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Behavioral deficits and exaggerated feedback control over raphe-hippocampal serotonin neurotransmission in restrained rats

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Abstract:
Serotonin (5-hydroxytryptamine, 5-HT), acting *via* the hippocampus, is thought to be critical for the neuroadaptation that alleviates the adverse effects of stress on emotion and behavior. It was hypothesized that a decrease in raphe-hippocampal serotonin neurotransmission caused by exaggerated feedback inhibition of 5-HT synthesis and release significantly contributes to stress-induced behavioral deficits. Acute exposure to 2 h of restraint stress increased 5-HT metabolism in the cortex and raphe region but had no such effect in the hippocampus. Exposure to 2 h of restraint stress elicited anxiety-like behavior, which was monitored in the light-dark transition test the next day. Animals sacrificed 24 h after termination of the stress period exhibited a decrease in the concentration of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus but not in the cortex and raphe. 8-Hydroxy-2-di-n-propylaminotetralin (8-OH-DPAT) injected at doses of 0.125, 0.25 and 0.5 mg/kg decreased 5-HT metabolism in the raphe, cortex and hippocampus of restrained and unrestrained animals, and the decreases in the raphe and hippocampus, but not those in the cortex, were greater in restrained than unrestrained animals. Exaggerated feedback control over raphe-hippocampal serotonin neurotransmission may be involved in the inability of the organism to cope with increased stress and elicits behavioral depression.

Key words:
light-dark transition test, 5-HT_{1A} receptor, cortex, hippocampus, raphe, 8-OH-DPAT, feedback control

Introduction

A dysfunctional serotonergic system is a risk factor for major depressive disorders and other forms of affective illness [29]. At least 14 different types and subtypes of serotonin (5-hydroxytryptamine, 5-HT) receptors have been identified [27]. A number of these receptors, such as 5-HT_2 [3, 12], 5-HT_3 [45] and 5-HT_{1A} [51], play a role in the pathogenesis of psychiatric illnesses. The 5-HT_{1A} receptor, which is a key mediator of serotonergic signaling in the central nervous system, is also implicated in the mechanism of action of selective serotonin reuptake inhibitors (SSRIs) [4].

Cell bodies of serotonin-containing neurons are located in the raphe nuclei in the brain stem. The 5-HT_{1A} receptor is widely distributed in regions that receive serotonergic input from the raphe nuclei: the frontal cortex, septum, amygdala, hippocampus and hypothalamus. It also serves as a somatodendritic autoreceptor in the raphe nuclei [57], reducing the firing rate of serotonergic neurons [23, 26].

Accumulating evidence implicates hippocampal serotonin in responses to stress, depression and antidepressant agents [21, 43]. The hippocampus is thought to play a role in responses to stress because acute exposure to a 2 h episode of restraint stress increased 5-HT turnover in the hypothalamus, midbrain and cortex, but such in-

creases did not occur in the hippocampus [24]. Conversely, repeated daily exposure to restraint for 2 h/day, which produced behavioral adaptation, only increased 5-HT turnover in the hippocampus and not in other brain regions. An increase in serotonin neurotransmission *via* the hippocampus may be involved in adaptation to stress [24]. More recent studies have also consistently shown that the hippocampus may mediate adaptation to severe inescapable stressors *via* the facilitation of serotonergic neurotransmission. Acute exposure to an elevated platform enhanced 5-HT overflow in the prefrontal cortex but not the dorsal hippocampus, whereas repeated daily exposure to the same stressor increased extracellular 5-HT in the dorsal hippocampus but not the prefrontal cortex [55]. In another study, rats received inescapable foot shock and were tested in a shuttle box 24 h later. Pre-stressed animals exhibited impairment of escape responses. This effect was prevented by bilateral intra-hippocampal injection of zimelidine, a serotonin reuptake blocker, but not by desipramine, a noradrenaline reuptake blocker [30]. Rats receiving acute restraint stress [41] a variety of chronic unpredictable mild stressors for 3 weeks [38] showed depression-like behavioral changes that were suppressed or blocked by intra-hippocampal injection of 5-HT [38].

These lines of evidence led to the hypothesis that reduced 5-HT neurotransmission in the hippocampus significantly contributes to restraint-induced behavioral deficits. To test this hypothesis, the present study investigates the metabolism of 5-HT in the hippocampus of restrained rats immediately and 24 h after termination of the restraint period. To further explore the mechanism, we determined the effects of 8-hydroxy-2-di-n-propylaminotetralin (8-OH-DPAT), a 5-HT agonist known to modulate the availability of 5-HT at nerve terminals, on the metabolism of 5-HT in the hippocampus, cortex and raphe of restrained rats. 8-OH-DPAT stimulated 5-HT_{1A} and 5-HT_7 receptors [54], but its effects in decreasing 5-HT metabolism were largely produced because of the stimulation of somatodendritic 5-HT receptors [21], which are of the 5-HT_{1A} type [57].

Materials and Methods

Animals

Locally bred male albino Wistar rats weighing 180–220 g were purchased from HEJ Research Institute, Karachi, Pakistan, and were housed individually under a 12-h light and dark cycle (lights on at 6:00 a.m.) with free access to a standard rodent diet and tap water 5 days before starting the experiment. All animal experiments were conducted in accordance with NIH guidelines and approved by the institutional Ethics and Animal Care Committee.

Drugs

(±)-8-OH-DPAT-HBr, purchased from SIGMA, was dissolved in saline and injected subcutaneously at a dose of 0.125, 0.25 and 0.5 mg/kg body weight. Control animals were injected with saline at a volume of 1 ml/kg.

EXPERIMENTAL PROTOCOL

Experiment 1: Effect of 2 h of acute restraint stress on 5-HT metabolism

Twelve rats were randomly assigned to unrestrained and restrained groups of 6 each. The animals assigned to the restrained group were immobilized on wire grids for 2 h (between 09:00 and 11:00 a.m.). The animals assigned to the unrestrained group were left in their home cages during this period. Animals in the restrained group were sacrificed immediately after the termination of the restraint period. Animals in the unrestrained group were also sacrificed at the same time. Brains were removed immediately after decapitation, and the desired regions (hippocampus, raphe and cortex) were dissected out and stored at –70°C for the subsequent estimation of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by high-performance liquid chromatography (HPLC-EC).

Experiment 2: Effects of 2 h of restraint stress on the activity of rats in the light-dark transition test and 5-HT metabolism 24 h after termination of the stress period

Twelve rats were randomly assigned to unrestrained and restrained groups of 6 each. The animals assigned to the restrained group were immobilized on wire grids for 2 h (between 09:00 and 11:00 a.m.). The animals assigned to the unrestrained group were left in their home cages during this period. The behavior of rats (both unrestrained and restrained) in the light-dark transition test was monitored the next day be-

tween 09:00 to 10:00 a.m. (i.e., 22 h after termination of the stress period). Animals were sacrificed between 11:00 and 12:00 a.m. (i.e., 24 h after the termination of stress period and 2 h after conducting the transition test) to collect and store desired brain regions for the later estimation of 5-HT and 5-HIAA.

Experiment 3: Effects of 8-OH-DPAT on 5-HT metabolism in unrestrained and restrained animals

Forty-eight rats were randomly assigned to unrestrained and restrained groups of 24 each. The animals assigned to the restrained group were immobilized on wire grids for 2 h (between 09:00 and 11:00 a.m.). The animals assigned to the unrestrained group were left in their home cages during this period. The effects of 8-OH-DPAT on 5-HT metabolism were monitored the next day. Groups of unrestrained and restrained animals (six animals in each group) received the drug as an injection at doses of 0.125, 0.25 and 0.5 mg/kg. A group of restrained animals and another group of unrestrained animals (six animals in each group) received a saline injection. The injections were made between 9:00 to 11:00 a.m., and animals were sacrificed 1 h after the injection to collect and store desired brain regions for the later estimation of 5-HT and 5-HIAA.

Restraining procedure

The animals were restrained as described previously [24] by taping them to a 10" × 9" wire grid fitted with a 9" × 6.5" Perspex plate. Restraint stress was produced by pressing the forelegs of the rat through the gaps in the metal grid and taping them together with zinc oxide plaster. Hind limbs were also taped, and the head of the animal rested on the Perspex plate.

Light-dark transition test

The test procedure was essentially the same as described earlier [7, 35]. The apparatus used in the present investigation was a two compartment light-dark box. Both the light compartment (made out of transparent plastic) and dark compartment (made out of black plastic) measured 26 × 26 × 26 cm. Access between the compartments was provided by a 12 × 12 cm passageway. The experiment was performed in a quiet, air-conditioned room, and the apparatus was placed under white light. An animal was introduced into the apparatus *via* the light compartment. Cumulative time spent in the light compartment and the number of entries into the light compartment were monitored for a period of 5 min.

Brain dissection technique

The technique used was essentially the same as described earlier [23, 24]. A fresh brain was rinsed with ice cold saline and placed with its dorsal side up in the molded cavity of a brain slicer. A fine-wire fishing line was inserted into the slots of the slicer to obtain 1-mm-thick slices of 1 mm. The slices were transferred to a Petri dish kept on ice, and the hippocampus, cortex and raphe were identified with the aid of a stereotaxic atlas. From the slice containing cortex, the olfactory bulb was discarded. Hippocampal tissue (CA1-4 fields + subiculum + dentate gyrus) was dissected out with a sharp scalpel. Raphe tissue was obtained by punching out discreet (0.5 mm diameter) tissue plugs from slices containing dorsal and dorsal plus median raphe.

Neurochemical analysis

The concentrations of 5-HT and 5-HIAA in brain regions were determined by HPLC-EC as described previously [35]. A 5 μm shim-pack ODS separation column with an internal diameter of 4.5 mm, and a length of 15 cm was used. The mobile phase was 0.1 M phosphate buffer at pH 2.9, containing 14% methanol, 0.023% OSS and 0.005% EDTA. Electrochemical detection was carried out at an operating potential of 0.8 V (glassy carbon electrode *vs.* Ag/AgCl reference electrode).

Statistical analysis

Data on the effects of restraint stress on the levels of 5-HT and 5-HIAA were analyzed by the *t*-test. Effects of restraint on the behavior of rats in the light-dark transition test were also analyzed by the *t*-test. Effects of 8-OH-DPAT on the levels of 5-HT and 5-HIAA in unrestrained and restrained animals were analyzed by two-way ANOVA; p values of less than 0.05 were taken as significant.

Results

The acute effects of 2 h of restraint stress on 5-HT and 5-HIAA concentrations in the cortex, hippocampus and raphe are shown in Figure 1. By the *t*-test, 5-HT as well as 5-HIAA concentrations were significantly increased in the raphe. Only 5-HIAA levels were significantly increased in the cortex, whereas there was no effect on 5-HT or 5-HIAA concentration in the hippocampus.

The effect of 2 h of restraint stress on the behavior of rats in the light-dark transition test, conducted 22 h after the termination of the stress period, is shown in Figure 2. Restrained animals exhibited a large and significant decrease in the number of entries into the light compartment (by the *t*-test). Time spent in the light compartment was also significantly less in restrained than unrestrained animals.

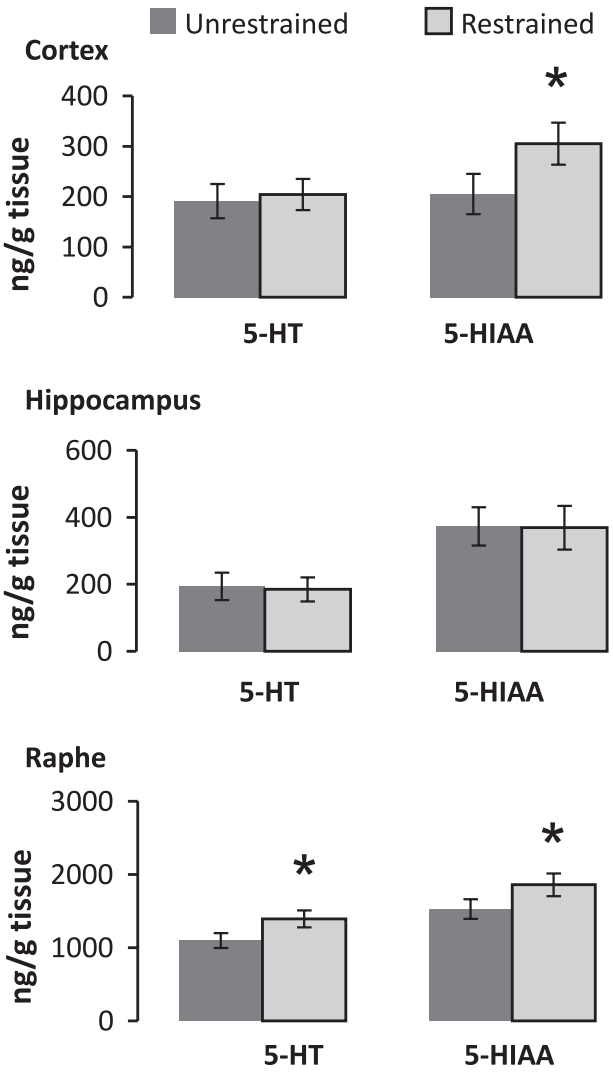

Fig. 1. Effect of 2 h of restraint stress on the levels of 5-HT and 5-HIAA in the cortex, hippocampus and raphe. Values (determined immediately after termination of the stress period) are given as the means ± SD (n = 6). * Significantly different ($p < 0.01$) from unrestrained control animals by the *t*-test

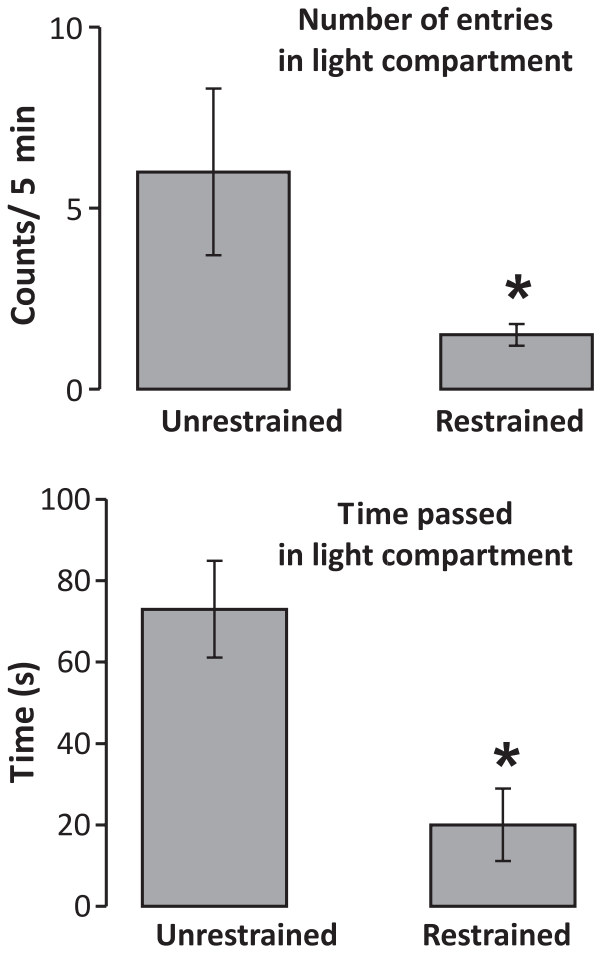

Fig. 2. Effect of 2 h of restraint stress on the behavior of rats in the light-dark transition test. Values (determined 22 h after termination of the stress period) are given as the means ± SD (n = 6). * Significantly different ($p < 0.01$) from unrestrained control animals by the *t*-test

The effects of 2 h of restraint on the metabolism of 5-HT in the cortex, hippocampus and raphe, 24 h after the termination of stress period, are shown in Figure 3. Restrained and unrestrained animals exhibited comparable values of 5-HT and 5-HIAA in the cortex and raphe region. The levels of 5-HT and 5-HIAA were smaller in the hippocampus of restrained than unrestrained animals.

The effects of 8-OH-DPAT at doses of 0.125, 0.25 and 0.5 mg/kg on the levels of 5-HT and 5-HIAA in

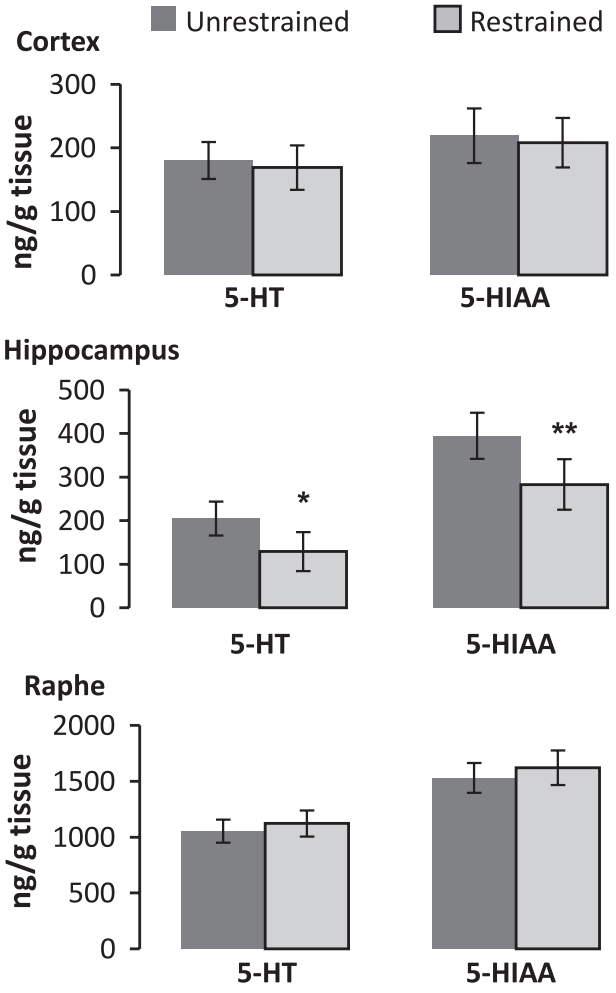

Fig. 3. Effects of 2 h of restraint stress on the levels of 5-HT and 5-HIAA in the cortex, hippocampus and raphe. Values (determined 24 h after termination of the stress period) are given as the means ± SD (n = 6). *, ** Significantly different at $p < 0.05$ (*) and $p < 0.01$ (**) from unrestrained animals by the *t*-test

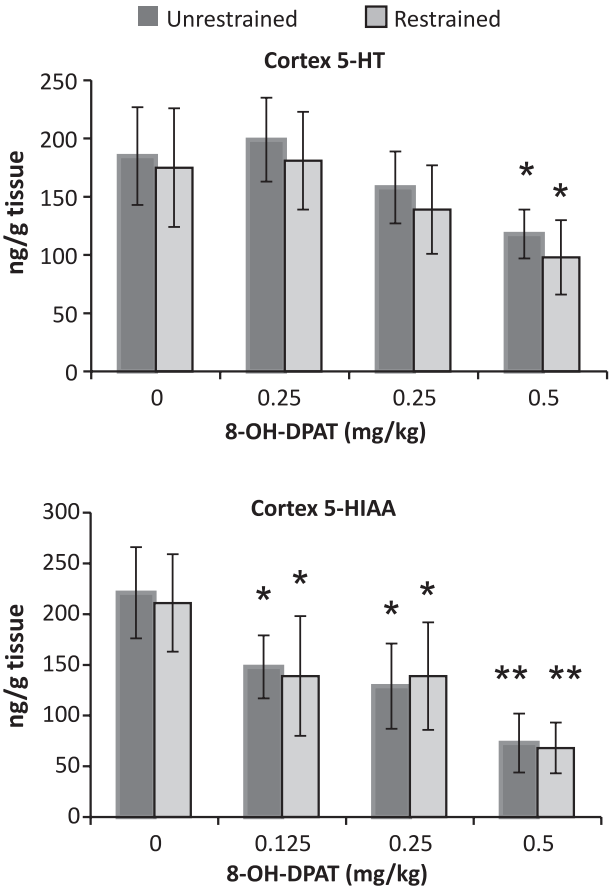

Fig. 4. Effects of 8-OH-DPAT on the levels of 5-HT and 5-HIAA in the cortex of unrestrained and restrained animals. Values (determined 24 h after termination of the stress period and 1 h after the administration of 8-OH-DPAT or saline) are given as the means ± SD (n = 6). *, ** Significantly different at $p < 0.05$ (*) and $p < 0.01$ (**) from the corresponding group of unrestrained or restrained saline-injected animals by two-way ANOVA with the Newman-Keuls *post-hoc* test

the cortex are shown in Figure 4. Statistical analysis by two-way ANOVA showed significant effects of 8-OH-DPAT (df 3,40) on 5-HT ($F = 3.9$ $p < 0.05$) and 5-HIAA ($F = 10.9$ $p < 0.01$) concentrations. Stress effects (df 1,40) on the level of 5-HT ($F = 1.8$ $p > 0.05$) or 5-HIAA ($F = 1.02$ $p > 0.05$) were not significant. The interaction between stress and 8-OH-DPAT (df 3,40) was also not significant for 5-HT ($F = 1.9$ $p > 0.05$) or 5-HIAA ($F = 2.1$ $p > 0.05$). *Post-hoc* testing showed that administration of 8-OH-DPAT at doses of 0.125 and 0.25 mg/kg decreased 5-HIAA levels comparably in restrained and unrestrained animals. Decreases in 5-HT were not significant at these doses of 8-OH-DPAT. Both 5-HT and 5-HIAA levels were decreased following the administration of 8-OH-DPAT at a dose of 0.5 mg/kg, and the decreases were comparable in restrained and unrestrained groups.

Effects of 8-OH-DPAT at doses of 0.125, 0.25 and 0.5 mg/kg on the levels of 5-HT and 5-HIAA in the hippocampus are shown in Figure 5. Statistical analysis by two-way ANOVA showed significant effects of 8-OH-DPAT (df 3,40) on 5-HT ($F = 13.8$ $p < 0.01$) and 5-HIAA ($F = 12.6$ $p < 0.01$) concentrations. Stress effects (df 1,40) on levels of 5-HT ($F = 10.8$ $p < 0.01$) and 5-HIAA ($F = 9.01$ $p < 0.01$) were significant. Interaction between stress and 8-OH-DPAT (df 3,40) was also significant both for 5-HT ($F = 4.1$ $p < 0.05$) and 5-HIAA ($F = 3.2$ $p < 0.05$). *Post-hoc* testing showed that administration of 8-OH-DPAT at a dose of 0.125 mg/kg decreased 5-HT and 5-HIAA concentrations in re-

strained but not in unrestrained animals. At doses of 0.25 and 0.5 mg/kg 5-HT and 5-HIAA were significantly decreased both in unrestrained and restrained animals, and the decreases were more pronounced in restrained than unrestrained animals. The levels of 5-HT and 5-HIAA were smaller in saline-injected restrained than in saline-injected unrestrained animals, and the differences were greater in 8-OH-DPAT-injected restrained rats than in 8-OH-DPAT-injected unrestrained animals.

The effects of 8-OH-DPAT at doses of 0.125, 0.25 and 0.5 mg/kg on the levels of 5-HT and 5-HIAA in the raphe region are shown in Figure 6. Statistical analysis by two-way ANOVA showed significant effects of 8-OH-DPAT (df 3,40) on 5-HT ($F = 8.3$ $p < 0.01$) and 5-HIAA ($F = 9.6$ $p < 0.01$) concentrations. Stress had significant effects (df 1,40) on 5-HT ($F = 7.01$ $p < 0.05$) but not on 5-HIAA levels ($F = 3.2$ $p > 0.05$). Interaction between stress and 8-OH-DPAT (df 3,40) was significant for 5-HT ($F = 2.9$ $p < 0.05$) and 5-

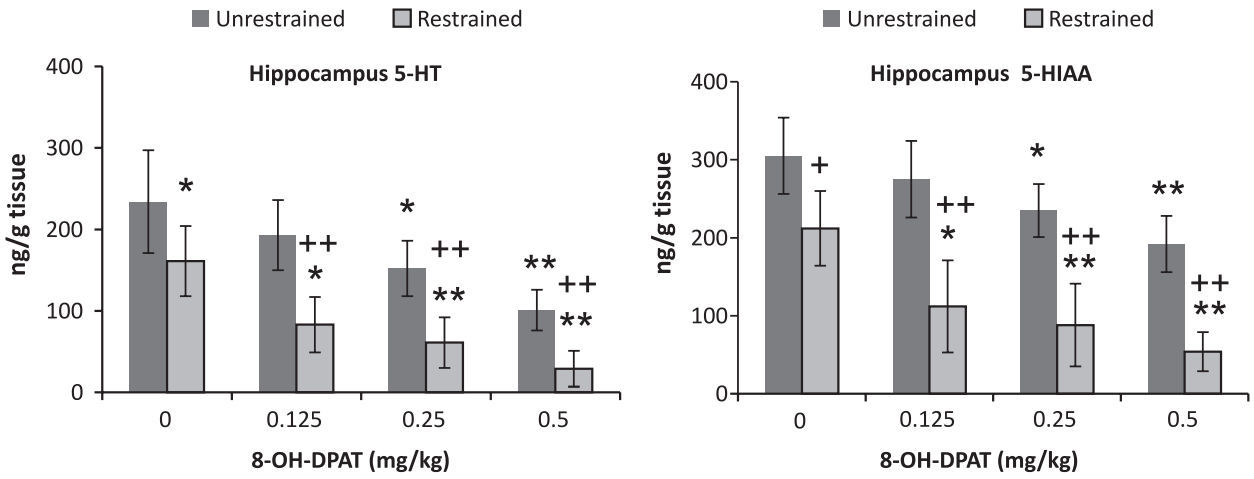

Fig. 5. Effects of 8-OH-DPAT on the levels of 5-HT and 5-HIAA in the hippocampus of unrestrained and restrained animals. Values (determined 24 h after termination of the stress period and 1 h after the administration of 8-OH-DPAT or saline) are given as the means ± SD (n = 6). *, ** Significantly different at $p < 0.05$ and $p < 0.01$ (**) from the corresponding group of unrestrained or restrained saline- injected animals by two-way ANOVA with the Newman-Keuls *post-hoc* test. $^{+}$, $^{++}$ Significance at $p < 0.05$ ($^{+}$) and $p < 0.01$ ($^{++}$) *vs.* the corresponding group of drug-injected unrestrained animals by two-way ANOVA with the Newman-Keuls *post-hoc* test

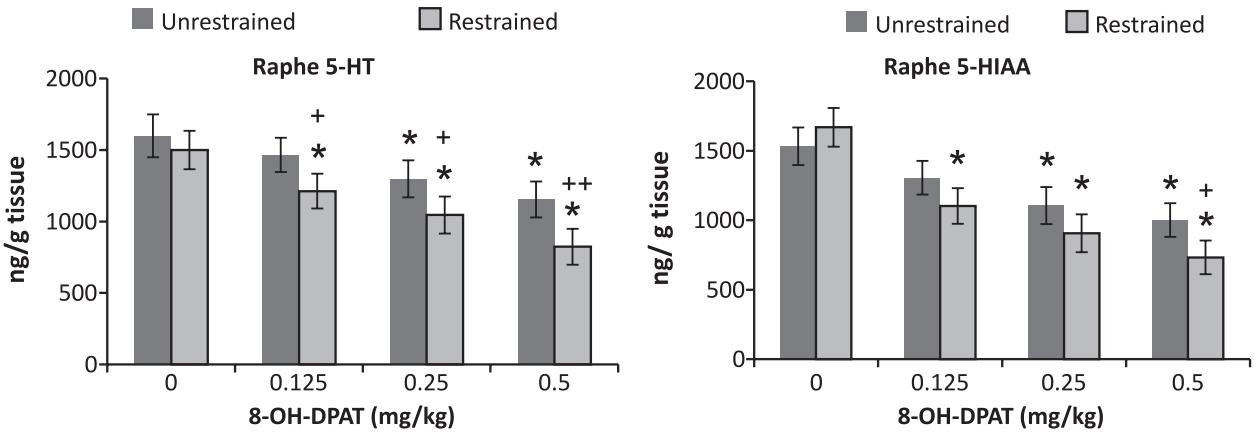

Fig. 6. Effects of 8-OH-DPAT on the levels of 5-HT and 5-HIAA in the raphe of unrestrained and restrained animals. Values (determined 24 h after termination of the stress period and 1 h after the administration of 8-OH-DPAT or saline) are given as the means ± SD (n = 6). * Significantly different ($p < 0.05$) from the corresponding group of unrestrained or restrained saline-injected animals by two-way ANOVA with the Newman-Keuls *post-hoc* test. $^{+}$, $^{++}$ Significantly different at $p < 0.05$ ($^{+}$) and $p < 0.01$ ($^{++}$) from the corresponding group of drug-injected unrestrained animals by two-way ANOVA with the Newman-Keuls *post-hoc* test

HIAA ($F = 3.1$ $p < 0.05$). *Post-hoc* testing showed that administration of 8-OH-DPAT at doses of 0.25 and 0.5 mg/kg decreased 5-HT and 5-HIAA levels in unrestrained and restrained animals and that the decreases in both 5-HT (0.25 and 0.5 mg/kg) and 5-HIAA (0.5 mg/kg) were greater in restrained than unrestrained animals. At a dose of 0.125 mg/kg, 5-HT and 5-HIAA levels were decreased in restrained but not in unrestrained animals. Saline-injected unrestrained rats and saline-injected restrained animals exhibited comparable values of 5-HT and 5-HIAA. The decreases in 5-HT were greater in 8-OH-DPAT-injected restrained rats than in 8-OH-DPAT-injected unrestrained animals. The decreases in 5-HIAA elicited by 0.5 mg/kg 8-OH-DPAT were also greater in restrained than unrestrained animals.

Discussion

The present study shows that rats restrained for 2 h, when subjected to the light-dark transition test 22 h later, exhibited a decrease in time spent in the light compartment (Fig. 2). The number of entries into the light compartment was also decreased. The results suggest that exposure to restraint stress produces behavioral deficits comparable to other uncontrollable stressors.

Based upon the clinical evidence that links stressful life events to depressive episodes [6], several animal models exhibiting stressor controllability and learned helplessness have been developed [40, 49, 58, 59]. The most common animal model of 'stress and coping' is that of 'learned helplessness' [39, 52]. In this model, animals are exposed to either controllable or uncontrollable stressors and later, they are tested in a follow-up task in which all animals are given the opportunity to control the stressor, usually by escape. In most reports, animals that are exposed to uncontrollable stressors do not learn to escape during testing on the new task [46, 52]. This behavior has been equated with a sense of 'giving up' experienced by humans with major depression [44].

The learned helplessness paradigm was not developed to provide an animal model of depression or anxiety but was shown in later studies to be sensitive to both antidepressants [18] and anxiolytics [53]. The paradigm is widely used to study neural mechanisms and the degree of behavioral adaptation in response to an uncontrollable stressor [11, 42, 49, 58].

Animals exposed to other unpredictable and uncontrollable stressors (e.g., restraint stress, elevated platform test and forced swimming) also show coping deficits in aversive but escapable situations [22, 24, 32, 55]. Chronic mild stress also causes behavioral changes in animals that parallel symptoms of depression [15, 38].

Coping deficits following exposure to a stress-inducing situation may also lead to anxiety-like behavior. The light-dark transition test is a biobehavioral test used to monitor the anxiolytic effects of drugs in preclinical investigations [7, 9]. The test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors. Classic anxiolytics and newer anxiolytics (serotonin anxiolytics) can reinstate novelty-induced and stress-induced deficits of behavior in this test [9]. The present results show that exposure to restraint stress induces anxiety-like behavioral deficits that can be seen in the light-dark transition test (Fig. 2).

Other authors have shown that 5-HT turnover is enhanced following exposure to various stressors such as exercise and foot shock, although brain levels of 5-HT are not always altered [10, 14]. It has been also shown that stress-induced increases in brain serotonin are caused by an increase in the availability of tryptophan, the precursor of 5-HT, [10, 33] or an increase in the activity of tryptophan hydroxylase, the rate-limiting enzyme of 5-HT biosynthesis [24]. Microdialysis also showed an increase in extracellular levels of serotonin in different areas of the brain following exposure to different types of stressors [2, 17]. In the present study, rats sacrificed immediately after the termination of the 2 h restraint period exhibited an increase in 5-HT metabolism in the cortex and raphe, but increases of this nature did not occur in the hippocampus (Fig. 1). In addition, the present study shows that rats sacrificed 24 h after termination of the stress period did not show an increase in 5-HT metabolism in the cortex and raphe, whereas 5-HT levels were decreased in the hippocampus (Fig. 3), suggesting that a decrease in 5-HT, particularly in the hippocampus, is involved in restraint-induced behavioral deficits (Fig. 2), which were monitored 22 h after the termination of stress period.

In a previous study, when unrestrained and restrained animals injected with saline or 8-OH-DPAT were sacrificed immediately after monitoring the behavioral and hyperphagic effects of the drug for 4 h, a decrease in 5-HT metabolism was not observed in the hippocampus [50]. The increases in 5-HT metabo-

lism in the hippocampus and other brain regions of restrained animals, as observed in a different experimental paradigm in the previous study [50], are largely explainable as the result of a 2nd exposure to a stress-inducing situation.

It is important to note that serotonin is involved in the pathophysiology of depression as well as anxiety. Preclinical research shows that post-stress facilitation of serotonin neurotransmission in the dorsal hippocampus prevented learned helplessness development [30]. Depression-like behavioral changes induced by chronic mild stressors were also blocked by the intra-hippocampal administration of 5-HT [38], whereas SSRIs that act by increasing the synaptic concentration of 5-HT are the most effective treatment for depression [4].

On the other hand, the hypothesized role of 5-HT in the pathogenesis of anxiety, which stems from observations of 5-HT antagonists in operant models [48], suggests that increasing 5-HT function is anxiogenic. The antianxiety effects of serotonin anxiolytics [34, 35] and benzodiazepines [34, 60] are explained in terms of a decrease in 5-HT neurotransmission in the hippocampus. The decreases of 5-HT observed in the hippocampus (Fig. 3) of rats exhibiting behavioral deficits in the light-dark transition test (Fig. 2) support the notion that the transition test may be used for monitoring stress-induced behavioral depression. The above notion is relevant in that symptoms of anxiety also exist in clinical depression, whereas SSRIs, buspirone and other newer serotonergic agents are effective treatments for both depression and anxiety [1, 3, 8, 36].

To understand the role of the 5-HT_{1A} receptor in restraint-induced decreases in 5-HT, it was hypothesized that somatodendritic 5-HT_{1A} receptors become more responsive in restrained animals and therefore the availability of 5-HT is decreased in terminal regions. The hypothesis was based on evidence from both human and animal studies. Thus, exposure to inescapable but not escapable stressors sensitized serotonergic neurons in the raphe region to subsequent input [40]. Rats exposed to different mild to moderate stressors every day, a procedure that made the daily stress exposure unpredictable, exhibited a significant decrease in 5-HT_{1A} mRNA and 5-HT_{1A} receptor binding in the hippocampus [37]. In contrast, rats adapted to a repetitive restraint stress schedule of 2 h/day for 5 days exhibited a decrease in the sensitivity of somatodendritic 5-HT_{1A} [19] and terminal 5-HT_{1B} [25] receptors, an antidepressant-like effect. The present results show that the effects of 8-OH-DPAT in decreasing 5-HT and 5-HIAA levels in the raphe region were greater in restrained than unrestrained animals (Fig. 6), suggesting an increase in the sensitivity of somatodendritic 5-HT_{1A} receptors that resulted in exaggerated feedback control over 5-HT [20].

The increase in the responsiveness of somatodendritic 5-HT_{1A} receptors would be expected to decrease the availability of 5-HT in terminal regions. The present study shows that 8-OH-DPAT-induced decreases of 5-HT and 5-HIAA, although greater in the hippocampus of restrained than unrestrained animals (Fig. 5), were no greater in the cortex (Fig. 4) of restrained animals. This would suggest that exaggerated feedback control over 5-HT availability *via* somatodendritic 5-HT_{1A} receptors is present in the hippocampus and not in the cortex. It is possible that postsynaptic 5-HT_{1A} receptors also control the synthesis and release of 5-HT *via* a feedback mechanism. The hippocampus is enriched with 5-HT_{1A} receptors and receives serotonergic innervation from the median raphe [5]. Many innervated areas project back to raphe nuclei, and these are interconnected [28]. It is therefore possible that postsynaptic 5-HT_{1A} receptors also alter raphe nucleus 5-HT neuronal firing. It is also possible that the effects are mediated *via* the stress-induced release of corticosteroid hormones. The hippocampus is also enriched with high-affinity mineralocorticoid receptors and lower-affinity glucocorticoid receptors to which corticosteroids bind to alter 5-HT_{1A} receptor mediated responses [31].

In addition to revealing the mechanism involved in the above-reported differences in 5-HT levels in the hippocampus and cortex of restrained animals, the present results are clinically relevant that adult male and female patients with major depression exhibit attenuation of 5-HT_{1A} receptor-mediated neuroendocrine and hypothermic responses, reflecting a decrease in the effectiveness of somatodendritic 5-HT_{1A} receptors [13, 36]. A decrease in 5-HT_{1A} binding potential was initially observed in multiple brain areas, including the raphe region of patients with major depression and bipolar disorder [16, 51]. In another study, patients with major depression who had never received medication were found to have higher 5-HT_{1A} receptor binding compared to depressed patients with a history of medication and control [47], suggesting that medication affected the 5-HT_{1A} binding potential. Higher 5-HT_{1A} binding potential in the raphe and hippocampus in bipolar depressed males, but not in bipolar depressed females, has been also reported [56].

In conclusion, the present study shows that feedback control over raphe-hippocampal serotonin neurotransmission may have important consequences on the ability of an organism to cope with increased stress. It shows that stress-induced exaggerated feedback control, decreasing the availability of 5-HT in the hippocampus, may impair adaptation to and lead to behavioral depression. To elucidate the role of somatodendritic and/or postsynaptic 5-HT_{1A} receptors in the exaggerated feedback control over 5-HT turnover in restrained animals, it may be important to block the 5-HT_{1A} receptor by a selective antagonist and monitor acute and delayed effects of stress exposure on 8-OH-DPAT-induced decreases in 5-HT release using the particular technique of microdialysis.

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References:

1. Adell A: Lu-AA21004, a multimodal serotonergic agent, for the potential treatment of depression and anxiety. Drugs, 2010, 13, 900–910.
2. Adell A, Casanovas JM, Artigas F: Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. Neuropharmacology, 1997, 36, 735–741.
3. Andre K, Kampman O, Setälä-Soikkeli E, Viikki M, Poutanen O, Nuolivirta T, Mononen N et al.: Temperament profiles, *5-HT-2A* genotype, and response to treatment with SSRIs in major depression. J Neural Transm, 2010, 117, 1431–1434.
4. Artigas F, Bel N, Casanovas JM, Romero L: Adaptive changes of the serotonergic system after antidepressant treatments. Adv Exp Med Biol, 1996, 398, 51–59.
5. Azmitia EC, Gannon PJ, Kheck NM, Whitaker-Azmitia PM: Cellular localization of 5-HT_{1A} receptors in primate brain neurons and glial cells. Neuropsychopharmacology, 1996, 14, 35–46.
6. Bale TL: Stress sensitivity and the development of affective disorders. Horm Behav, 2006, 50, 529–533.
7. Bilkei-Gorzó A, Gyertyán I, Lévay G: MCPP-induced anxiety in the light-dark box in rats – a new method for screening anxiolytic activity. Psychopharmacology (Berl), 1998, 136, 291–298.
8. Blier P, Abbott FV: Putative mechanisms of action of antidepressant drugs in affective and anxiety disorder and pain. J Psychiatry Neurosci, 2001, 26, 37–43.
9. Bourin M, Hascoet M: The mouse light-dark box test. Eur J Pharmacol, 2003, 463, 55–65.
10. Chaouloff F, Berton O, Mormede P: Serotonin and stress. Neuropsychopharmacology, 1999, 21, 28S–32S.
11. Chourbaji S, Zacher C, Sanchis-Sequra C, Dormann C, Vollmayr B, Gass P: Learned helplessness: validity and reliability of depressive like states in mice. Brain Res Brain Res Protoc, 2005, 16, 70–78.
12. Christiansen L, Tan Q, Iachina M, Bathum L, Kruse TA, McGue M, Christensen K: Candidate gene polymorphisms in the serotonergic pathway: influence on depression symptomology in an elderly population. Biol Psychiatry, 2007, 61, 223–230.
13. Cowen PJ, Power AC, Anderson IM: 5-HT_{1A} receptor sensitivity in major depression: A neuroendocrine study with buspirone. Br J Psychiatry, 1994, 164, 372–379.
14. Curzon G, Joseph MH, Knott PJ: Effects of immobilization and food deprivation on rat brain tryptophan metabolism. J Neurochem, 1972, 19, 1967–1974.
15. Dalla C, Antoniou K, Drossopoulou G, Xagoraris M, Kokras N, Sfikakis A, Papadopoulou-Daifoti Z: Chronic mild stress: are females more vulnerable? Neuroscience, 2005, 135, 703–714.
16. Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ, Mathis C: Serotonin type-1A receptor imaging in depression. Nucl Med Biol, 2000, 27, 499–507.
17. Fujino K, Yoshitake T, Inoue O, Ibii N, Kehr J, Ishida J, Nohata H, Yamaguchi M: Increased serotonin release in mice frontal cortex and hippocampus induced by acute physiological stressors. Neurosci Lett, 2002, 320, 91–95.
18. Gambarana C, Scheggi S, Tagliamonte A, Pierluigi T, De Montis MG: Animal models for the study of antidepressant activity. Brain Res Protoc, 2001, 7, 11–20.
19. Haleem DJ: Attenuation of 8-OH-DPAT-induced decreases in 5-HT synthesis in brain regions of rats adapted to a repeated stress schedule. Stress, 1999, 3, 123–129.
20. Haleem DJ: Exaggerated feedback control over 5-HT and hyperactivity in a rat model of anorexia nervosa. Appetite, 2009, 52, 44–50.
21. Haleem DJ: Raphe hippocampal serotonin neurotransmission in the sex related differences of adaptation to stress: focus on serotonin-1A receptor. Current Neuropharmacology, 2011, 9, (in press).
22. Haleem DJ, Kennett GA, Curzon G: Adaptation of female rats to stress: shift to male pattern by inhibition of corticosterone synthesis. Brain Res, 1988, 458, 339–347.
23. Haleem DJ, Kennett GA, Curzon G: Hippocampal 5-HT synthesis is greater in females than in males and is more decreased by 5-HT-1A agonist 8-OH-DPAT. J Neural Transm, 1990, 79, 93–101.
24. Haleem DJ, Parveen T: Effects of restraint on rat brain regional 5-HT synthesis rate following adaptation to repeated restraint. NeuroReport, 1994, 5, 1785–1788.
25. Haleem DJ, Saify ZS, Siddiqui S, Batool F, Haleem MA: Pre and post synaptic responses to 1-(1-naphthylpiperazine) following adaptation to stress in rats. Prog Neuropsychopharmacol Biol Psychiatry, 2002, 26, 149–156.
26. Hjorth S, Auerbach SB: Further evidence for the importance of 5-HT-1A autoreceptors in the action of selective serotonin reuptake inhibitors Eur J Pharmacol, 1994, 260, 251–255.
27. Hoyer D, Hannon JP, Martin GR: Molecular pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav, 2002, 71, 533–554.

28. Jacobs BL, Azmitia EC: Structure and function of the brain serotonin system. Physiol Rev, 1992, 72, 165–229.
29. Jans LA, Riedel, WJ, Markus CR, Blokland A: Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry, 2006, 12, 522–543.
30. Joca SR, Zanelati T, Guimaraes FS: Post stress facilitation of serotonergic, but not noradrenergic, neurotransmission in the dorsal hippocampus prevented learned helplessness development in rats. Brain Res, 2006, 1087, 67–74.
31. Joels M: Functional actions of corticosteroids in the hippocampus. Eur J Pharmacol, 2008, 583, 312–321.
32. Kennett GA, Dickinson SL, Curzon G: Enhancement of some 5-HT dependent behavioral responses following repeated immobilization in rats. Brain Res, 1985, 330, 252–263.
33. Kennett GA, Joseph MH: The functional importance of increased brain tryptophan in the serotonergic responses to restraint stress. Neuropharmacology, 1981, 20, 39–43.
34. Khan A. Haleem DJ: Tolerance in the anxiolytic profile following repeated administration of diazepam but not buspirone is associated with a decrease in the responsiveness of postsynaptic 5-HT-1A receptor. Acta Biol Hung, 2007, 58, 354–357.
35. Khan A, Haleem DJ: Responsiveness of 5-HT_{2C} receptors in repeatedly diazepam injected rats: a behavioral and neurochemical study. Pharmacol Rep, 2008, 60, 716–724.
36. Lesch KP: 5-HT_{1A} receptor responsitivity in anxiety disorder and depression. Prog Neuropsychopharmacol Biol Psychiatry, 1991, 15, 723–733.
37. López JF, Chalmers D, Little KY, Watson SJ: Regulation of serotonin$_{1A}$, glucocorticoid and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. Biol Psychiatry, 1998, 43, 547–573.
38. Luo DD, An SC and Zhang X: Involvement of hippocampal serotonin and neuropeptide Y in depression induced by chronic unpredicted mild stress. Brain Res Bull, 2008, 77, 8–12.
39. Maier SF: Learned helplessness and animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry, 1984, 8, 435–446.
40. Maier SF and Watkin LR: Stressor controllability and learned helplessness: The roles of the dorsal raphe nucleus, serotonin, and corticotrophin releasing factor. Neurosci Biobehav Rev, 2005, 29, 829–841.
41. Manchanda RK, Jaggi AS and Singh N: Ameliorative potential of sodium cromoglycate and diethyldithiocarbamic acid in restraint stress-induced behavioral alterations in rats. Pharmacol Rep, 2011, 63, 54–63.
42. McArthur R, Borsini F: Animal models of depression in drug discovery: a historical perspective. Pharmacol Biochem Behav, 2006, 84, 436–452.
43. McEwen BS: The neurobiology of stress: from serendipity to clinical relevance. Brain Res, 2000, 886, 172–189.
44. Miller WR, Seligman ME: Depression and learned helplessness in man. J Abnorm Psychol, 1975, 84, 228–238.
45. Niesler B, Kapeller J, Hammer C, Rappold G: Serotonin type 3 receptor genes: *HTR3A, B, C, D, E*. Pharmacogenomics, 2008, 9, 501–504.
46. Overmier JB, Seligman ME: Effects of inescapable shock upon subsequent escape and avoidance responding. J Comp Physiol Psychol, 1967, 63, 28–33.
47. Parsey RV, Oquenda MA, Ogden RT, Olvet DM, Simpson N, Huang YY, Van Heertum R et al.: Altered serotonin 1A binding in major depression: a [carbonyl-C-11] WAY100635 positron emission tomography study. Biol Psychiatry, 2006, 59, 106–113.
48. Robichaud RC, Slede KL: The effects of p-chlorophenylalanine on experimentally-induced conflict in rats. Life Sci, 1969, 8, 965–969.
49. Rupniak NM: Animal models of depression challenges for a drug development perspective. Behav Pharmacol, 2003, 14, 385–390.
50. Samad N, Batool F, Haleem DJ: Neurochemical and behavioral effects of 8-OH-DPAT following exposure to restraint stress in rats. Pharmacol Rep, 2007, 59, 173–180.
51. Sargent PA, Kjaer KH, Bench CJ, Rabiner AE, Messa C, Meyer J, Gunn RN et al.: Brain serotonin-1A receptor binding measured by positron emission tomography with [^{11}C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry, 2000, 57, 174–180.
52. Seligman ME, Beagley G: Learned helplessness in the rat. J Comp Physiol Psychol, 1975, 88, 534–541.
53. Short KR, Maier SF: Stressor controllability, social interaction and benzodiazepine systems. Pharmacol Biochem Behav, 1993, 45, 827–835.
54. Sprouse J, Reynold L., Li X, Braselton J, Schmidt A: 8-OH-DPAT as a 5-HT-7 agonist: phase shift of the circadian biological clock through increases in cAMP production. Neuropharmacology, 2004, 46, 52–62.
55. Storey JD, Robertson DA, Beattie JE, Reid IC, Mitchell SN, Balfour DJ: Behavioral and neurochemical responses evoked by repeated exposure to an elevated open platform. Behav Brain Res, 2006, 166, 220–229.
56. Sullivan GM, Ogden RT, Oquendo MA, Kumar JS, Simpson N, Huang YY, Mann JJ, Parsey RV: Positron emission tomography quantification of serotonin-1A receptor binding in medication-free bipolar depression. Biol Psychiatry, 2009, 66, 223–230.
57. Verge D, Daval G, Patey A, Gozlan H, Mestikawy E, Hamon M: Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT-1A subtype. Eur J Pharmacol, 1985, 113, 463–464.
58. Vollmayr B, Henn FA: Learned helplessness in the rat: improvements in the validity and reliability. Brain Res Brain Res Protoc, 2001, 8, 1–7.
59. Willner P, Mitchell PJ: The validity of animal model of predisposition to depression. Behav Pharmacol, 2002, 13, 169–188.
60. Wright IK, Upton N, Marsden CA: Effects of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in the ventral hippocampus of rat during behavior on an elevated X-maze. Psychopharmacology (Berl), 1992, 109, 338–346.

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