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Increased 5-HT_2 receptor-mediated behavior 11 days after shock in learned helplessness rats

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Abstract

In the learned helplessness procedure, rats can be differentiated into two distinct groups. Learned helplessness (LH) rats do not learn to escape a controllable shock while non-learned helplessness (NLH) rats learn this response. This deficit in performance in LH rats lasted for 11 days. In LH rats, pretreatment with acute desipramine (15 mg/kg i.p.) or chronic diazepam (0.95 mg/kg/day p.o. for 7 days) did not produce recovery from this deficit of performance, but pretreatment with chronic desipramine (17.7 mg/kg/day p.o. for 7 days) or chronic mianserin (6.1 mg/kg/day p.o. for 7 days) led to recovery. Before presentation of uncontrollable shock, there was no difference between LH and NLH rats, but 11 days after the shock, head shakes induced by (±)-1-(2,5-demethoxy-4-iodophenyl)-2-aminopropane (DOI) in LH rats was significantly more frequent than those in NLH and naive rats without change of [^{3}H]ketanserin binding. The basal corticosterone level was higher in LH rats than in NLH rats. These findings suggest that the learned helplessness model is a reliable animal model of depression accompanied by 5-HT_2 receptor hypersensitivity.

Keywords: Learned helplessness; Antidepressant; 5-HT_2 receptor; Corticosterone

1. Introduction

The learned helplessness model is regarded as one of the most valid models for depression (Willner, 1984). Seligman and co-workers first demonstrated that exposure to inescapable and uncontrollable stress results in behavioral deficits described as 'learned helplessness' (Seligman and Maier, 1967). These behavioral deficits are similar to the vegetative symptoms of depression (Weiss and Glazer, 1975). The learned helplessness model has also been shown to have good predictive validity for the treatment of depression. The behavioral deficits in the learned helplessness model are reversed by chronic treatment with tricyclic antidepressants, as well as with atypical antidepressants and monoamine oxidase inhibitors (Sherman et al., 1982; Martin et al., 1990a). Edwards et al. (1986) proposed a modified version of Seligman and Maier's 'learned helplessness' model. They took hereditary vulnerability to stress into consideration and compared two distinct groups of rats which emerged when subjected to a mild course of inescapable shock; one group developed a performance deficit on a subsequent escape test (learned helplessness); another group under identical shock conditions performed like controls in the shock escape test (non-learned helplessness).

Recently, a variety of 5-HT receptor subtypes have been identified, and abnormalities of 5-HT_2 receptors have been reported in depressed patients. Thus, several studies showed that 5-HT_2 receptor sites were increased in the post-mortem frontal cortex of depressed patients and of suicide victims (Mann et al.,

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1986; McKeith et al., 1987; Arora and Meltzer, 1989a); however, these results were not confirmed by other investigators (Owen et al., 1986; Cheetham et al., 1988). Platelet 5-HT_2 binding sites were also increased in depressed patients (Biegon et al., 1987, 1990; Arora and Meltzer, 1989b; Pandey et al., 1990). 5-HT_2 receptor-mediated intracellular Ca^{2+} mobilization in platelets from patients with major depression was significantly increased (Kusumi et al., 1991; Mikuni et al., 1992). Recently, Kusumi et al. (1994) reported that this increase persisted after remission of depressive symptoms in unmedicated patients. These studies suggest that 5-HT_2 receptor-mediated responses may be increased in depression and this phenomenon may be a trait-dependent marker in depression.

It has been reported that 5-HT_2 receptor binding in hippocampus and cortex did not vary significantly among LH, NLH and naive-control rats 2 days after the inescapable shock (Martin et al., 1990b). However, recently the B_{max} for cortical 5-HT_2 receptor binding was found to be elevated in rats exposed to mild stress for 7 weeks, another animal model of depression (Papp et al., 1994).

In the present study, hereditary vulnerability to stress was taken into consideration to evaluate learned helplessness in rats. The aim of the study was to confirm the reliability of learned helplessness rats as a depression model and to investigate 5-HT_2 receptor function 3 days before and 11 days after the induction of learned helplessness. [^{3}H]Ketanserin binding was investigated 2 days after the behavioral study. Basal plasma corticosterone was also measured 11 days after the induction of learned helplessness.

2. Materials and methods

2.1. Animals

Male Wistar albino rats weighing 200–220 g at the beginning of the experiments were used. The animals were housed four per cage in a colony room with a 12 h light-dark cycle (light on 7:00 h, light off 19:00 h). A week for setting in and handling was allowed prior to any behavioral experiments. A total of 284 rats were used in these experiments.

2.2. Establishment of learned helplessness

Learned helplessness was produced according to the method of Edwards et al. (1986) with some modifications. All experimental animals were placed in an experimental chamber with an electrifiable grid floor. Each chamber was 18.5 cm long × 20 cm high × 9 cm wide with Plexiglas walls and covers. The floor was constructed of stainless-steel rods, 0.3 cm in diameter and spaced 0.6 cm apart. A disk was from the ceiling mounted 7 cm off the grid floor. A yellow cue light was placed 2 cm under the disk. A rat's tail extended through the rear door of the chamber and was taped to a Plexiglas rod. Unscrambled electric shock was delivered from an alternating-current shock generator (Model SGS-002, Muromachi Kikai Co., Japan). One electrode was positioned on the tail with electrode paste, the other was the floor grid.

Pre-shock escape testing

One day before the inescapable shock session, pre-shock escape testing was carried out. Each rat was fixed in the chamber 5 min before the experiment. The rats received pulsed (35 ms on and 35 ms off) 1 mA electric shocks with the yellow cue light on. Shock onset began a trial, which was terminated either by pulling and releasing the disk or the end of 60 s. Fifteen trials were given for a rat and intertrial latency was set at 15 s. Experimental events were programmed and failures to escape were recorded by an NEC PC-9801 VM microcomputer.

Inescapable shock training

On the next day, inescapable pulsed electric shocks (1 mA, 35 ms on and 35 ms off) were delivered via stainless steel grids and the tail in the chamber for a single 40-min session.

Post-shock escape testing

Two days after the inescapable shock, a post-shock escape test was conducted. The procedure for this test was the same as that for the pre-shock test.

Classification

The rats were divided into two groups according to the following criteria. Rats scoring fewer than ten escape failures in the pre-shock test with an increase of more than five failures in the post-shock test were considered as the learned helplessness (LH) rats. Rats scoring fewer than ten escape failures in the pre-shock test with no increase in failures in the post-shock test were considered the non-learned helplessness (NLH) rats. In our study, 30–35% of all the rats tested became LH rats and 15–20% of all the rats tested became NLH rats.

2.3. Escape testing before and after the inescapable shock and body weight gain in LH and NLH rats

A total of 50 rats were used in this experiment. The pre-shock test, the inescapable shock and the post-shock test were performed. Seventeen LH rats and seven NLH rats were obtained according to the criteria. Eleven days after the inescapable shock, escape testing was demonstrated in LH ($n = 17$) and NLH

($n = 7$) rats. One day before and 11 days after the inescapable shock, body weights of the rats were recorded.

2.4. Effect of acute and chronic administration in LH rats

Effect of acute desipramine administration on escape test with LH rats

The pre-shock test, the inescapable shock and the post-shock test were performed on the same schedule. At the end of the post-shock test the LH rats were further subdivided into two groups, matched for the results of body weight and pre- and post-escape tests. Twenty hours after the post-shock escape test, each subgroup then received saline (1 ml/kg i.p.) or desipramine dissolved in saline (15 mg/kg i.p.). Sixty minutes after injection, escape testing with 15 trials was carried out again.

Effect of chronic desipramine, mianserin or diazepam administration on escape test with LH rats

The pre-shock test, the inescapable shock and the post-shock tests were performed on the same schedule. The LH rats were further subdivided into two groups. Twenty hours after the post-shock escape test, each group was given food with or without 0.03% desipramine, 0.01% mianserin, or 0.002% diazepam for the next 7 days. The daily average intake of desipramine was about 17.7 mg/kg/day, that of mianserin was about 6.1 mg/kg/day and that of diazepam was about 0.95 mg/kg/day. A similar escape test with 15 trials was carried out with the desipramine-treated, mianserin-treated and diazepam-treated groups and with each control group 7 days later while drug administration continued.

2.5. DOI-induced head shake behavior

DOI-induced head shake behavior before inescapable shock in LH, NLH and the other rats

The pre-shock test, the inescapable shock and the post-shock tests were performed on the same schedule. Three days before the inescapable shock, the rats were placed in individual cages (38 cm × 26 cm × 13 cm) 5 min before behavioral testing. The frequency of head shake responses elicited by the subcutaneous administration of 1 mg/kg (±)-1-(2,5-demethoxy-4-iodophenyl)-2-aminopropane (DOI) was quantified for 30 min, commencing immediately after the injection. A preliminary study confirmed that DOI induced dose-dependent shaking behavior at low doses (0.25–3.0 mg/kg) and that a maximum response occurred at 3.0 mg/kg (data not shown). Therefore, DOI at 1.0 mg/kg was used in this experiment. Head shakes were characterized as rapid side-to-side twitches of the head and ears, as previously described by Bedark and Pycock (1977). Two days after the DOI administration, pre-shock escape testing was carried out. Inescapable shock and post-shock escape testing was also carried out on the same schedule.

DOI-induced head shake behavior in LH, NLH and naive rats 11 days after the inescapable shock

The pre-shock test, the inescapable shock and the post-shock tests were performed on the same schedule. Eleven days after the inescapable shock, LH rats ($n = 12$) and NLH rats ($n = 12$) were used in the behavioral experiment. Another naive group (NC, $n = 15$) without shock or escape test was also used. The frequency of head shake responses elicited by the subcutaneous administration of 1 mg/kg DOI was quantified for 30 min, commencing immediately after the injection.

2.6. [^{3}H]Ketanserin binding 13 days after the inescapable shock

Thirteen days after the inescapable shock, 24 h after DOI administration, LH and NLH rats were decapitated. The prefrontal cortex was dissected on ice and stored at −80° C until assayed for receptor binding. The binding of [^{3}H]ketanserin ([ethylene-^{3}H]ketanserin hydrochloride, specific activity, 60.08 Ci/mmol; New England Nuclear, USA) was demonstrated by the method of Leysen et al. (1982) with minor modifications. Briefly, homogenates of the prefrontal cortex were prepared in 50 vols. (w/v) of 50 mM Tris-HCl buffer (pH 7.4 at 25° C) with a Polytron. The homogenates were centrifuged twice (20 min, 40 000 × g) at 4° C with resuspension of the intermediate pellet in the same buffer. The final pellets were resuspended in the same buffer and used for the binding assay. Membranes were incubated for 15 min at 37° C with six different concentrations (0.25–5 nM) of [^{3}H]ketanserin. Non-specific binding was defined with 1 μM methysergide. The samples were rapidly run through Whatman GF/B glass filters and washed 3 times with 5 ml of ice-cold buffer using a 24-channel cell harvester (Brandell, USA). The maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_D) for each subject were determined by non-linear regression analyses of Scatchard plots. The protein concentration was estimated by the method of Bradford (1976).

2.7. Basal corticosterone levels in LH, NLH and naive control 11 days after the inescapable shock

The pre-shock test, the inescapable shock and the post-shock test were performed on the same schedule. Eleven days after the inescapable shock, between 9:00 and 10:00 a.m. 25 μl blood samples were collected in

heparinized capillary tubes by the tail tip method. Plasma corticosterone was measured in 25 μl blood sample with 1 ml ethanol. After centrifugation (2300 × g for 30 min), the solvent was decanted off (100 μl or 50 μl) and dried. The dried extracts were assayed in duplicate by adding 100 μl diluted antiserum (UCB-Bioproducts, Belgium), 100 μl of [1,2,6,7,-^{3}H(N)]-corticosterone (NEN, spec. act. = 72.5 Ci/mmol) and assay buffer. The tubes were incubated at 37° C for 30 min, followed by incubation at 4° C for 16 h. Separation of bound and free hormone fractions was achieved by adding 500 μl of Dextran (0.025%)-coated charcoal (0.25 g%) suspension. The method has a sensitivity of 4 pg/tube.

2.8. Drugs

Desipramine-HCl, mianserin-HCl, methysergide-HCl and diazepam were kindly donated by Nippon Ciba-Geigy (Japan), Nihon Organon (Japan), Sandoz (Switzerland) and Yamanouchi Pharmaceuticals Co. (Japan), respectively. (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) was purchased from Research Biochemicals (USA). The other drugs were of analytical grade.

2.9. Statistical analysis

Results are usually given as means with S.E.M. The statistical significance of behavioral changes was calculated by means of Mann-Whitney *U*-tests or Wilcoxon matched-pairs tests. The DOI treatment experiments (three groups) were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney *U*-tests in group-to-group comparisons. The statistical significance of body weight gain and biochemical data was compared using unpaired two-tailed Student's *t*-tests. The correlation between number of head shakes and B_{max} or K_D values of [^{3}H]ketanserin binding was calculated with Spearman's rank correlation coefficient. $P < 0.05$ was chosen as the minimum level of significance.

3. Results

3.1. LH and NLH in escape test 11 days after the inescapable shock

Fig. 1 shows the means ± S.E.M. of escape failures in LH ($n = 17$) and NLH ($n = 7$) rats. One day before the inescapable shock, the numbers of failures in the pre-shock escape test were not different in LH and NLH rats (LH, 4.0 ± 1.1; NLH, 4.1 ± 1.5). Two days after the shock, the numbers of failures in the post-shock escape test were increased in LH rats ($P < 0.01$); in contrast, those in NLH rats were decreased compared to those in the pre-shock test (LH, 11.8 ± 1.3; NLH, 1.3 ± 0.9; $P < 0.05$). Eleven days after the shock, the numbers of failures in the post-shock escape test were still significantly increased in LH rats compared to those in the pre-shock test ($P < 0.05$) and were significant higher compared to those in NLH rats ($P < 0.01$) (LH, 8.8 ± 1.8; NLH, 2.0 ± 1.1). In addition, the numbers of failures in NLH rats 11 days after the shock were still significantly lower than those in NLH rats before the shock ($P < 0.05$).

The mean weight gain in LH rats was significantly lower than that in NLH rats (LH, 54.0 ± 1.3; NLH, 81.5 ± 1.7 g; $P < 0.01$).

3.2. Effect of acute and chronic administration in LH rats

Effect of acute desipramine administration on escape test in LH rats

The results of acute and chronic administration of drugs are summarized in Table 1. The numbers of

Table 1
Effects of acute desipramine treatment and chronic desipramine, mianserin or diazepam treatment for 7 days on escape test in learned helplessness rats

Treatment	Group	*n*	Pre-treatment (failures)	Post-treatment (failures)
Acute	Control	6	13.8 ± 0.7	14.6 ± 0.2
	Desipramine	6	14.1 ± 0.5	12.9 ± 0.7
Chronic	Control	12	12.7 ± 0.6	13.0 ± 0.3
	Desipramine	12	12.3 ± 0.6	4.0 ± 1.4 [a]
	Control	6	11.9 ± 0.9	13.3 ± 0.8
	Mianserin	6	10.3 ± 0.9	5.1 ± 1.8 [a]
	Control	7	10.9 ± 0.9	8.7 ± 1.4
	Diazepam	7	11.0 ± 0.8	8.4 ± 1.1

In the acute treatment experiment, learned helplessness rats received saline (1 ml/kg i.p) or desipramine (15 mg/kg). After 60 min of injection escape testing with 15 trials was carried out. In the chronic treatment experiment, learned helplessness rats received vehicle food, desipramine (0.03%), mianserin (0.01%) or diazepam (0.002%) for 7 days. The average intake of desipramine, mianserin or diazepam was 17.7, 6.1, 0.95 mg/kg/day, respectively. Numbers of failures are expressed as means ± S.E.M. of 15 trials. [a] Significant difference at $P < 0.01$ from control (Mann-Whitney *U*-test) or the same group (Wilcoxon test) before treatment.

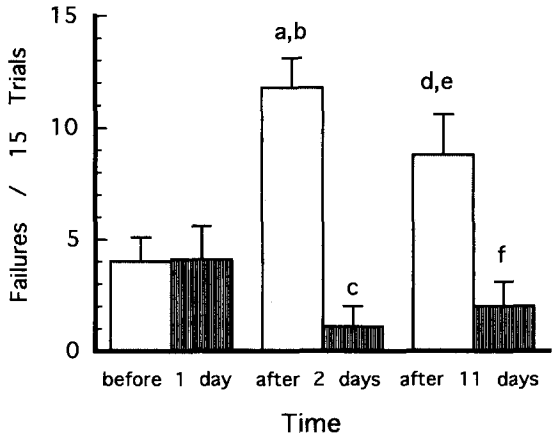

Fig. 1. Effects of the inescapable shock on escape test in learned helplessness rats (LH) (left column) or non-learned helplessness rats (NLH) (right column) before or after the shock. The escape tests were performed one day before and 2 and 11 days after the inescapable shock. Values are means ± S.E.M. of the total number of failures with 15 trials. [a]$P < 0.01$ versus LH before the shock (Wilcoxon test). [b]$P < 0.01$ versus NLH 2 days after the shock (Mann-Whitney U-test). [c]$P < 0.01$ versus NLH before the shock (Wilcoxon test). [d]$P < 0.05$ versus LH before the shock (Wilcoxon test). [e]$P < 0.01$ versus NLH 11 days after the shock (Mann-Whitney U-test). [f]$P < 0.05$ versus NLH before the shock (Wilcoxon test).

escape failures in saline-treated rats (control) and acute desipramine-treated rats were similar before the injection. The numbers of failures were not significantly affected by a single dose of desipramine (15 mg/kg i.p.) given 60 min before.

Effect of chronic desipramine, mianserin or diazepam administration on escape test in LH rats

The numbers of failures in all control groups and all chronic treated groups were not different in the post-shock escape test. Chronic administration of desipramine for 7 days significantly decreased the number of escape failures ($P < 0.01$) (Table 1). The number of failures after the chronic desipramine treatment was significantly lower than that of the control group ($P < 0.01$) (Table 1). The number of failures after the chronic mianserin treatment was also significantly decreased ($P < 0.01$) and was significantly lower than the mean number of failures of the control group ($P < 0.01$) (Table 1). On the other hand, there was no difference between the number of failures in control and diazepam-treated groups after the treatment. There was also no difference between the number of failures in the diazepam-treated group before and after the treatment.

3.3. DOI-induced head shake behavior

DOI-induced head shake behavior 3 days before inescapable shock in LH, NLH and the other rats

Fig. 2 shows DOI-induced head shake frequency in LH ($n = 9$), NLH ($n = 7$) and the other rats ($n = 8$).

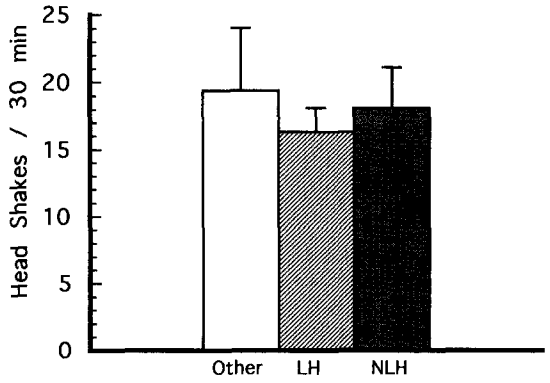

Fig. 2. DOI-induced head shakes 3 days before the shock in learned helplessness (LH), non-learned helplessness (NLH) and the other rats. Values are means ± S.E.M. of the total number of head shakes occurring in the 30-min observation period following DOI (1 mg/kg s.c.) injection.

The numbers of head shakes in LH, NLH and the other rats (LH, 16.9 ± 1.8; NLH, 18.3 ± 2.6; the others, 18.3 ± 2.6) were not significantly different.

DOI-induced head shake behavior 11 days after the inescapable shock in LH, NLH and naive rats

As shown in Fig. 3, DOI-induced head shake frequency was significantly increased in LH rats as compared to NLH and naive control rats (LH, 22.4 ± 2.3; NLH, 14.2 ± 1.4; control, 13.3 ± 1.6; $P < 0.01$, LH versus control; $P < 0.01$, LH versus NLH).

3.4. [^{3}H]Ketanserin binding 13 days after the inescapable shock

As shown in Table 2, neither the B_{max} nor the K_D values of [^{3}H]ketanserin binding were different between LH and NLH rats. There was no significant

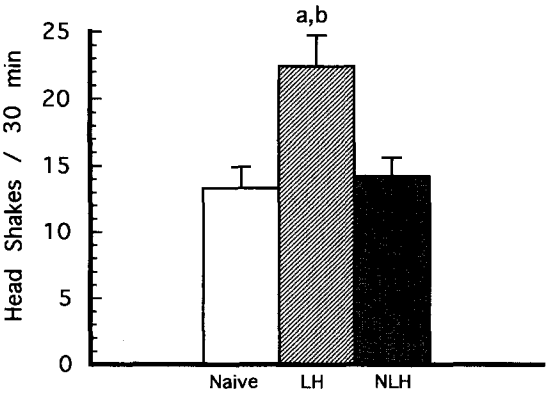

Fig. 3. DOI-induced head shakes 11 days after the shock in naive control (Other), learned helplessness (LH) and non-learned helplessness (NLH) rats. Values are means ± S.E.M. of the total number of head shakes occurring in the 30-min observation period following DOI (1 mg/kg s.c.) injection. [a]$P < 0.01$ versus Other (Mann-Whitney U-test). [b]$P < 0.01$ versus NLH (Mann-Whitney U-test).

Table 2
B_{max} and K_D values for [^{3}H]ketanserin binding in the prefrontal cortex of learned helplessness rats and non-learned helplessness rats 13 days after the inescapable shock

Group	n	B_{max} (fmol/mg protein)	K_D (nM)
Learned helplessness	9	159.1 ± 8.9	0.633 ± 0.038
Non-learned helplessness	9	148.2 ± 7.6	0.574 ± 0.029

Thirteen days after the inescapable shock, 24 h after DOI administration, LH and NLH rats were killed. The prefrontal cortex was dissected and stored. Membranes were assayed with six concentrations (0.25–5 nM) of [^{3}H]ketanserin as described in Materials and methods. The results are given as means ± S.E.M.

Table 3
Basal serum corticosterone levels in learned helplessness, non-learned helplessness and control rats 11 days after the inescapable shock

Group	n	Corticosterone level (μg/100 ml)
Control	5	2.16 ± 0.73
Learned helplessness	8	3.82 ± 1.02 [a]
Non-learned helplessness	6	1.43 ± 0.50

Eleven days after the inescapable shock, 25 μl blood samples were collected by the tail tip method. The basal corticosterone levels were measured. The results are given as means ± S.E.M. [a] $P < 0.05$ compared to non-learned helplessness (Student's t-test).

correlation between the number of DOI-induced head shakes and the B_{max} ($r = 0.12$) or K_D ($r = -0.21$) values of [^{3}H]ketanserin binding in LH and NLH rats.

3.5. Basal corticosterone levels 11 days after the inescapable shock in LH, NLH and naive rats

The basal plasma corticosterone levels in each group are shown in Table 3. The mean corticosterone level was significantly higher in LH than in NLH rats ($P < 0.05$). There was no difference between naive controls and the LH or NLH group.

4. Discussion

After exposure to uncontrollable shock, Wistar rats can be classified into LH and NLH rats according to their response. This would lend support to the findings of Edwards et al. (1986) who reported that sub-populations of Sprague-Dawley rats were classified into helpless and non-helpless ones. Individual differences were also reported for apomorphine-susceptible and apomorphine-unsusceptible Wistar rat lines (Cools et al., 1993) and for short attack latency and long attack latency in male mice (Compaan et al., 1993).

The results show that learned helpless behavior is not reversed by acute treatment with desipramine or chronic treatment with diazepam, but is reversed by chronic treatment with desipramine or mianserin. The results are consistent with a previous report (Sherman et al., 1982) that showed chronic administration of tricyclic antidepressants or atypical antidepressants to be effective to reverse learned helplessness, but anxiolytics were not. Weight gain in LH rats was also suppressed compared to NLH rat weights. The behavioral deficits lasted for at least 11 days after the inescapable shock. Van Dijken et al. (1992) reported that a brief session of inescapable foot shocks induced a long-lasting change in behavioral responses to novel environmental stimuli. In addition, the basal corticosterone level was significantly higher in LH than in NLH rats 11 days after the inescapable shock. Haracz et al. (1988) and Greenberg et al. (1989) reported that helpless rats showed impaired feedback regulation in the hypothalamic-pituitary-adrenal (HPA) axis. These results indicate that this learned helplessness rat is a reliable and valid animal model for depression.

The 5-HT$_2$-mediated head shake response is thought to be centrally mediated, because head shake responses in rats were induced by intracerebroventricular injection of serotonin (Oliveria and Campos, 1993). In the present study, DOI-induced head shake behaviors before and after the induction of learned helplessness were seen in independent groups of animals, because 5-HT$_2$-mediated behavioral changes after a single dose of DOI persisted for at least 6 days in mice (Darmani et al., 1992a). In our model, 5-HT$_2$-related behavior was not different between LH, NLH and the other rats before the inescapable shock, but 5-HT$_2$ receptor-related behavior was enhanced in LH rats compared to NLH, and compared to naive rats after the shock. If these results are extrapolated to the clinical situation, it is possible that 5-HT$_2$ receptor function may be elevated after the induction of depression.

[^{3}H]Ketanserin binding was also evaluated, but there was no difference in B_{max} or K_D values between LH and NLH rats, in apparent contradiction with our behavioral data. There was no significant correlation between the numbers of head shakes induced by DOI and B_{max} or K_D values for the [^{3}H]ketanserin binding. Several studies showed changes of 5-HT$_2$ receptor-mediated behavior without any change in 5-HT$_2$ receptor sites (De Souza et al., 1986; Wieland et al., 1990; Darmani et al., 1992b; Javaid et al., 1993). De Souza et al. (1986) reported that chronic treatment with a 5-HT$_{1A}$ receptor agonist produced a modest enhancement of the 5-HT$_2$ receptor-mediated head-twitch behavioral response in contrast to a decreased 5-HT$_2$ receptor number in the frontal cortex of mice. Therefore, it is conceivable that 5-HT$_2$ receptor-mediated responses may be enhanced independently of the 5-HT$_2$ receptor sites.

Previous studies with learned helplessness rats

showed that presynaptic serotonin mechanisms were changed by inescapable shock. Serotonin uptake and release were increased in the hippocampus, while 5-HT uptake and release were decreased in the hypothalamus of learned helplessness rats compared to non-learned helplessness and naive-control rats (Edwards et al., 1992). The B_{max} for 5-HT_{1B} receptors in the cortex, hippocampus and septum is increased in LH rats (Edwards et al., 1991). These reports suggested that presynapic serotonergic mechanisms may be increased in septum or cortex after the induction of learned helplessness. However, in the present study postsynaptic 5-HT_2-mediated behavior was increased 11 days after the induction of learned helplessness. Agonists of 5-HT_2 receptors rapidly down-regulate 5-HT_2 receptors (Leysen et al., 1989). In addition, pre- and postsynaptic manipulations of 5-HT failed to up-regulate 5-HT_2 receptors (Leysen et al., 1983, Sander-Bush, 1990). Therefore the hyperfunction of 5-HT_2-mediated behavior may be independent of the regulation of serotonergic systems. There is evidence for a tight control of central serotonergic systems by corticosterone (see Chaouloff, 1993 for review). Subchronic adenocorticotropic hormone (ACTH) treatment significantly increased the B_{max} values for [^{3}H]ketanserin binding in neocortex of rat forebrain and DOI-induced head shake behavior (Kuroda et al., 1992). Lesch and Lerer (1991) suggested that the differential regulation of 5-HT_{1A} and 5-HT_2 function, leading to a relative 5-HT_2-phosphoinositide complex supersensitivity may maintain HPA hyperactivity during the course of depression. In our study the basal corticosterone level of LH rats was higher than that of NLH rats, so that the hyperfunction of the serotonergic system may be caused by a dysregulation of corticosterone.

On the other hand, serotonergic hyperfunction in learned helplessness is also important, since serotonin depletion by *p*-chlorophenylalanine prevents the development of learned helplessness (Edwards et al., 1986). These results indicate that the interaction of corticosterone and the serotonergic system plays an important role in the induction of learned helplessness and long-lasting behavioral deficits.

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References

Arora, R.C. and H.Y. Meltzer, 1989a, Serotonergic measures in the brains of suicide victims: 5-HT_2 binding sites in the frontal cortex of suicide victims and control subjects, Am. J. Psychiatry 146, 730.

Arora, R.C. and H.Y. Meltzer, 1989b, Increased serotonin$_2$ (5-HT_2) receptor binding as measured by ^{3}H-lysergic acid diethylamide (^{3}H-LSD) in the blood platelets of depressed patients, Life Sci. 44, 725.

Bedark, P and C.J. Pycock, 1977, 'Wet-dog' shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity, Neuropharmacology 16, 663.

Biegon, A., A. Weizman, L. Karp, A. Ram, S. Tiano and M. Wolff, 1987, Serotonin 5-HT_2 receptor binding on blood platelets; a peripheral maker for depression?, Life Sci. 41, 2485.

Biegon, A., A. Grinspoon, B. Blumenfeld, A. Bleich, A. Apter and R. Mester, 1990, Increased serotonin 5-HT_2 receptor binding on blood plateles of suicidal men, Psychopharmacology 100, 165.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Chaouloff, F., 1993, Physiopharmacological interactions between stress hormones and central serotonergic systems, Brain Res. Rev. 18, 1.

Cheetham, S.C., M.R. Crompton, C.L.E. Katona and R.W. Horton, 1988, Brain 5-HT_2 receptor binding sites in depressed suicide victims, Brain Res. 443, 272.

Compaan, J.C., G. Van Wattum, A.J. De Ruiter, G.A. Van Oortmerssen, J.M. Koolhaas and B. Bohus, 1993, Genetic differences in female house mice in aggressive response to sex steroid hormone treatment, Physiol. Behav. 54, 899.

Cools, A.R., J. Dierx, C. Coenders, D. Heeren, S. Ried, B.G. Jenks and B. Ellenbroek, 1993, Apomorphine-susceptible and apomorphine-unsusceptible Wistar rats differ in novelty-induced changes in hippocampal dynorphin B expression and two-way hippocampal-accumbens axis, Behav. Brain Res. 55, 213.

Darmani, N.A., B.R. Martin and R.A. Glennon, 1992a, Behavioral evidence for different adaptation of the serotonergic system following acute and chronic treatment with (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI) or ketanserin, J. Pharmacol. Exp. Ther. 262, 692.

Darmani, N.A., B.R. Martin and R.A. Glennon, 1992b, Repeated administration of low doses of cocaine enhances the sensitivity of 5-HT_2 receptor function, Pharmacol. Biochem. Behav. 41, 519.

De Souza, R.J., G.M. Goodwin, A.R. Green and D.J. Heal, 1986, Effect of chronic treatment with 5-HT_1 agonist (8-OH-DPAT and RU 24969) and antagonist (ipsapirone) drugs on the behavioural responses of mice to 5-HT_1 and 5-HT_2 agonists, Br. J. Pharmacol. 89, 377.

Edwards, E., J. Johnson, D. Anderson, P. Turano and F.A. Henn, 1986, Neurochemical and behavioral consequences of mild, uncontrollable shock: effects of PCPA, Pharmacol. Biochem. Behav. 25, 415.

Edwards, E., K. Harkins, G. Wright and F.A. Henn, 1991, 5-HT_{1b} receptors in an animal model of depression, Neuropharmacology 30, 101.

Edwards, E., W. Kornrich, P.V. Houtten and F.A. Henn, 1992, Presynaptic serotonin mechanisms in rats subjected to inescapable shock, Neuropharmacology 31, 323.

Greenberg, L., E. Edwards and F.A. Henn, 1989, Dexamethazone supression test in helpless rats, Biol. Psychiatry 26, 530.

Haracz, J.L., T.R. Minor, J.N. Wilkins and E.G. Zimmerman, 1988, Learned helplessness: an experimental model of the DST in rats, Biol. Psychiatry 23, 388.

Javaid, J.I., S.K. Sahni, S.C. Pandey and J.M. Davis, 1993, Repeated cocaine administration does not affect 5-HT receptor subtypes

(5-HT_{1A}, 5-HT_2) in several rat brain regions, Eur. J. Pharmacol. 238, 425.

Kuroda, Y., M. Mikuni, T. Ogawa and K. Takahashi, 1992, Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT_2 receptor binding sites in neocortex of rat forebrain and 5-HT_2 receptor-mediated wet-dog shake behaviors, Pscyhopharmacology 108, 27.

Kusumi, I., T. Koyama and I. Yamashita, 1991, Serotonin-stimulated Ca^{2+} response is increased in the blood plateles of depressive patients, Biol. Psychiatry 30, 310.

Kusumi, I., T. Koyama and I. Yamashita, 1994, Serotonin-induced platelet intracellular calcium mobilization in depressed patients, Psychopharmacology 113, 322.

Lesch, K.P. and B. Lerer, 1991, The 5-HT receptor-G-protein-effector system complex in depression. I. Effect of glucocorticoids, J. Neural. Transm. [GenSect] 84, 3.

Leysen, J.E., P. Van Gompel, M. Verwimp, C.J.E. Niemegeers and P.M. Laduron, 1982, [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Binding properties, brain distribution and functional role, Mol. Pharmacol. 21, 301.

Leysen, J.E., P. Van Gompel, M. Verwimp and C.J.E. Niemegeers, 1983, Role and localization of serotonin 2 (S2)-receptor-binding sites: effect of neuronal lesions, in: CNS Receptors – From Molecular Pharmacology to Behavior, eds. P. Mandel and F.V. DeFeudis (Raven Press, New York) p. 373.

Leysen, J.E., P.F. Janssen and C.J. Niemegeers, 1989, Rapid desensitization and down regulation of 5-HT_2 receptors by DOM treatment, Eur. J. Pharmacol. 163, 145.

Mann, J.J., M. Stanley, P.A. McBride and B.S. McEwen, 1986, Increased serotonin$_2$ and beta-adrenergic receptor binding in the frontal cortices of suicide victims, Arch. Gen. Psychiatry 43, 954.

Martin, J.V., E. Edwards, J.O. Johnson and F.H. Henn, 1990a, Monoamine receptors in an animal model of affective disorder, J. Neurochem. 55, 15.

Martin, P., P. Soubrie and A.J. Pueuch, 1990b, Reversal of helpless behavior by serotonin uptake blocker, Psychopharmacology 101, 403.

McKeith, I.G., E.F. Marshall, I.N. Ferrier, M.M. Armstrong, W.N. Kennedy, R.H. Perry, E.K. Perry and D. Eccleston, 1987, 5-HT receptor binding in post-mortem brain from patients with affective disorder, J. Affect. Disord. 13, 67.

Mikuni, M., A. Kagaya, K. Takahashi and H.Y. Meltzer, 1992, Serotonin but not norepinephrine-induced calcium mobilization of platelets is enhanced in affective disorders, Psychopharmacology 106, 311.

Oliveria, M.P. and M.F. Campos, 1993, Behavioral effects of the intraventricular administration of 5-HT and dopamine in the common marmoset (callithrix jacchus), Pharmacol. Biochem. Behav. 46, 21.

Owen, F., D.R. Chambers, S.J. Cooper, T.J. Crow, J.A. Johnson, R. Lofthouse and M. Poulter, 1986, Serotonergic mechanisms in brains of suicide victims, Brain Res. 362, 185.

Pandey, G.N., S.C. Pandey, P.G. Janicak, R.C. Marks and J.M. Davis, 1990, Platelet serotonin-2 receptor binding sites in depression and suicide, Biol. Psychiatry 28, 215.

Papp, M., V. Klimek and P. Willner, 1994, Effects of imipramine on serotonergic and beta-adrenergic receptor binding in a realistic animal model of depression, Psychopharmacology 114, 309.

Sander-Bush, E., 1990, Adaptive regulation of central serotonin receptors linked to phosphoinositide hydrolysis, Neuropsychopharmacology 3, 411.

Seligman, M.E.P. and S.F. Maier, 1967, Failure to escape traumatic shock, J. Exp. Psychol. 74, 1.

Sherman, A.D., J.L. Sacquitne and F. Petty, 1982, Specificity of the learned helplessness model of depression, Pharmacol. Biochem. Behav. 16, 449.

Van Dijken, H.H., J.A.M. Van Der Heyden, J. Mos and F.J.H. Tilders, 1992, Inescapable footshocks induced progressive and long-lasting behavioural changes in male rats, Physiol. Behav. 51, 787.

Weiss, J.M. and H.I. Glazer, 1975, Effects of acute exposure to stressors on avoidance-escape behavior and on brain norepinephrine, Psychosom. Med. 37, 522.

Wieland, S., M.S. Kreider, P. McGonigle and I. Lucki, 1990, Destruction of the nucleus raphe obscurus and potentiation of serotonin mediated behaviors following administration of the neurotoxin 3-acetylpyridine, Brain Res. 520, 291.

Willner, P., 1984, The validity of animal models of depression, Psychopharmacology 83, 1.