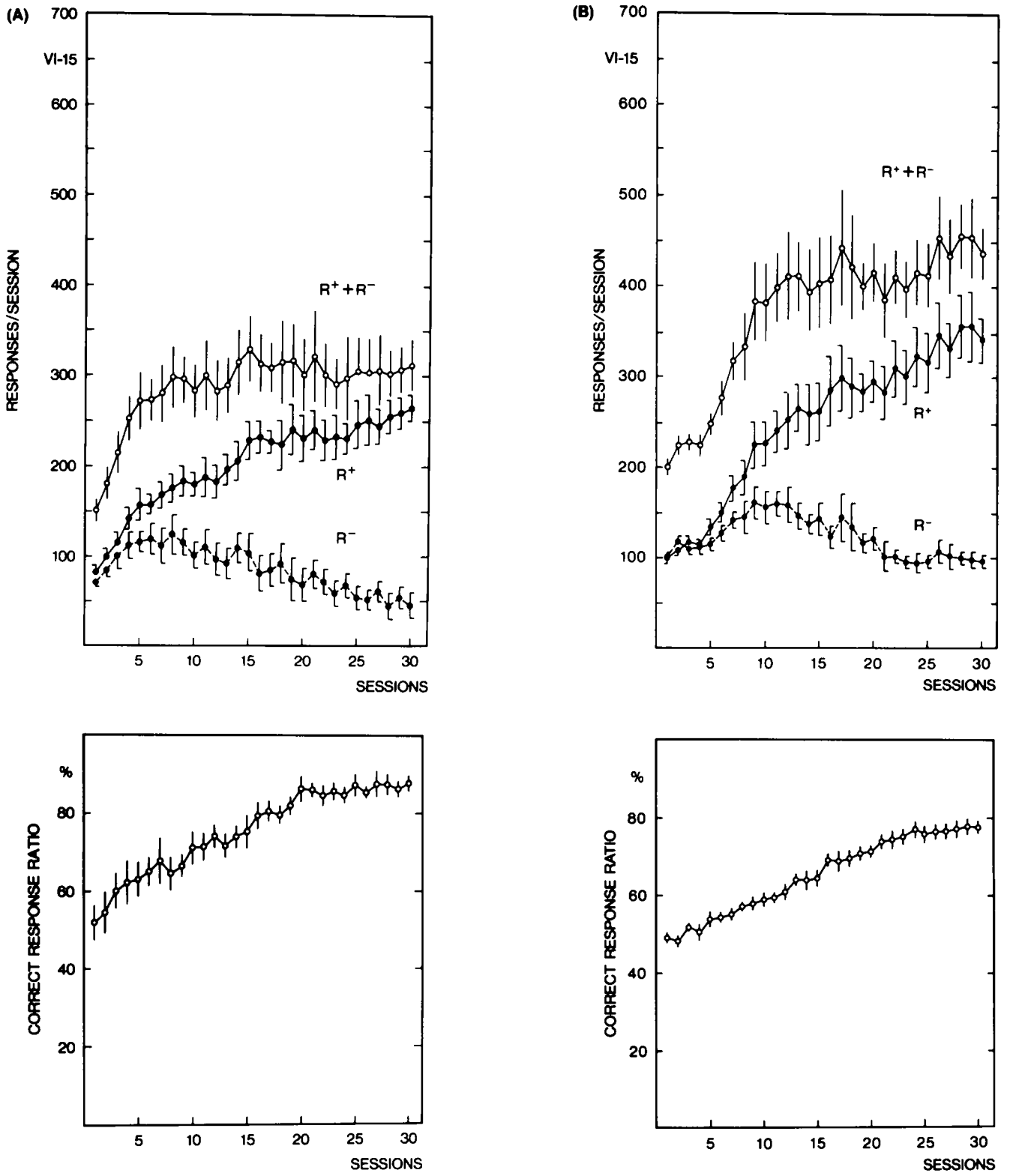

FIG. 1. Results of the discrimination learning tests for rats pressing a level to obtain food delivered in multiple-variable intervals of 15 s (VI 15). (A) Data for 10 rats fed with a normal diet; (B) data for another group of 10 rats fed a tryptophan-deficient diet. All rats were trained to distinguish light periods (S+, 20 s) from dark periods (S−, 20 s). The responses for pressing the level in the S+ periods were reinforced in the preparation courses. R+ represents the number of responses during the S+ periods (mean ± SE) and R− represents the number of responses during the S− periods (mean ± SE). In the preparatory learning courses, R+ was reinforced using the VI 15 schedule but R− was not reinforced (extinction schedule). Lighting during the S+ period was 1,000 times brighter than in the S− period. Each S+ or S− period was repeated following the Gellermann sequence 20 times in a session. The percentages of correct responses (R+) to total number of responses (R+ plus R−) are shown on the lower part of each figure.

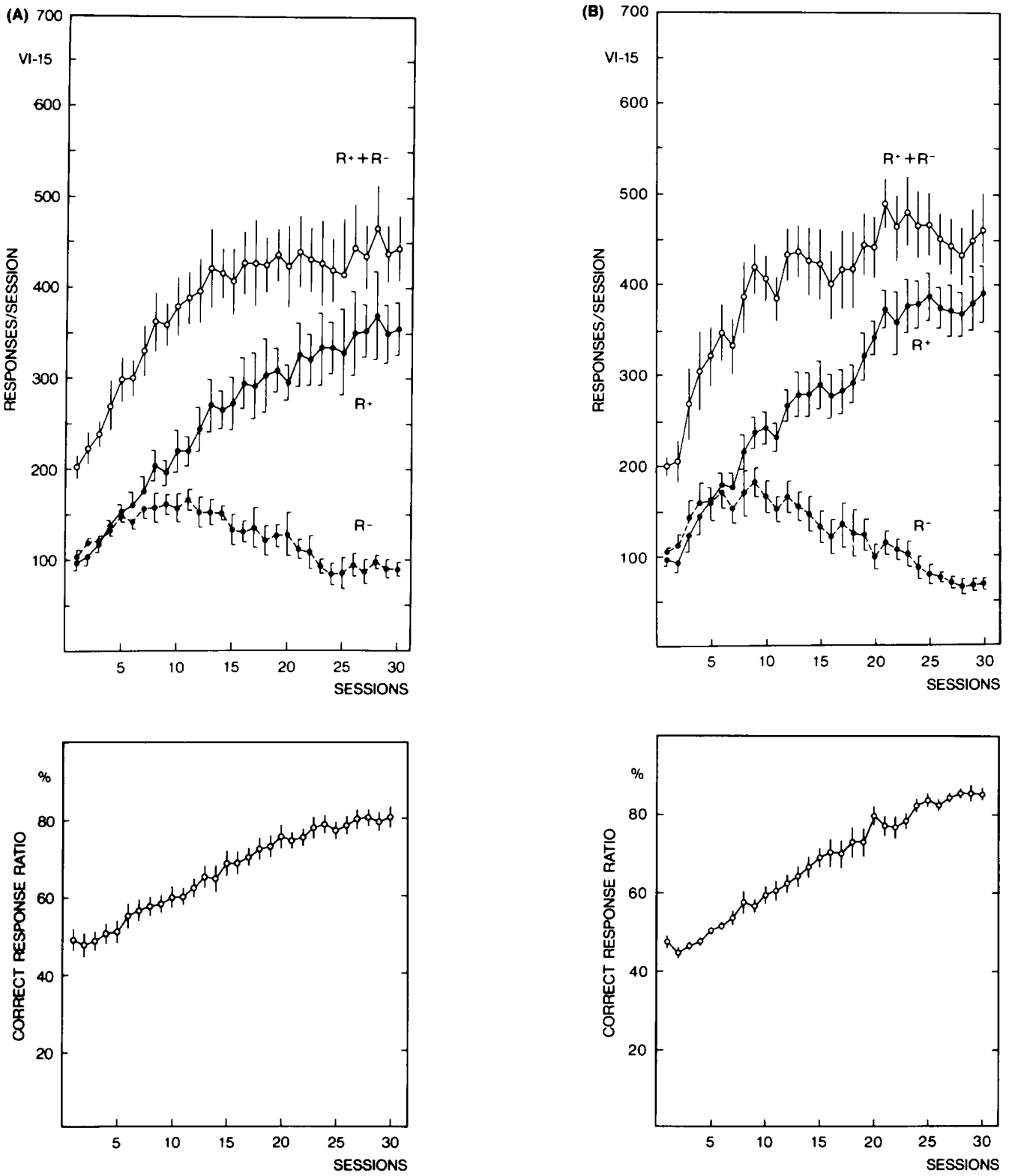

FIG. 2. Results of the discrimination learning tests for rats pressing a lever for food in the VI 15 schedule. Rats were fed a tryptophan-deficient diet. (A) Data on rats that received befemelane hydrochloride 10 mg/kg; (B) data on other rats that received befemelane hydrochloride 50 mg/kg. All other experimental conditions were the same as described in Fig. 1.

The total number of responses increased steadily up to day 19 (80%) and was higher than 80% after day 24.

Reversal Learning Experiments

Normal group. Figure 3A summarizes the results. The R+ was low immediately after the completion of the primary learning experiments. It increased from 110 on day 1 to 194 on day 10 and 266 on day 30. On the other hand, the R− decreased from 273 on day 1 to 58 on day 30. The numbers of the R+ and R− reversed on day 7. The total number of responses reflected the decreased number of the R−, and decreased from 383 on day 1 to 320 on day 30. It could be said that rats clearly distinguished the different learning practices from around day 13. The ratio of the R+ to the total number of responses sharply increased from 28% on day 1 and exceeded 80% on day 26.

Control group. The results are summarized in Fig. 3B. The R+ increased abruptly during the first 10 days, and then remained between 300–350. On the other hand, the R− showed continuous decreases, from 400 on day 1 to 125 on day 26. The ratio of the R+ to the total number of responses increased sharply up to day 10 but thereafter increased by a slower rate. As a result, it reached only 73% on day 30.

10-mg group. Figure 4A shows the results. The R+ increased in the same way as the control group, and the levels remained at around 300–350 after day 10. On the other hand, the R− decreased constantly from 380 on day 1 to 120 on day 30. The ratio of R+ to the total number of responses never reached 80% during this study period.

50-mg group. Figure 4B shows the results. The increases of the R+ were similar to the control and 10-mg groups. However, the R− decreased more sharply than the control and 10-mg groups for the first 4 days. The R− changed from 396 on day 1 to 86 on day 30. The ratio of R+ to the total number of responses also showed steeper increases for the first 4 days, and reached 80% on day 28.

Analyses of the Curves

Changes in R+ among groups.

Primary learning experiments. In the normal group, there were four stages in the learning experiments: The first 6 days showed steep increases in the slope. After a period when it became stable, it started to increase sharply, but then later only increased slowly. On the other hand, the control and 10-mg groups had two stages of learning: a steeper slope for the first 10–13 days and slower increases for the rest of the period. It is interesting to see that the 50-mg and normal groups showed four stages. The higher activity in the 50-mg group as compared to the normal group reflected the tryptophan-deficient conditions in the 50-mg group.

ANOVA showed that there were significant differences among the groups, $F(3, 36) = 3.030$, $p < 0.05$. When ANOVA was performed to determine differences in the R+ responses during a 30-day period, it was $F(29, 1044) = 92.556$, $p < 0.001$, showing that rats' performances were significantly different as the number of sessions increased. When ANOVA was performed to analyze interactions of these two factors (group and day), the results were $F(87, 1044) = 2.133$, $p < 0.001$. In short, we can conclude that there were significant differences among the groups and as the number of sessions increased.

G-H tests among the groups revealed that rats of the normal group made more R+ responses than all other groups ($p < 0.05$).

Reverse learning experiments. The R+ increased sharply for the first 6–8 days and then slowly increased for the rest of the period in all groups. There were no extreme differences in the shapes of the slopes among the groups.

ANOVA for the R+ data in the reverse learning experiments showed no significant overall group difference, $F(3, 36) = 2.138$, $p > 0.05$. However, results obtained on the training day revealed that there was a significant difference in rats' performance as the number of sessions increased, $F(29, 1044) = 43.526$, $p < 0.01$. When interactions of these two factors (group and day) were analyzed, ANOVA showed no significant overall differences, $F(87, 1044) = 0.914$, $p > 0.05$. Since there were no significant differences in overall data, G-H tests for any two groups were not performed.

Changes in R- among groups.

Primary learning experiments. The pattern of curves in each group was almost the same: R− increased for the first few days, became stable, and then decreased. The first increasing period was longer in the control and 10-mg groups (7–10 days), whereas it was shorter in the normal and 50-mg groups (about 5 days).

ANOVA on R− data showed there was a significant overall group difference, $F(3, 36) = 4.266$, $p < 0.05$, as well as a significant difference in performance as the session number increased, $F(29, 1044) = 25.505$, $p < 0.001$. However, when these two factors (group and day) were considered no significant interaction was observed, $F(87, 1044) = 1.250$, $p > 0.05$.

G-H tests revealed that the number of R− in the normal group was lower than the other groups ($p < 0.05$).

Reverse learning experiment. The R− decreased sharply for the first 5–8 days and then slowed in all groups during the rest of the study period. There were no extreme differences in the shapes of the slopes among the groups except for the normal group, in which the curve started at 300. In the other groups, it started at around 400. On day 30, the R− number was higher than 100 in the control and 10-mg groups, while it was lower than 100 in the 50-mg group.

ANOVA on the R− data obtained from the reverse learning experiment showed that there was no significant overall group difference, $F(3, 36) = 2.758$, $p > 0.05$, even though there was a significant difference as the number of sessions increased, $F(29, 1044) = 78.819$, $p < 0.001$. There was no significant interaction between the two factors (group and day), $F(87, 1044) = 0.615$, $p > 0.05$. Therefore, the G-H tests were not conducted.

Changes of the R+ ratios in the total number of responses among groups

Primary learning experiments. In the primary learning period, the R+ ratios were always higher in the normal group than in the others.

Among the Control, 10-mg, and 50-mg groups, the control and 10-mg groups had almost the same curve slopes, whereas that of the 50-mg group had a steeper slope and exceeded the 80% level after day 23. In the normal group, the ratio exceeded 80% after day 17.

ANOVA showed a significant overall group difference for the R+ ratio, $F(3, 36) = 7.879$, $p < 0.001$, as well as for the training day, $F(29, 1044) = 180$, $p < 0.001$. No significant interaction between the two factors (group and day) was observed, $F(87, 1044) = 1.263$, $p > 0.05$. The G-H tests

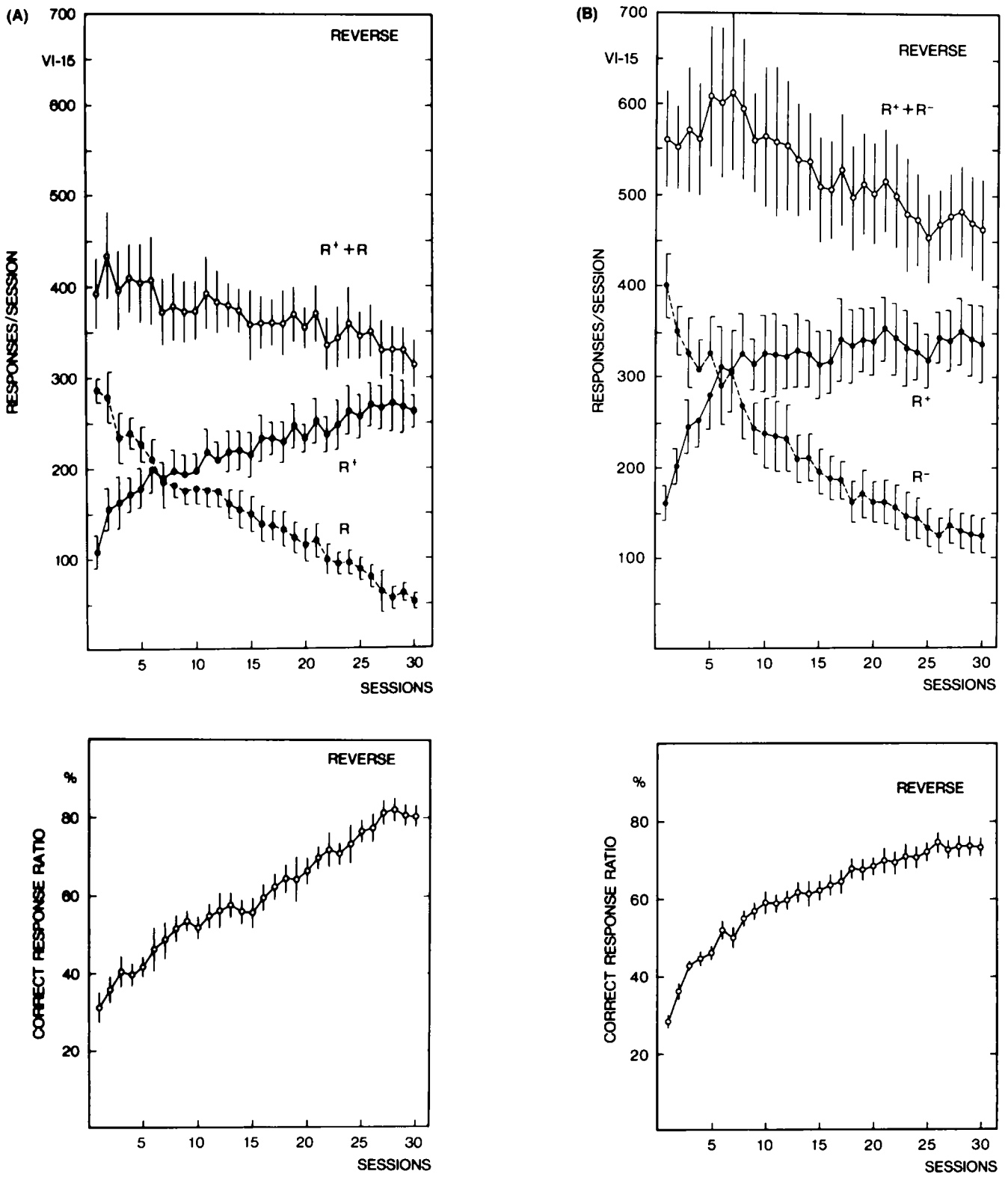

FIG. 3. Results of reverse learning tests for rats pressing a lever for food in the VI 15 schedule. (A) Results for rats fed a normal diet; (B) results for rats fed a tryptophane-deficient diet. In this reverse learning test, rats were able to get food pellets when pressing the lever in the S− period. All other conditions were the same as described in Fig. 1.

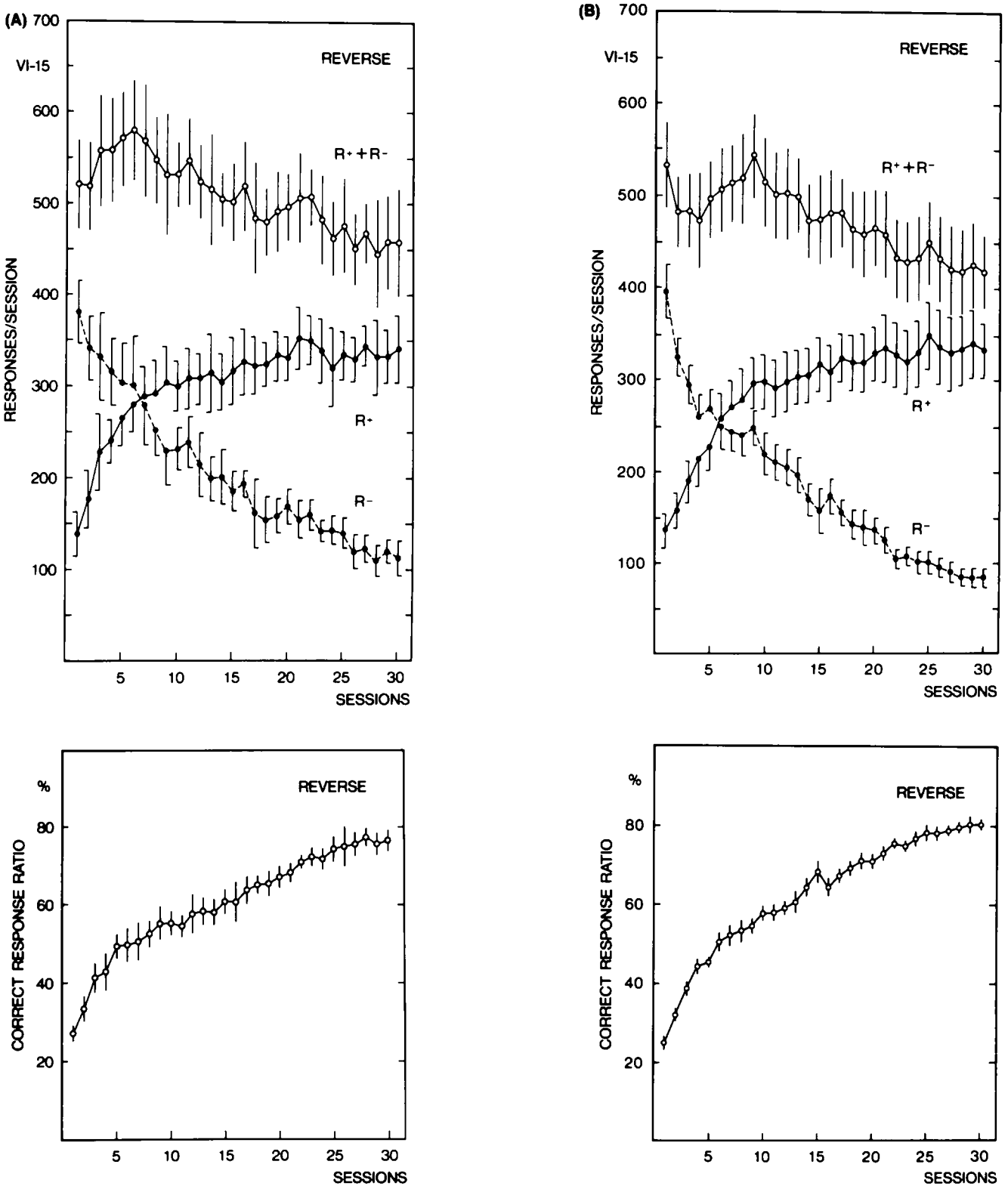

FIG. 4. Results of the reverse discrimination learning tests for rats receiving bifemelane hydrochloride 10 mg/kg (A) or 50 mg/kg (B). Rats were fed tryptophan-deficient diets. The other conditions of the tests were the same as described in Fig. 1.

showed that the R+ ratio was significantly higher in the normal group than in the others ($p < 0.05$), and the ratio of the 50-mg group was also significantly higher than the control group ($p < 0.05$). We also calculated the difference in the R+ ratio for each session in the primary learning tests for the 50-mg and control groups (Fig. 5). As a result, significant differences of $p < 0.05$ were constantly obtained for the experiments after day 25.

Reverse learning experiments. In the period of reverse learning experiments, the R+ ratio was always higher in the normal group compared to the others (Fig. 6). Among the control, 10-mg, and 50-mg groups, the control and 10-mg groups had almost the same curve slopes, whereas the slope of the 50-mg group was steeper and reached the level of 80% after day 28.

ANOVA on the R+ ratio data from the reverse learning experiments showed no significant overall group difference, $F(3, 36) = 0.353, p > 0.05$. However, there was a significant difference as the number of sessions increased, $F(29, 1044) = 197.29, p < 0.001$, and also for the interaction between the two factors (group and day), $F(87, 1044) = 1.504, p < 0.01$.

Changes of total number of responses.

Primary learning experiments. There was a significant difference in the total number of responses among the groups. The normal group had the lowest numbers of the four groups.

ANOVA on the total number of responses showed a significant overall group difference, $F(3, 36) = 3.676, p < 0.05$, as well as a significant difference as the number of sessions increased, $F(29, 1044) = 34.030, p < 0.001$. ANOVA also showed a significant interaction between the two factors (group and day), $F(87, 1044) = 1.518, p < 0.05$. The G-H tests showed that the number of total responses was significantly lower than the other groups ($p < 0.05$), and the number of the control group was significantly higher than the 50-mg group.

Reversal learning experiments. There was a significant difference in the total number of responses among the groups, of which the normal group had the lowest number.

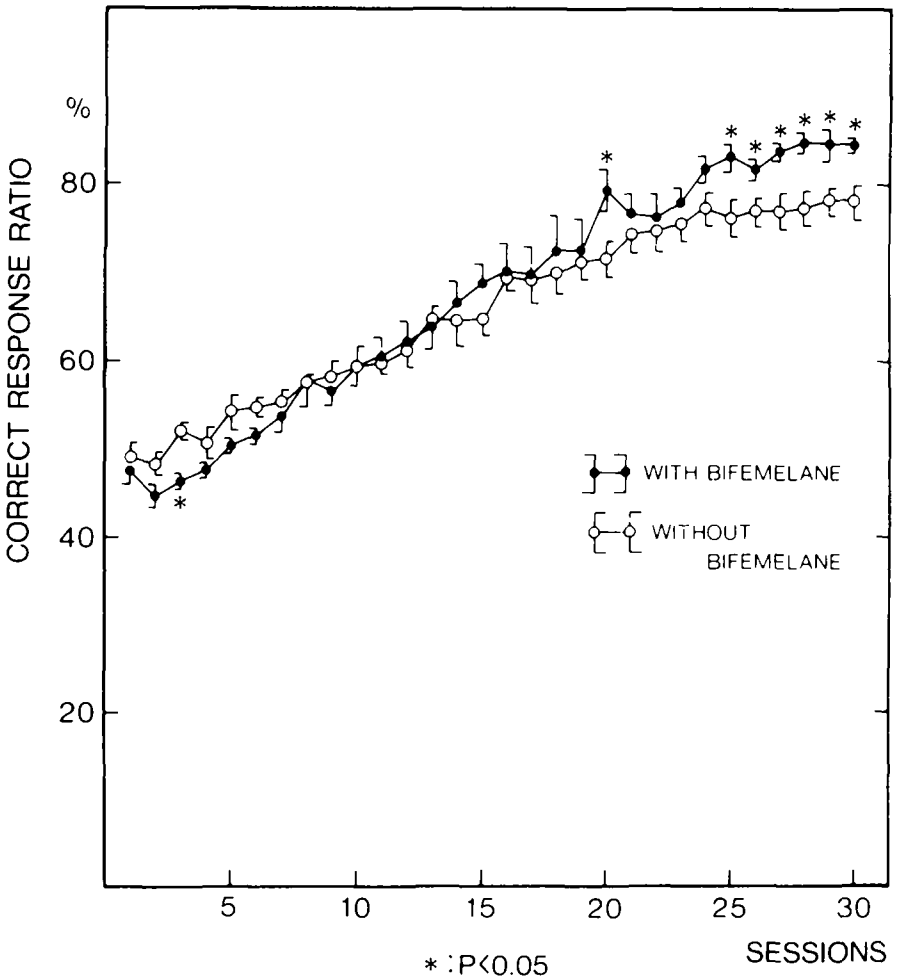

FIG. 5. Ratio of correct responses in the primary learning tests using the VI 15 schedule on the control group (tryptophan-deficient diet only) and 50-mg group (tryptophan-deficient diet + bifemelane hydrochloride 50 mg/kg).

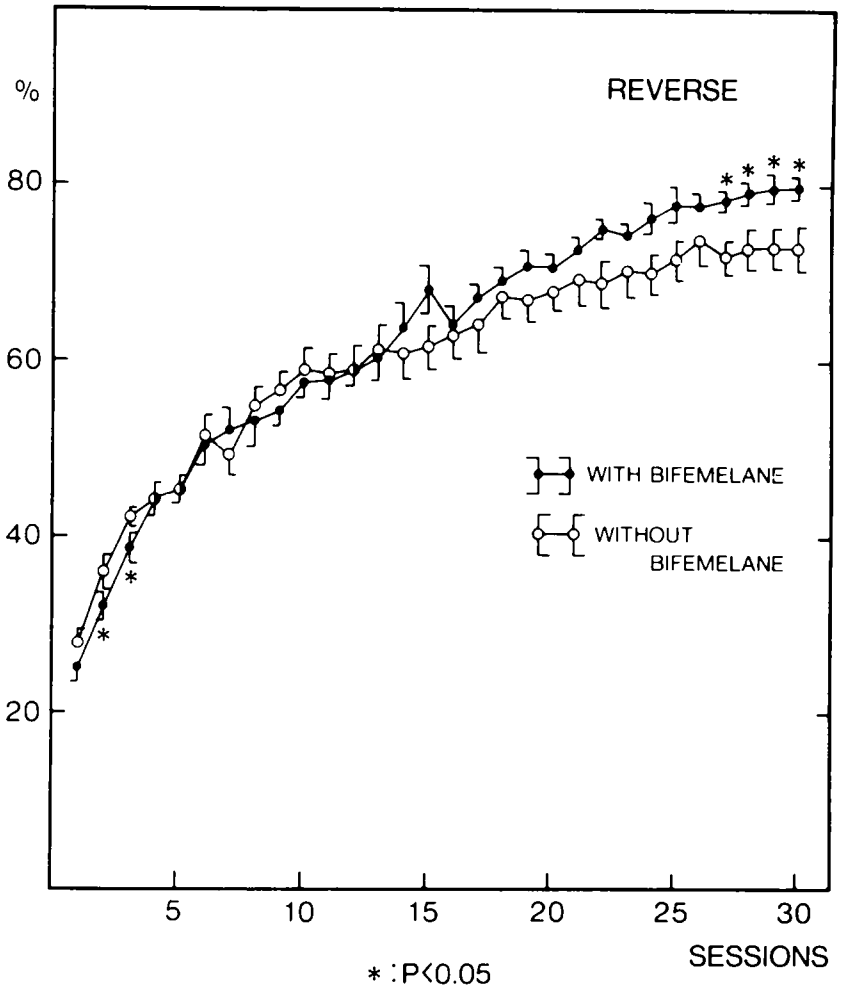

FIG. 6. Ratio of correct responses in the reverse learning tests using the VI 15 schedule on the control group (tryptophan-deficient diet only) and 50-mg group (tryptophan-deficient diet + bifemelane hydrochloride 50 mg/kg).

ANOVA on the total response numbers obtained from reverse learning experiments showed no significant group differences, $F(3, 36) = 2.711$, $p > 0.05$. However, there was a significant difference as the number of sessions increased, $F(29, 1044) = 7.803$, $p < 0.01$. There was no significant interaction between group and day, $F(87, 1044) = 0.516$, $p > 0.05$.

DISCUSSION

The nature of serotonin involvement in neurofunction can be investigated by preparing animals that have different intracerebral serotonin content and observing the corresponding changes in their behavior. However, if the raphe nuclei, which are regarded as the central nuclear sites of the serotonergic system, are destroyed animals show different behaviors when they receive administration of such substances as *p*-chlorophenylalamine (PCPA), 5,6-dihydroxytryptamine, or 5,7-dihydroxytryptamine. For example, in one-way avoidance behavior tests responses such as rod-climbing and jumping onto a platform were delayed in animals with destroyed raphe nuclei (6), although these responses tended to be enhanced by the administration of PCPA (29,42). However, animals with destroyed raphe nuclei showed higher levels of activity when they were placed in a new environment (40,41), and they started to move actively with a delay (50 min after transfer to the new environment) when PCPA was administered. In this way, when raphe nuclei were destroyed no common effects of such substances as PCPA on behavior have been determined (13), and it is difficult to evaluate the results from experiments using such rats.

To date, no investigators have studied the relationships between serotonergic neurons and memory or learning behavior. In this study, experiments were designed to study the relationships between serotonin and learning behavior and the effect of bifemelane on learning achievements in serotonin-deficient rats (which were reared on a tryptophan-deficient diet). It is known that cerebral serotonin and 5-hydroxyindole acetic acid (5-HIAA) levels in these animals are less than 70% of the levels in normal rats (1,31,33). The raphe nuclei are not damaged by a tryptophan-deficient diet.

Changes in animal behavior are commonly investigated either by operant type positive reinforcement learning tasks (32,35) or negative reinforcement learning tasks (2,38,44). In

the positive learning tasks, the responses of animals are studied under natural conditions, and these responses can be regarded as showing the pure responses of animals (25,30,46). These are useful in studying inherent behavioral patterns. On the other hand, in the negative tasks the behavior of animals is observed by applying such negative tasks as electrical shocks. Therefore, observation of inherent behavioral patterns is rather difficult even though serotonin is believed to be intimately related to algesthesia (27,45). Escape or avoidance behavior experiments are relatively easy to conduct, require no training of animals (2,22), for example, lever pressing, and as a result are widely used in investigations (16,20,26). However, the interpretation of those results is also very difficult. We must note that these experiments need to be conducted and analyzed with extreme caution because the results differ greatly according to the types of learning tasks (25,37). In this study, positive learning tasks were given to serotonin-deficient rats to investigate the effect of bifemelane hydrochloride on learning behavior and also to consider the effect of bifemelane on the serotonin system in the brain. In positive learning experiments, differences in the results can be regarded as showing true differences among groups (22,25).

The overall comparison with the normal group (animals that received normal diets) and other animals that received tryptophan-deficient diets showed higher levels of activities in the latter group. It is quite natural that decreased serotonin levels lead to suppression of serotonergic neurons, and as a result test animals showed higher activity levels. If bifemelane acts on the serotonin system, the activity of serotonin-deficient rats would be lower. In the reverse learning experiments, the total number of responses in the 50-mg group was lower than that of the control group, while the primary learning experiment did not show any differences (3). Therefore, it could be said that bifemelane has some effect on the serotonin system of serotonin-deficient rats even though the impact would be limited.

In terms of the ratio of correct responses (R+) to the total number of responses (R+ plus R−), the control and 10-mg groups clearly showed lower learning achievements as compared to the normal group, whereas the ratios in the 50-mg group were almost the same as in the normal group in the final few sessions. Significant differences were obtained for individual sessions between the control and 50-mg groups (Fig. 5). Therefore, a high dose of bifemelane was evaluated to be effective in improving learning achievements of serotonin-deficient rats.

Recent studies using rats with cerebral ischemia (28,39,47) and cerebral infarction (21), as well as quantitative biochemical assays of active substances in the brain (19,34), indicated the importance of serotonin in cerebral functions. In this study, the control, 10-mg, and 50-mg groups could be regarded as having the same physical conditions, including the cerebral serotonin levels, because rats were reared and fed under identical conditions. In other words, the cerebral serotonin levels, acetylcholine metabolisms, monoaminergic systems, and other neurotransmitters of these three groups are considered to be the same (1,13).

What is the mechanism of bifemelane in improving learning achievements in these rats? Bifemelane is believed to improve cerebral noradrenaline metabolic turnover (10), promote recovery in gerbils from cerebral serotonin metabolic impairment caused by ischemia associated with cerebral infarction (10,34,39), and prevent as well as aid in recovery from acetylcholine depletion (10,34,39). It is also known that bifemelane improves the cerebral energy metabolism and enhances cerebral serotonin metabolism (34). Therefore, in considering the results of this study the effect of bifemelane on learning achievement could have resulted from a) the functional activation of the serotonergic nervous system and b) by changes in acetylcholine levels, noradrenaline turnover, and/or overall energy metabolism in the brain. In this study, definite conclusions cannot be stated as the levels of neurotransmitters were not measured. However, it is interesting to note that the 50-mg group showed a better improvement in learning achievement than the 10-mg group, and the level of activity was lower in the 50-mg group than in the other serotonin-deficient groups.

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