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Effects of 5-Hydroxytryptamine on Defecation in Open-Field Behavior in Rats

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KAMEYAMA, T., M. SUZUKI AND T. NABESHIMA. *Effects of 5-hydroxytryptamine on defecation in open-field behavior in rats.* PHARMAC. BIOCHEM. BEHAV. 12(6) 875–882, 1980.—An attempt was made to elucidate the role of the serotonergic nervous system in defecation resulting from environmental stimulation in rats. The open-field (OF) test and shuttle box method were used to study the defecation. 5-Hydroxytryptophan (5-HTP) significantly decreased the number of fecal boluses excreted in both emotional situations, namely, in both OF and shuttle box. The fecal excretion was significantly reduced compared with the controls after intraventricular injection of 5-hydroxytryptamine (5-HT). Animals pretreated with p-chlorophenylalanine (pCPA) and 5,6-dihydroxytryptamine (5,6-DHT) tended to show a slight increase in the OF defecation. 5-HTP was equally effective in diminishing the OF performance of pCPA-treated rats. The inhibitory effects of 5-HTP on the defecation were also observed after depletion of biogenic amines by reserpine treatment. Home cage defecation was increased after 5-HTP administration, decreased under pretreatment with pCPA and not influenced by intraventricular injection of 5-HT. These results suggested that the defecation after environmental stimuli was due to a change in 5-HT levels in the brain.

Open-field Shuttle box Defecation Emotionality 5-Hydroxytryptamine 5-Hydroxytryptophan p-Chlorophenylalanine Reserpine 5,6-Dihydroxytryptamine

HALL [17] has first used the term "emotionality" to conceptualize behavioral and peripheral changes presumed to accompany high sympathetic nervous activity, and a number of test situations have been used since then to measure the relative emotionality of animal subjects (generally rats and mice). These tests include several different types of "novel environment" tests, in which the animal is simply placed into an unfamiliar situation, and "timidity tests" in which the latency to emerge or move from a more familiar or sheltered environment is recorded. Measures taken in active or passive avoidance conditioning situations have also been related to the concept of emotionality [6].

Most commonly, the open-field (OF) test has frequently been used for many years to study emotional behavior in experimental animals [1, 6, 17, 42]. The principle of the test in that the novel situation of the OF evokes a pattern of behavior characterized by defecation, urination, ambulation, rearing and grooming in the animals.

Defecation in the unfamiliar environment has been characterized "emotional defecation" as that which occurred at a higher rate than home cage defecation by Hall [17]. Thus, the term "emotional defecation" has been widely used in experimental studies of emotionality [1, 4, 5, 6, 17, 18, 20, 28, 33]. Recently, Royce summarized OF defecation as primarily an index of autonomic balance [42]. Emotional defecation has been utilized in attempts to measure psychotropic activity of various compounds [20,21]. It was reported earlier that a variety of psychotropic drugs decrease OF defecation in rats and mice [4, 5, 8, 20, 21, 28, 32, 33, 34, 43, 48, 49]. For example, antidepressant drugs such as tricyclic compounds [8] and monoamine oxidase inhibitors [4] reduce the number of fecal boluses excreted in the OF test.

In our previous investigations, it was suggested that the greater the content of 5-HT in the telencephalon the less frequent the defecation, and that the greater the 5-HT content in non-telencephalic areas the greater the ambulation and more frequent the defecation occurred in the OF [23]. In the open-field the animals, when treated with p-chorophenylalanine (pCPA), a 5-HT depletor, show an increase in defecation [51]. The higher defecation rate is also observed during confrontation in pCPA treated mice [30]. On the other hand, the animals frequently defecate after injection of 5-hydroxytryptophan (5-HTP), a 5-hydroxytryptamine (5-HT) precursor, because of strong contractions of the intestinal muscles. The diarrhea which follows injection of 5-HTP could be completely prevented by prior treatment of animals with an antimetabolite of 5-HT [53]. In view of the inconsistency, it is suggested that 5-HT regulates the defecation in home cage and OF in different manners.

In the present investigation an attempt was made to elucidate the role of the serotonergic nervous system in OF defecation in relation to home cage defecation and other OF behavior of rats. This was performed (1) by a selective increase of the 5-HT with 5-HTP, (2) by intraventricular injection with 5-HT, and (3) by an inactivation of the serotonergic neuronal system with pCPA [25] and 5,6-dihydroxytryptamine (5,6-DHT) [2].

METHOD

Male albino rats of Wistar strain, having an average body

weight of about 200 g served as subjects. Animals were acclimated to laboratory conditions (room temperature 23 ± 1°C, humidity 55 ± 5%) for one week prior to study and were housed in groups of five. Food and water were permitted ad lib except during OF test.

The OF test was carried out according to the method reported by Kameyama and Shigehisa [22]. The apparatus consisted of a wooden open-field, 180 cm in diameter; surrounded by a metal wall 60 cm high, with inside surface painted mat dark gray. The field was demarcated by 30 cm squares, with 3 mm wide black lines, and was lit evenly by a 100-W lamp from 70 cm above. The apparatus was placed in a ventilated darkened semi-sound-proofed room. Inside the apparatus, the sound pressure level was ca. 48dB (with white noise) at the center of the field and the brightness ca. 55 cd/m^2 (with achromatic light) on the surface of the floor of the field. The animal was placed gently in a square beside the wall, with its nose facing away from the wall by 90°, and it was allowed to walk about and explore freely during a 3-min period in the OF. Defecation (the number of fecal boluses), ambulation (the number of times the animal crossed the demarcation line), rearing (the number of times the animal stood up on hind legs), and grooming (the number of times the animal washed itself) scores were recorded during a 3-min period. Since the behavioral scores (defecation, ambulation and grooming scores) on first exposure to the OF were variable [52], 50–100 rats were first acclimated to the OF apparatus for two periods of 3-min each two days before the trials. Behavioral scores were measured during a 3-min period in the OF, one day prior to testing. These scores were stable for repeated tests during the experimental period (Fig. 6A). In our procedure described above a habituation phenomenon to the OF was considerable. However, rats repeatedly tested in the OF situation, defecated more than did rats in home cages (Fig. 2, 4, 6). Therefore, the defecation which we observed in our experimental procedure mentioned above was used as an emotional index. On the basis of the defecation and ambulation data, 30–40% of the animals which were acclimated to the OF apparatus before trials, were selected and divided into two or three groups of 7–10 each.

The number of fecal boluses produced under shuttle box conditions was counted also by the method described by Kameyama and Nabeshima [21]. A rat shuttle box system (Lehigh Balley Electronics, U.S.A.) which was enclosed in a sound-attenuated chamber was employed. This box (20×45×20 cm) was separated into two compartments by a 4.5 cm high hurdle which was electrified when rats were trained to avoid a shock. Rats were conditioned to avoid a painful electric shock. The animals were first habituated to the two-compartment conditioning apparatus for two consecutive days (15 min/day) prior to the conditioning trial. Daily sessions in the shuttle box consisted of 20 trials, with presentation of a light and sound signal for 5 sec and an intertrial interval of 40 sec. If the animals did not avoid the signals by moving to the opposite chamber of the shuttle box during presentation of the signals, a scrambled shock of 2.0 mA was delivered to the cage floor. Rats usually learned to avoid a shock in about one week. After conditioning was completed, the assay procedure was begun. On the test day, each rat was first given a ten-trial control test in the apparatus. Animals which did not perform at least nine conditioned responses were excluded from further participation in the experiment, as they did not evidence satisfactory avoidance conditioning. The animals which met this criterion were divided randomly into 2 groups, control group and 5-HTP-treated group. The number of avoidance failures and intertrial responses (the crossing of the hurdle during the intertrial intervals) were recorded for all trials throughout the entire experiment. The number of fecal boluses excreted during daily sessions was also counted for each animal. The frequency of urination was recorded also in each animal.

In the home cages, rats were randomly divided into two or three groups of 7–8 each in isolated housing and in groups of 25 in grouped housing. Since the number of fecal boluses eliminated was very few during a 3-min period in home cage, it was recorded every 30 min after drug-treatment. The number of fecal boluses eliminated and the amount of food consumed during a 6-hr period were also measured every 6 hr after depletor-treatment experiments involving circadian rhythms. Drugs were administered into the right lateral ventricle through permanently implanted cannula without anaes-

TABLE 1

EFFECTS OF MONOAMINE-RELATED DRUGS ON THE 5-HYDROXYTRYPTAMINE CONCENTRATION (μg/g WET TISSUE) AT VARIOUS BRAIN SITES IN RATS

Treatment [number of sample]		Brain sites				
		Telencephalon	Remaining brain areas			
		Cortex	Striatum	Hypothalamus	Midbrain	Medulla oblongata
Control	[6]	0.44 ± 0.01	0.73 ± 0.02	1.22 ± 0.08	1.13 ± 0.08	0.92 ± 0.03
5-HTP (30 mg/kg)	[6]	0.76 ± 0.02‡	1.36 ± 0.05‡	1.73 ± 0.10†	1.69 ± 0.16†	1.51 ± 0.09‡
pCPA (3 × 100 mg/kg)	[5]	0.14 ± 0.03‡	0.36 ± 0.03‡	0.11 ± 0.02‡	0.25 ± 0.03‡	0.11 ± 0.01‡
RSP (2 mg/kg) + 0.3% CMC	[5]	0.23 ± 0.02‡	0.37	0.63 ± 0.02‡	0.53 ± 0.04†	0.29 ± 0.02‡
RSP (2 mg/kg) + 5-HTP(60 mg/kg)	[6]	0.64 ± 0.03‡	1.65 ± 0.27†	1.37 ± 0.10	1.30 ± 0.06‡	1.23 ± 0.12‡
pCPA (300 mg/kg) + 0.3% CMC	[5]	0.27 ± 0.04‡	0.28 ± 0.01‡	0.30 ± 0.64‡	0.25 ± 0.03‡	0.17 ± 0.02‡
pCPA (300 mg/kg) + 5-HTP(60 mg/kg)	[4]	0.85 ± 0.05‡	1.43 ± 0.10‡	1.73 ± 0.11‡	1.55 ± 0.13*	1.23 ± 0.12*
Saline containing 0.1% ascorbic acid	[7]	0.64 ± 0.05			0.57 ± 0.04	
5,6-DHT (30 μg/rat)	[7]	0.53 ± 0.03			0.51 ± 0.08	

Each drug was administered as described in Fig. 1–6. After the open-field test, each animal was sacrificed immediately by decapitation. The 5-HT content of brain tissue was determined as described in Materials and Methods.

*$p<0.05$, †$p<0.01$, ‡$p<0.001$ compared with control.

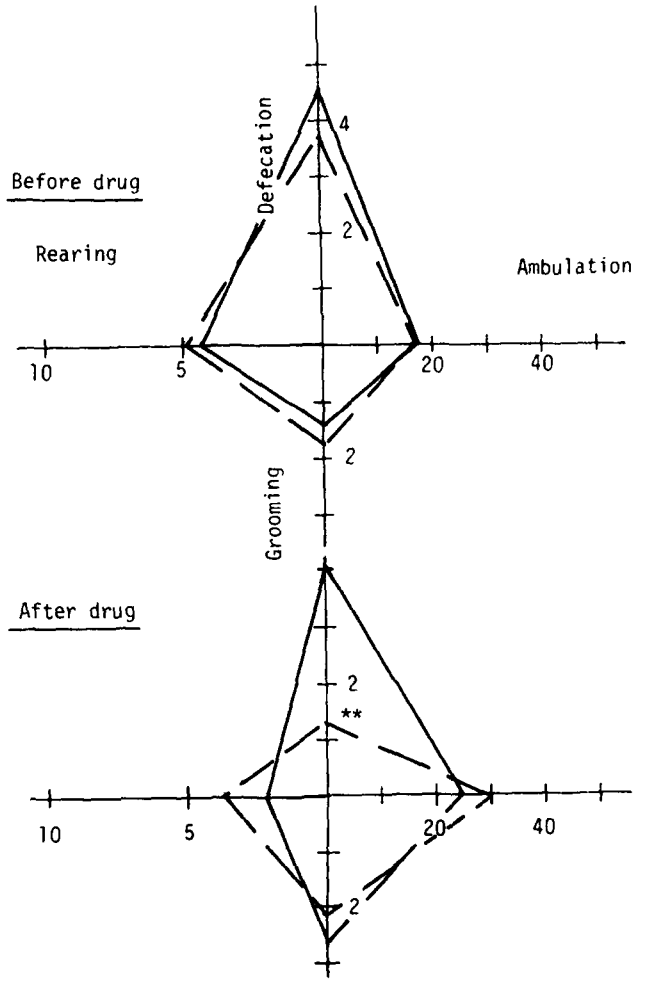

FIG. 1. Effects of 5-hydroxytryptophan (5-HTP) on the open-field behavior in rats. The open-field behavior (defecation, ambulation, grooming and rearing) was observed during a 3-min period, one hr after intraperitoneal injection of 5-HTP (30 mg/kg). Each point represents a mean value of at least seven rats. ———: 0.3% CMC-group, — — —: 5-HTP-group, $^{**}p<0.01$ compared with the control.

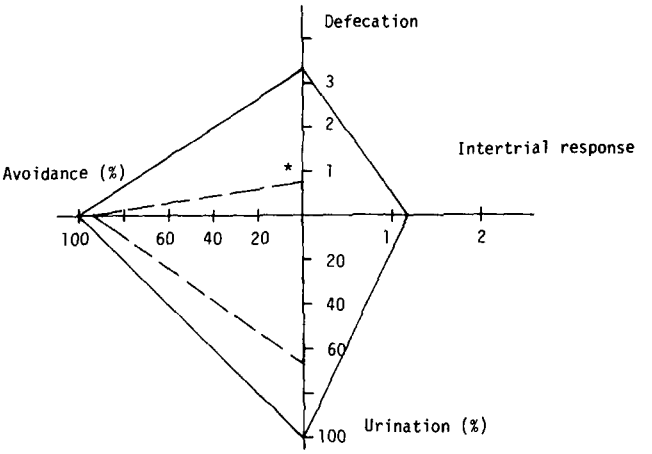

FIG. 2. Effects of 5-hydroxytryptophan (5-HTP) on the shuttle box behavior in rat. The shuttle box behavior (avoidance, intertrial response, defecation and urination) was observed during ten trials, one hr after intraperitoneal injection of 5-HTP (30 mg/kg). Avoidance (%)=[(the number of avoidance)/(number of trials)]×100, Urination (%)=[(number of rats urinating)/(the number of rats in the experimental group)]×100, Intertrial response=unpunished crossing of the hurdle during intertrial intervals. ———: 0.3% CMC-group, — — —: 5-HTP-group, $^{*}p<0.05$ compared with the control. Number of animals=6.

thesia as described in Fujimori and Iwamoto [13]. All experiments were performed one week after the operation to minimize any effects produced by surgery. The injected volumes were 0.01 ml intraventricularly and 2 ml/kg intraperitoneally.

Drug solutions were prepared as follows: 5-HTP (Kyowa Fermentation) and pCPA (Nakarai Chemical Industries) were suspended in 0.3% carboxymethylcellulose (CMC). Reserpine (RSP, Serpasil, Ciba Geigy) and 5-HT creatinine sulfate (Sigma) were dissolved in an isotonic saline solution. 5,6-DHT creatinine sulfate (Sigma) was dissolved in an isotonic saline solution containing 0.1% ascorbic acid. Control rats were given the vehicle only. Doses of the drugs used in most experiments usually had no effects on the OF behavior, except in the case of defecation.

After the behavioral measurements were made, each animal was sacrificed immediately by decapitation, and the brain was carefully removed and quickly frozen in dry ice. The guidelines given by Glowinski and Iversen [16] were followed for dissection of the brain. The 5-HT content of brain tissue was measured fluorometrically according to the method of Snyder *et al.* [47]. Determinations were based on four to six samples per group with seven to ten brains pooled per sample. Statistical evaluation of results was performed by the *t*-test. The drugs-treated groups were compared with the vehicle-treated groups.

RESULTS

Effects of 5-Hydroxytryptophan (5-HTP) on Emotional Defecation and Home Cage Defecation in Rats

Open-Field Test

The effects of higher levels of brain 5-HT on OF defecation were investigated. 5-HTP, a precursor of 5-HT, 30 mg/kg, was injected into rats IP one hr before the OF test. As shown in Table 1, there is a significant increase in the 5-HT content at various sites in brain by this treatment. At this dose, 5-HTP significantly decreased defecation, but other OF behavior was not affected by the drug (Fig. 1).

Shuttle Box Situation

Hunt and Otis [18] suggested that emotional defecation is a conditioned "anxiety" response. Therefore, the possibility was investigated that the reduction in defecation after 5-HTP administration was due to a psychotropic action of the compound. That is, experiments were carried out to investigate effects on defecation in the shuttle box situation.

5-HTP, 30 mg/kg, significantly decreased the number of fecal boluses produced in conditioned rate compared with controls ($p<0.05$). The frequency of urination, which was used as the index of emotionality [17], was decreased also with the drug in this situation (Fig. 2). 5-HTP decreased the intertrial response (number of response=0) which was used as the index of locomotor activity, but did not affect the avoidance response.

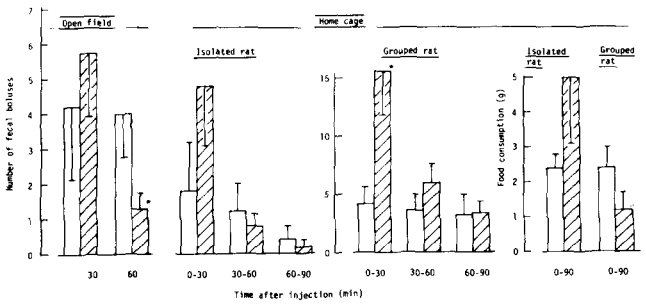

FIG. 3. Effects of 5-hydroxytryptophan (5-HTP) on defecation and food consumption in rats under differing conditions. The number of fecal boluses excreted during a 30-min period in home caged animals and during a 3-min period in an open-field was counted every 30 min after intraperitoneal injection of 5-HTP (30 mg/kg). Food consumption (g) was measured during the experimental period. Rats were housed one (isolation-housed rat) or five (group-housed rat) per cage in home cages. □ : 0.3% CMC-group, ▨ : 5-HTP-group, $*p<0.05$ compared with the vehicle-treated group.

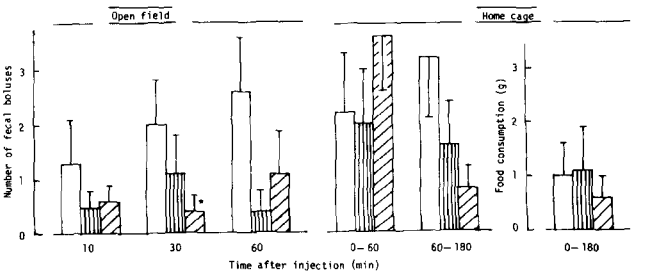

FIG. 4. Effects of intraventricular injection of 5-hydroxytryptamine (5-HT) on defecation and food consumption in rats under differing conditions. 5-HT was administered into the right lateral ventricle, and then the number of fecal boluses excreted and the amount of food consumed were recorded as described in Fig. 1. □ : saline-group, ▥ : 5-HT-group (0.5 μg/rat), ▨ : 5-HT-group (5.0 μg/rat), $*p<0.05$ compared with the vehicle-treated group.

Home Cage Defecation

In order to investigate the possibility that a reduction in defecation after 5-HTP administration was due to the emotional situation, experiments were carried out to investigate the effects of the drug on non-emotional defecation, that is, defecation in rats which had become accustomed to their home cages.

As shown in Fig. 3, 5-HTP increased the number of fecal boluses eliminated, compared with the controls, 0–30 min after injection in the home cage. The increase of fecal boluses deposited in group-housed rats was significant ($p<0.05$). Defecation occurring 30–90 min after injection in home cage was similar to that of the vehicle-treated control group.

In contrast with these results, 5-HTP significantly reduced defecation one hr after injection in OF. There was little possibility that the significant decrease of defecation 60 min after 5-HTP in OF was ascribable to the increase of it 30 min after the drug was administered, because defecation occurring 30–60 min after 5-HTP in home cage was similar to that of the vehicle-treated control group in spite of the significant increase of defecation 0–30 min after the drug.

Effects of Intraventricular Injection with 5-Hydroxytryptamine (5-HT) on Defecation and Food Consumption in Rats under Different Experimental Condition

In order to determine whether reduction in defecation after 5-HTP administration was due to a peripheral action of the compound, experiments were designed to investigate the effects of intraventricular injection of 5-HT on fecal excretion in the OF.

Each rat was submitted to the OF test 10, 30 and 60 min after 5-HT injection (0.5 and 5.0 μg/0.01 ml/rat). Animals treated with 5-HT tended to show a decrease in the number of fecal boluses excreted in OF. At a dose of 5.0 μg, boluse elimination was decreased significantly compared with controls (Fig. 4). On the other hand, 5-HT did not influence home cage defecation and food consumption (Fig. 4).

Effects of p-Chlorophenylalanine (pCPA) on Emotional Defecation in Rats

The effect of lower level of brain 5-HT on defecation in OF were investigated. When pCPA (100 mg/kg each day for three days) was given to rats, there was a significant increase in defecation at 72 hr after the first pCPA administration. This dose of pCPA decreased brain 5-HT to 9–50% of control levels (Table 1 and Fig. 5A).

On the other hand, pCPA (300 mg/kg) tended to produce an increased number of fecal boluses deposited, although there was no significant increase in scores (Fig. 5B). Rats treated with pCPA (100 mg/kg each day for three days) showed a more reduced 5-HT content than rats treated with pCPA (300 mg/kg) (Table 1).

5-HTP, 60 mg/kg, was equally effective in diminishing the OF performance of pCPA-pretreated rats. That is, 5-HTP significantly decreased defecation compared with the pCPA-treated group. In addition, there were significant decreases in rearing and grooming scores, compared with the controls at this drug dose (Fig. 5B).

Effects of an Inactivation of the 5-Hydroxytryptaminergic Neuronal System with p-Chlorophenylalanine (pCPA) on Defecation and Food Consumption in Rats under Different Experimental Conditions

Figure 6 shows the effects of pCPA on defecation (Fig. 6A) and on food consumption (Fig. 6B) under different conditions. Home caged animals having vehicle only showed a decrease in both scores during the days, but an increase in these scores at night. Rats treated with pCPA tended to show diminishing effects in circadian rhythm. That is, the pCPA-treated animals defecated no more than the home caged controls. The group-housed rats in home cages were influenced by this treatment more than the isolation-housed rats.

Treated rats, when tested in the OF after pCPA injection, tended to show an increase of defecation throughout the four-day experimental period, although defecation and food consumption were decreased during several periods in home caged rats. At 60 hr after pCPA injection, defecation in the OF was significantly increased compared with the control ($p<0.01$).

Effects of Intraventricular Injection of 5,6-Dihydroxytryptamine (5,6-DHT) on Emotional Defecation in Rats

The effects of 5,6-DHT, the other depletor of brain 5-HT on defecation in the OF were observed (Fig. 7). 5,6-DHT (30 μg/0.01 ml/rat) was injected into the right lateral ventricle 13 days before the OF test. 5,6-DHT tended to produce a slight

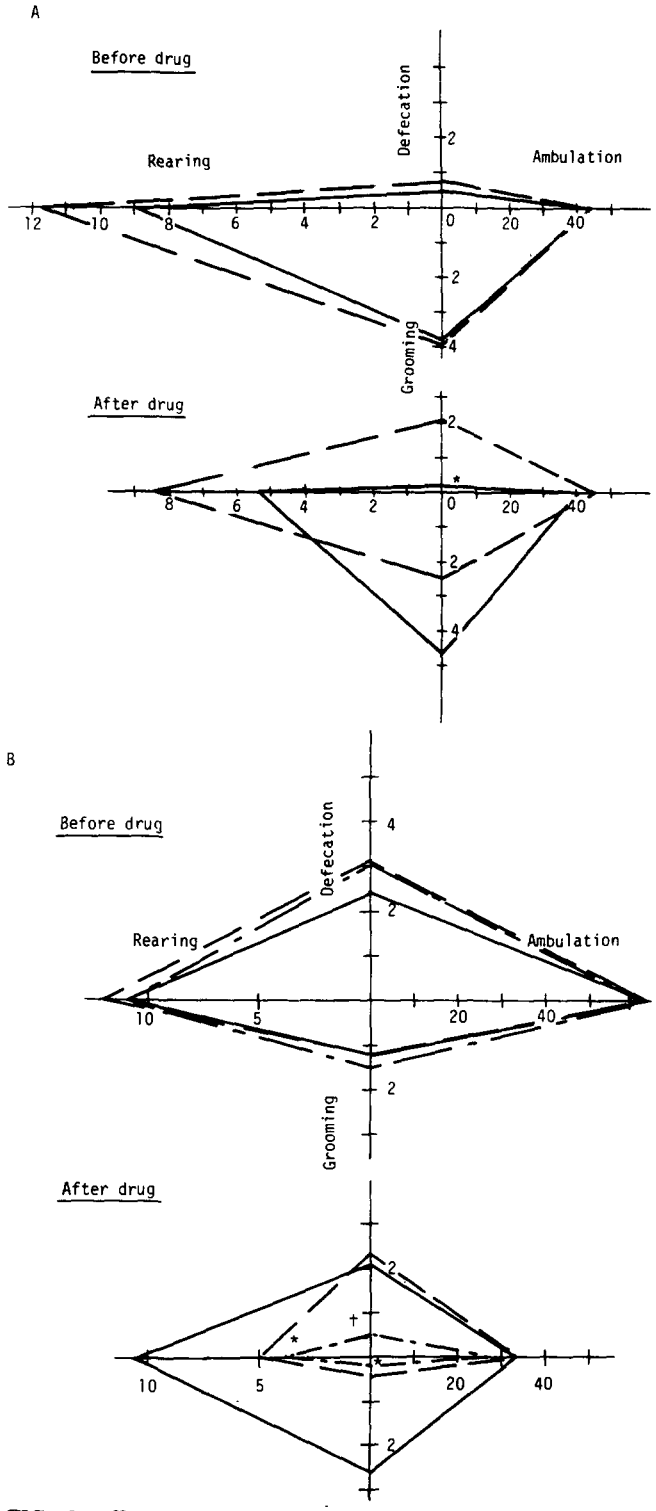

FIG. 5. Effects of p-chlorophenylalanine (pCPA) and 5-hydroxytryptophan (5-HTP) on the open-field behavior in rats. Fig. 5A: The open-field behavior was observed 72 hr after injection with pCPA (100 mg/kg each day for three days). Fig. 5B: pCPA (300 mg/kg) was injected 24 hr before the open-field test. 5-HTP (60 mg/kg) was given 23 hr after pCPA treatment then rats were subjected to the open-field test. Each point represents a mean value of at least seven rats. ———: (0.3% CMC+0.3% CMC)-group, — — —: (pCPA+0.3% CMC)-group, — - —: (pCPA+5-HTP)-group, *$p<0.05$ compared with the control. †$p<0.05$ compared with the pCPA-treated group.

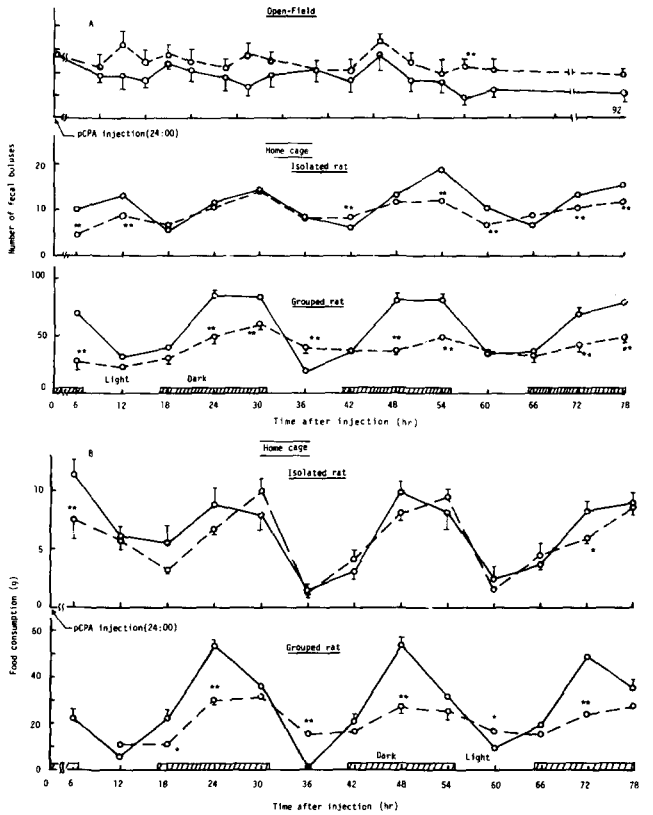

FIG. 6. Effects of p-chlorophenylalanine (pCPA) on the defecation and food consumption in rats under differing conditions. After intraperitoneal injection with pCPA (300 mg/kg), the number of fecal boluses excreted (A) and the amount of food consumed (B) during a 6-hr period were measured every 6 hr in the home cages. In the open-field, the number of fecal boluses deposited was counted during a 3-min period every 3 or 4 hr. ———: 0.3% CMC-group, — — —: pCPA-group, *$p<0.05$, **$p<0.01$ compared with the vehicle-treated group.

increase in defecation. These effects of 5,6-DHT were similar to those produced by pCPA. The depleting effects of 5,6-DHT on 5-HT levels are seen in Table 1. Brain 5-HT concentrations were reduced to 70–90% of control levels.

Effects of Reserpine (RSP) on Emotional Defecation in Rats

The effects of RSP, a depletor of brain 5-HT and catecholamines, on defecation in the OF are shown in Fig. 8. RSP (2 mg/kg) was injected into rats 4 hr before the OF test. Although there was significant decrease in ambulation and rearing scores, there was only a slight decrease in defecation compared with controls. The inhibitory effects of 5-HTP, 60 mg/kg, on defecation were observed also after depletion of biogenic amines by RSP pretreatment. The effects of RSP and/or 5-HTP on the levels of 5-HT at various brain sites are shown in Table 1.

DISCUSSION

The behavioral effects of many psychoactive drugs are supposedly mediated through 5-HT in the central nervous system. Several attempts have been made to determine the specificity of behavioral patterns as mediated through 5-HT [14, 19, 26, 27, 50].

It has been reported that a variety of 5-HT-related drugs

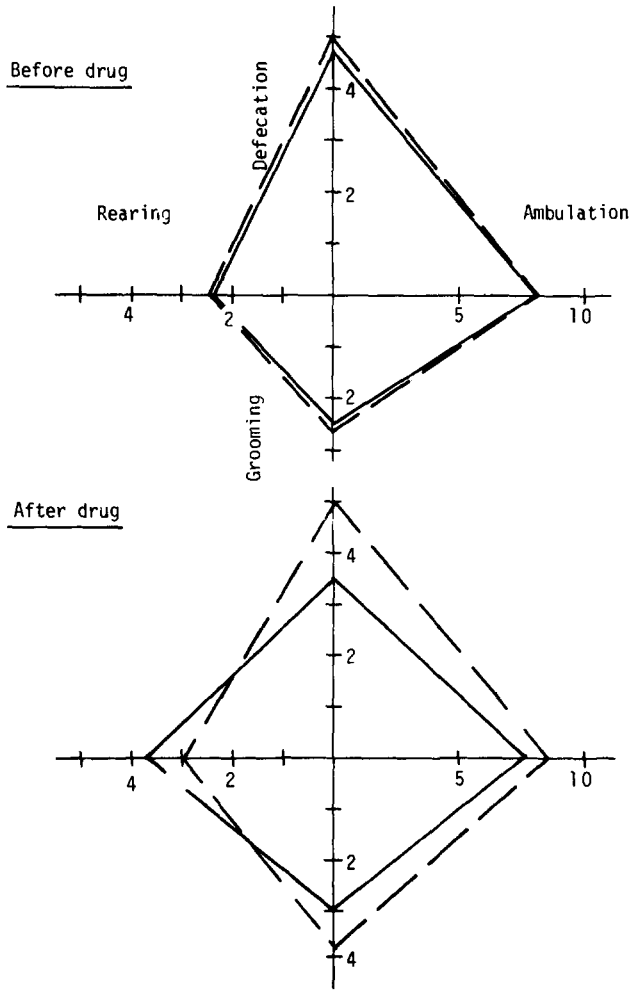

FIG. 7. Effects of intraventricular injection of 5,6-dihydroxytryptamine (5,6-DHT) on the open-field behavior in rats. The open-field behavior was observed 13 days after pretreatment with 5,6-DHT. Each point represents a mean value of at least seven rats.——: saline-group, — — —: 5,6-DHT-group (30 μg/rat).

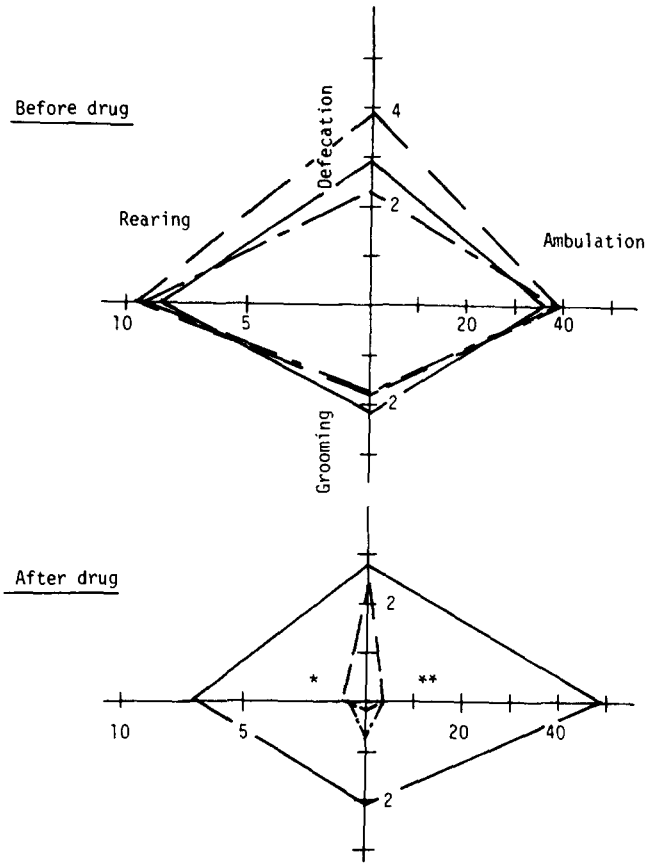

FIG. 8. Effects of reserpine (RSP) and 5-hydroxytryptophan (5-HTP) on the open-field behavior in rats. RSP (2 mg/kg, IP) was injected 4 hr before the open-field test. 5-HTP (60 mg/kg) was given 3 hr after RSP-treatment, and then rats were subjected to the open-field test. Each point represents a mean value of at least seven rats. ——: (saline+0.3% CMC)-group, — — —: (RSP+0.3% CMC)-group, — - —: (RSP+5-HTP)-group, $*p<0.05$, $**p<0.01$ compared with the control.

modify the OF behavior in rats. Ellison and Bresler [12] and Diaz *et al.* [10] studied locomotion and rearing of 5-HT-depleted rats in an OF, and the influence of pCPA on the grooming behavior was compared with that of RSP by Rohte and Müntzing [37]. Rosecrans [39] has indicated that female rats and mice both have a more functional 5-HT system in the forebrain and a higher rate of rearing. Moreover, he has reported that in the forebrain 5-HT turnover is depressed in high defecating rats [40].

However, few detailed investigations on the behavioral effects of 5-HT in OF defecation have yet been reported. We have shown that brain 5-HT may play an important role in OF defecation [23]. In the present study, administration of 5-HTP, a 5-HT precursor, significantly decreased fecal boluses excreted in the OF. Moreover, the same effects were observed on defecation studied in the shuttle box after 5-HTP-pretreatment. Hunt and Otis [18] suggested that defecation can serve to indicate conditioned emotional disturbance of the "fear" or "anxiety" type. In fact, as a result of the conditioning procedure the CS actually acquired the power to "elicit" defecation as one component of the general emotional response. In the shuttle box procedure, the conditioned avoidance stimulus elicits fear in the conditioned subject since it is followed by punishment. Thus, in avoidance learning, the subject at first is motivated by pain but later learns to avoid this pain by responding appropriately when the eliciting stimulus is presented [31]. The defecation in the shuttle box was increased with repeated conditioning. Since the defecation occurred at a higher rate in the shuttle box than in the home cage, the defecation in the shuttle box and those in OF may be correlated positively. But, it would be difficult to extract a single emotionality state from the various measures [1]. Thus, further investigation would be necessary to relate between defecation in shuttle box and those in OF.

On the other hand, Woolley showed the animal frequently defecated after injection of 5-HTP, because of strong contractions of the intestinal muscles [53]. Ndika suggests that the inhibition of 5-HTP-induced diarrhea by methylphenidate is a direct result of a specific anti-5-HT action of this drug in the gastrointestinal tract [35]. Therefore, home cage defecation was observed whether the reduction in OF defecation by 5-HTP is based on a peripheral constipation after 5-HTP-induced diarrhea. 5-HTP increased defecation compared with controls, 0–30 min after injection in home cage. However, fecal elimination in this familiar situation 30–90

min after injection was not affected by 5-HTP. Therefore, the reduction in OF defecation by 5-HTP is unlikely to be due to a peripheral constipating effect.

In addition, fecal excretion in the OF was significantly decreased, compared with controls, 30 min after intraventricular injection of 5-HT at a dose of 5.0 μg/rat. In spite of intraventricular injection of 5-HT, the defecation of home cage was similar to that of the vehicle-treated control group. There is a possibility that the 5-HT turnover in forebrain is increased after administration of 5-HTP and 5-HT. This is supported by the research of Rosecrans [40], who indicated that forebrain 5-HT turnover is depressed in high defecation rats.

In contrast with these results, rats pretreated with pCPA and 5,6-DHT, the depletors of 5-HT, tended to show a slight increase in the number of fecal boluses eliminated in the OF. pCPA has noradrenergic as well as serotonergic effects, however, in pCPA treated animals norepinephrine (NE) metabolism has returned to normal within 24 hr following drug treatment [45,46]. Therefore, in order to minimize NE influences on behavior produced by pCPA, animals were tested 24 hr or longer after treatment. When pCPA (100 mg/kg/day for three days) was given to rats, there was a significant increase in defecation, but a marked decrease in brain 5-HT levels. Therefore, the slight increase in defecation 24 hr after treatment with pCPA (300 mg/kg) in spite of decrease of food consumption, and 13 days after administration of 5,6-DHT, may be due to a slight decrease in brain 5-HT. In relation to our findings it has been reported that pCPA treated animals defecate more than control up to 30 days after drug administration in OF [51] and also fecal bolus counts are significantly increased under pCPA in wild mice during a confrontation [30]. However, home cage defecation and food consumption were significantly decreased in pCPA treated rats. These results were similar to Saller and Stricker's data that intraventricular injections of 5,7-DHT, combined with systemic administration of desmethylimipramine produced large depletion of brain 5-HT without affecting NE levels and inhibited gastrointestinal motility more than 5,7-DHT single injection which produced large depletion of brain 5-HT and NE [44].

These results suggest that the levels and activity of 5-HT may influence differences in defecation between OF and home caged groups. It is well known and in agreement with our data, that psychotropic drugs can produce different effects according to the emotional level of the experimental animals [22, 36, 41].

The principle of the OF test is based upon a novel situation whereby the OF evokes a pattern of behavior characterized by defecation, urination, ambulation, rearing, and grooming in animals [5]. In general, rats with a low emotionality were defined by a low defecation rate and high ambulation scores in OF tests [6, 40, 52]. In relation to our findings, it has been reported that the behavior in the OF of rats having extensive 5-HT depletion are vigilant and have fearful behavior characteristics of an animal at the periphery of its territory. Such animals are cautious, have slow locomotion, frequently react to stimuli, and avoid open spaces [10]. Ellison and Bresler [12] have similarly observed that animals receiving pCPA showed decreased locomotion accompanied by "freezing" in quiet novel environments.

On the other hand, 5-HT and 5-HTP in low or moderate doses produce behavioral signs of calm and sleep [15], perhaps because 5-HT is involved in central inhibitory processes [7]. 5-HTP, like imipramine, has been reported to possess clinically useful antidepressant properties [16]. It has been well known that anti-depressant drugs reduce the number of fecal boluses excreted in the OF [5,29]. Thus, lowered functional levels of 5-HT in the brain resulting from emotional stimulation of animals may be responsible for increased defecation in the OF.

In fact, a number of authors have shown decreased concentrations of brain 5-HT during stress. Eleftheriou and Church [11] found a lower concentration of brain 5-HT in the mouse after exposure to aggression and defeat. Rosecrans [38] found that acute oscillation stress caused a decrease of 5-HT synthesis in the rat brain. Curzon and Green [9] found a reduction in brain 5-HT in the rat after immobilization, and Bliss and coworkers [3] found an increased brain 5-HT breakdown in rats after foot shock. Rosecrans [40] found that forebrain 5-HT turnover was depressed on high defecating rats in the OF. Furthermore, Kameyama *et al.* [23] found that there was a significant "negative" correlation with telencephalic/non-telencephalic ratio of 5-HT and defecation in OF. There is a possibility, therefore, that responsiveness to stresses differ at specific brain sites. However, regional differences at brain sites of 5-HT content are complex and difficult to interpret in terms of the physiological events occurring from our present results.

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