5-HT$_3$ receptors in the central amygdala mediate the modulation of thymus function in rats

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Abstract: The aim of the present study was to investigate the modulatory role of activated 5-HT$_3$ receptors in the central amygdala (CeA) on mitogen concanavalin A (ConA)-stimulated proliferative response of thymocytes in rats and the underlying neuroendocrine regulation circuits. 1-phenylbiguanide (PBG), a putative selective 5-HT$_3$ receptor agonist, was administered by intraperitoneal (i.p.), bilateral intracerebroventricular (i.c.v.), and bilateral intracentral amygdala (i.c.a.) injection. In addition, thymocytes isolated from untreated rats were incubated with PBG (at a range of concentrations of $1\times10^{-8}$-$1\times10^{-5}$ mol/L) in vitro in the presence and absence of ConA, in order to investigate any direct effect of PBG on the proliferation in vitro. MTT method was applied to demonstrate the effect of PBG on the proliferative response of thymocytes. An immunohistochemical SABC assay was used to describe the expression profiles of c-Fos-positive cells in different brain regions including the CeA, hippocampus, cortex, hypothalamus and periaqueductal gray (PAG) at 1, 2, 4 and 8 h after bilateral single-administration of PBG by i.c.a. (1.0 µg/side). Results showed that PBG ($1\times10^{-8}$-$1\times10^{-5}$ mol/L) had no significant influence on the proliferative responses of the isolated thymocytes in vitro, no matter ConA was present or not. The proliferation of thymocytes stimulated by ConA was not significantly changed when PBG was administered by i.p. (0.5 mg/kg per day, for consecutive 5 d), whereas it was remarkably enhanced after bilateral i.c.v. injection of PBG (10 µg/side per day, for consecutive 5 d). Similarly, when PBG was injected bilaterally by i.c.a. (1.0 µg/side per day, for 1 d or consecutive 3, 5 and 7 d), a significantly enhanced proliferation occurred on the 1st day and continued until reaching its peak on the 5th day before decreasing on the 7th day. All of the promoting effects of PBG on the ConA-stimulated proliferation of thymocytes were reversed by pretreatment with the 5-HT$_3$ receptor antagonist tropisetron (TRP) 5 min prior to the administration of PBG. Interestingly, compared to the treatment with normal saline or TRP + PBG, after a bilateral single-administration of PBG (1.0 µg/side) by i.c.a., the number of c-Fos-positive cells in different brain regions significantly increased at 1 h in the CeA, 1-2 h in the hippocampus, 1-2 h in the cortex, 4 h in the hypothalamus and 8 h in the PAG, respectively, with each maximum response at 1 h in the CeA, 2 h in the hippocampus and cortex, and 4 h in the hypothalamus. Subsequently, the number of cells expressing c-Fos gradually reduced to the minimum at 4 h in the CeA, and at 8 h in the hippocampus, cortex and hypothalamus. In conclusion, the 5-HT$_3$ receptors in the CeA of rats mediate the modulation of thymus function, at least partly, through the neuroendocrine circuit of the limbic system-cortex-hypothalamus-PAG.

Key words: 5-HT$_3$ receptor; 1-phenylbiguanide; central amygdala; thymocyte; c-Fos; neuroimmunomodulation; rat
The thymus gland is a central lymphoid organ in which bone marrow-derived T cell precursors undergo the complex processes of differentiation and maturation. It plays an important role in maintaining the homeostasis of the neuro-endocrine-immune network by mediating crosstalk between the immune, endocrine, and central nervous systems (IS, ES and CNS, respectively). The thymus continuously impacts on the functions of the CNS and ES via the synthesis and release of various hormones and cytokines. Innervation of the thymus is prominently mediated via sympathetic and parasympathetic nerves through a variety of receptors distributing on the surface of thymocytes. Thus, it is clear that the CNS and ES play a crucial role in the modulation of thymus function. It is well established that neuropsychiatric disorders such as stress, anxiety and depression are capable of inducing the apoptosis of thymocytes. There is substantial evidence that the hypothalamus-pituitary-adrenal (HPA) axis and 5-HT3 receptors, along with the sequential expression of c-Fos in the nervous cells beginning in the hippocampus, then the cortex and hypothalamus, finally ending at the periaqueductal gray (PAG). Such phenomena suggest that 5-HT3 receptors in the limbic system may mediate the neuroimmunomodulation, at least partly, through the limbic system-hypothalamus-PAG pathway. Therefore, the present study was designed to investigate the role of 5-HT3 receptors in the central amygdala (CeA) in relation to the proliferative ability of concanavalin A (ConA)-stimulated thymocytes, and to demonstrate the underlying modulatory circuit in rats.

1 MATERIALS AND METHODS

1.1 Animals
Healthy male Sprague-Dawley rats [(220±20) g] were obtained from the Laboratory Animal Centre, Medical College, Southeast University. Before the experimental intervention, all rats were habituated to the new environment for one week after arrival. The animals were housed one per cage in a humidity-controlled (60%-70%) room at 18-20 °C with a 12 h:12 h light/dark cycle (light from 08:00 am to 08:00 pm) and given free access to clean water and a standard diet.

1.2 Chemicals and reagents
1-phenylbiguanide (PBG) was purchased from RBI (USA) and tropisetron (TRP) was donated by Shanghai Institute of Materia Medica, Chinese Academy of Sciences. ConA was purchased from Sigma (USA). All of the chemicals were stored at 4 °C and dissolved in normal saline (NS) at appropriate concentrations prior to use.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA), RPMI 1640 culture medium containing HEPEs and L-glutamine (Gibco BRL, USA), penicillin and streptomycin (Generun, USA), fetal bovine serum (FBS, Hangzhou Sijiqing Biological Engineering Material Co., Ltd., China), SABC kit, DAB kit and 4% paraformaldehyde (Wuhan Boster Bioengineering Co., Ltd., China), dimethylsulfoxide (DMSO, Amresco, USA), as well as 0.02 mol/L PBS buffer, 0.01 mol/L citrate buffer and Hank’s buffer (pH 7.2-7.4, Nanjing Jiancheng Bioenginee-
uring Institute, China) were all commercial products.

1.3 Apparatus
Stoelting 51600 Brain Stereotaxic Apparatus (USA), CM1900 Freezing Microtome (Leica, Germany), Napco 6500 Incubator (USA), Microplate Reader 550 (Bio-Rad, USA), FACSCalibur Flow Cytometry (BD Bioscience, USA), and microsyringe (Shanghai Medical Instruments Factory, China) were used in the study.

1.4 Experimental design and treatment

1.4.1 Thymocyte proliferation test
Eighteen rats were randomly divided into 3 treatment groups (n=6). The animals were administered either NS (control), 0.01% PBG or 0.02% TRP plus 0.02% PBG via intraperitoneal (i.p.) injection (5.0 mL/kg per day, for consecutive 5 d). 0.02% TRP was administered 5 min prior to 0.02% PBG in TRP + PBG treatment rats (Table 1).

An incision was made and two holes were drilled into the skull at stereotaxic coordinates referring to the atlas of Paxinos-Watson (CeA: AP -2.8 mm, RL ±4.4 mm, H 8.0 mm; CV: AP -1.4 mm, RL ±2.0 mm, H 3.4 mm)\(^{13}\). A set of stainless steel guide cannulas (outer diameter 0.78 mm) were embedded in both sides of the brain and fixed with dental cement. An inner cannula (outer diameter 0.38 mm), 0.5 mm longer than the guide cannula, was inserted into the guide cannula and left in place until injections were made. After a week, the conscious rat was carefully injected with the testing agent at a constant velocity (0.1 µL/min) by a microsyringe through the inner cannula. The dose volume in each lateral injection was 1.0 µL in the CV and 0.1 µL in the CeA. The injection position was confirmed by administration of an equal volume of methylene blue at the end of the study followed by histological microscopic observation.

1.6 Thymocyte proliferation

1.6.1 Preparation of thymocyte suspension
The animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) on the day after the last injection. The thymus gland was removed under sterile conditions and ground on a 200-mesh nylon sieve. After washing with Hank’s buffer (4 ºC) twice, the thymocyte suspension was collected by a pipette after forcing the homogenate through fine gauze. The suspension was then centrifuged (1 000 r/min, 5 min) and the deposit was rinsed twice with complete culture medium (pH 7.2-7.4) containing RPMI 1640, 25 mmol/L HEPES, 2 mmol/L L-glutamine, 10% FBS, 100 IU/mL penicillin and 100 IU/mL streptomycin. The final concentration of thymocytes was adjusted to 8×10^6 cells/mL with complete culture medium.

1.6.2 MTT colorimetric assay
An aliquot (100 µL) of thymocyte suspension was added to a 96-well plate together with 100 µL of ConA solution (final concentration of 2.5 µg/mL) or an equal volume of complete culture medium as a ConA-absent control. After incubation for 72 h at 37 ºC in a humidified atmosphere containing 5% CO₂, MTT solution (final concentration of 0.5 mg/mL) was added to the suspension mixture, followed by a 4-hour incubation. 150 µL of DMSO solution (final concentration of 5.0 mg/mL) was then added to the mixture, and vortex mixed for 10 min. Finally, the optical density (OD) was measured at 570 nm by a microplate reader. The index of proliferation stimulated by ConA (SI) was calculated for estimation of proliferative ability of thymocytes using the following equation:

\[
SI = (OD_{\text{ConA-stimulated}} - OD_{\text{ConA-absent}}) \times 100\% / OD_{\text{ConA-absent}}
\]
1.7 c-Fos-positive cell expression
The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) at 1, 2, 4 and 8 h after i.c.a. administration of PBG. The thorax was incised and the ascending aorta was cannulated. After a rapid flush with 200 mL of NS (4 °C), the brain was perfused with 400 mL of 4% paraformaldehyde (4 °C). The brain was removed from the head and submerged in 4% paraformaldehyde for 2-4 h, and then moved into 30% saccharose (4 °C) for dehydration until the brain sank to the bottom of the container. One week later, the brain was sliced (40 µm thick) coronally using a freezing microtome and the expression profiles of c-Fos-positive cells in different brain regions were determined by the SABC method. The number of c-Fos-positive cells per mm² was counted in at least six high power visual fields (100×). For negative control purpose the same streptavidin-biotin technique was used on tissue sections in which 1% BSA in PBS was substituted for the primary antibody. Positive control pictures were provided by Sigma. Data were analysed by the CellQuest software.

1.8 Statistical analysis
All data were presented as mean±SD. Statistical analysis was performed using analysis of variance (ANOVA) or Student’s t test, as appropriate. P<0.05 was considered statistically significant.

2 RESULTS
2.1 Effects of PBG on the proliferation of isolated thymocytes in vitro
PBG at different concentrations (1×10⁻⁸-1×10⁻⁵ mol/L) had no considerable influence on the proliferation of incubated thymocytes in vitro, no matter ConA was present or not (Fig.1).

Fig.1. Effects of PBG on the proliferation of isolated thymocytes in vitro. There were no significant effects of PBG (1×10⁻⁸-1×10⁻⁵ mol/L) on the proliferation of isolated thymocytes, no matter ConA was present or not. n=6.

2.2 Effects of PBG (i.p. or i.c.v.) on the ConA-stimulated proliferation of thymocytes
PBG (0.5 mg/kg per day, i.p., for consecutive 5 d) had no obvious effect on the proliferation of thymocytes stimulated by ConA, whereas PBG (10 µg/side per day, i.c.v., for consecutive 5 d) significantly enhanced the proliferation of thymocytes stimulated by ConA and this enhancing effect was reversed by the pretreatment with TRP (10 µg/side per day, i.c.v., for consecutive 5 d) (Fig.2).

Fig.2. Effects of PBG (0.5 mg/kg per day, i.p., for consecutive 5 d; or 10 µg/side per day, i.c.v., for consecutive 5 d) on the ConA-stimulated proliferation of thymocytes. *P<0.01 compared with the NS group, **P<0.01 compared with the PBG group. n=6.

2.3 Effects of PBG (i.c.a.) on the ConA-stimulated proliferation of thymocytes
PBG (1.0 µg/side per day, i.c.a., for 1 d or consecutive 3, 5 and 7 d) significantly enhanced the proliferation of thymocytes stimulated by ConA which occurred on the 1st day and continued until reaching a peak on the 5th day, followed by a reduction on the 7th day. TRP (1.0 µg/side per day, i.c.a., for 1 d or consecutive 3, 5 and 7 d) pretreatment reversed the enhancing effect (Fig.3).

Fig.3. Effects of PBG (1.0 µg/side per day, i.c.a., for 1 d or consecutive 3, 5 and 7 d) on the ConA-stimulated proliferation of thymocytes. *P<0.05, **P<0.01 compared with the NS group; ***P<0.05 compared with the PBG group. n=6.
2.4 c-Fos expression profiles in different brain regions

2.4.1 Cortex
One to two hours after bilateral injection of PBG (1.0 µg/side, i.c.a.), a large number of c-Fos-positive cells were shown in the cortex, with the peak number reaching at 2 h. Although the expression began to reduce later, there still remained some c-Fos-positive cells at 8 h after the injection of PBG. At the time points of 1, 2, 4 and 8 h, the number of c-Fos-positive cells per mm² in the PBG group was significantly higher than that in the NS group. The effect of PBG was reversed by the pretreatment with TRP (1.0 µg/side, i.c.a.) (Fig.4 and 5).

2.4.2 Hippocampus
One to two hours after bilateral injection of PBG (1.0 µg/side, i.c.a.), a large number of c-Fos-positive cells were shown in the cortex, with the peak number reaching at 2 h. Although the expression began to reduce later, there still remained some c-Fos-positive cells at 8 h after the injection of PBG. At the time points of 1, 2, 4 and 8 h, the number of c-Fos-positive cells per mm² in the PBG group was significantly higher than that in the NS group. The effect of PBG was reversed by the pretreatment with TRP (1.0 µg/side, i.c.a.) (Fig.4 and 5).

![Fig.4: c-Fos expression profiles in the cortex of rat brain after bilateral single-injection of PBG (1.0 µg/side, i.c.a.). *P<0.05 compared with the NS group, #P<0.05 compared with the PBG group. n=8.](image)

![Fig.5: Expression profiles of c-Fos in the cortex of rat brain (immunohistochemical SABC method). Brown dots represent c-Fos-positive cells. The brain tissue for A, B, C or D was taken at 1, 2, 4 or 8 h after bilateral single-injection of PBG (1.0 µg/side, i.c.a.), respectively. The tissue for E and F was taken at 2 h after single-bilateral injections of TRP + PBG or NS only (i.c.a.), respectively. n=8. Scale bar, 50 µm.](image)
side, i.c.a.), there were a large number of c-Fos-positive cells presented in the hippocampus, followed by extensive reduction of the number of c-Fos-positive cells at 4 and 8 h. At 1, 2 and 4 h, the number of c-Fos-positive cells per mm² in the PBG group was significantly higher than that in the NS group. At 8 h there was no significant difference in the number of c-Fos-positive cells between the PBG and NS groups. The effect of PBG was reversed by the pre-treatment with TRP (1.0 µg/side, i.c.a.) (Fig.6 and 7).

2.4.3 CeA, hypothalamus and PAG

After bilateral injection of PBG (1.0 µg/side, i.c.a.), the number of c-Fos-positive-cells significantly increased at 1 h in the CeA, 4 h in the hypothalamus, and at 8 h in the PAG compared with that in the NS group (Table 2). However, there were no significant differences in the number of c-Fos-positive cells between the PBG and NS groups. The effect of PBG was reversed by the pre-treatment with TRP (1.0 µg/side, i.c.a.) (Fig.6 and 7).

![Fig.6. c-Fos expression profiles in the hippocampus of rat brain after bilateral single-injection of PBG (1.0 µg/side, i.c.a.). * P<0.05 compared with the NS group, # P<0.05 compared with the PBG group. n=8.](image)

**Table 2. The number of c-Fos-positive cells in the CeA, hypothalamus and PAG after bilateral injections of NS, PBG or TRP + PBG**

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Time after i.c.a.</th>
<th>NS</th>
<th>PBG</th>
<th>TRP + PBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CeA</td>
<td>1 h</td>
<td>8.75±2.51</td>
<td>77.24±11.75**</td>
<td>18.75±5.12**</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>4 h</td>
<td>41.75±7.27</td>
<td>208.31±10.05**</td>
<td>90.25±12.84**</td>
</tr>
<tr>
<td>PAG</td>
<td>8 h</td>
<td>3.75±0.95</td>
<td>54.75±6.45**</td>
<td>16.10±7.53**</td>
</tr>
</tbody>
</table>

**P<0.01 compared with the NS group, ***P<0.01 compared with the PBG group. n=8.**
there was not a significantly increased number of c-Fos-positive cells at 4 h in the CeA and at 8 h in the hypothalamus compared with that in the NS group. The effect of PBG was reversed by the pretreatment with TRP (1.0 µg/side, i.c.a.).

3 DISCUSSION

In the present study, using MTT method we have demonstrated that i.p. administration (0.5 mg/kg per day, for 5 d) of PBG, a putative selective agonist of 5-HT3 receptors, has no significant influence on the proliferation of thymocytes, no matter they are stimulated by ConA or not. After peripheral administration of PBG this phenomenon is likely to be mediated via a peripheral pathway, rather than a central route involving the 5-HT3 receptors lying in the brain, considering that PBG is unable to penetrate the blood-brain barrier due to insufficient lipid-solubility[16,17]. As we did not further sort thymocytes, one conceivable explanation may be that PBG has different impacts on the proliferation of various subsets of thymocytes. As a consequence, we may have observed an overall lack of effect. Another possible explanation may be that PBG is incapable of penetrating the blood-thymus barrier and it’s unable to subsequently bind with the 5-HT3 receptors in thymocytes. The last but not the least explanation may be that the 5-HT3 receptor is absent on the surface of thymocytes so that there is no pharmacological target for PBG. In order to clarify the feasibility of these possibilities, we performed an experiment using suspension of the isolated thymocytes in vitro. When incubated with PBG at a range of concentrations (1×10⁻⁸-1×10⁻⁵ mol/L), the proliferation of isolated thymocytes was unaffected no matter ConA was present or not. These data suggest that the 5-HT3 receptor is not present on thymocytes in rats. Interestingly, this supposition is supported by a study which employed a RT-PCR method and showed that the mRNA of 5-HT3 receptor is only expressed in pokeweed mitogen (PWM) and ConA-stimulated splenic cells, whereas it is not expressed in resting splenic cells, thymus cells, and peripheral blood lymphocytes in Sprague-Dawley rats and Wistar rats[19].

Using MTT method, we have recently demonstrated that the proliferation of splenic T lymphocytes stimulated by ConA and lipopolysaccharide (LPS) is inhibited when PBG is administered i.p. to rats, and it can be reversed when the 5-HT3 receptor antagonist TRP is injected prior to PBG as a pretreatment (unpublished data). This finding supports a previous study which employed the ³H-TdR incorporation test and showed that the ConA-stimulated proliferation of isolated splenic T lymphocytes of ICR mice tended to be enhanced by PBG[11]. These data not only show that there is a satisfactory agreement between the MTT method and ³H-TdR incorporation test, but also provide direct pharmacological evidence for the existence of 5-HT3 receptors on the surface of splenic T lymphocytes in rats and mice, and are in agreement with similar results reported by Choquet, Khan and Mayniel et al[18-21]. Thus, PBG has entirely different effects on the proliferative responses of the thymocytes maturing in the thymus and splenic T lymphocytes that originally migrate from the thymus. We believe that the lack or presence of the 5-HT3 receptors in these two kinds of immunocytes should account for such a surprising discrepancy. As a result, there are several areas that should be focused on in future research, such as what kind of factors induce, mediate and impact on the mRNA expression of silent 5-HT3 receptor genes in splenic T lymphocytes during their migration from thymus; whether it is just a strategic cell developmental and differential fate or not; and whether this phenomenon occurs uniquely for the 5-HT3 receptor (the only ligand-gated cation channel receptor in the 5-HT receptor family) and thymocytes or it is generally exhibited by other kinds of receptors and immunocytes.

Since the 5-HT3 receptors are absent on the surface of thymocytes, as shown by both in vivo and in vitro experiments, and penetration through the blood-brain barrier by PBG is difficult, it is reasonable to assume that our results reflect the effects mediated by the central 5-HT3 receptors when PBG is administered in the CeA or in the CV, although we cannot rule out the possibility that sufficient PBG leaks from the brain tissue into the peripheral circulation where it may affect thymocytes. In fact, when PBG is injected by i.c.a. (1.0 µg/side per day, for 7 d) or i.c.v. (10 µg/side per day, for 5 d), the proliferation of thymocytes stimulated by ConA is enhanced; however, this enhancement is abolished by the TRP pretreatment. These data suggest that there are some underlying mechanisms that modulate these effects via nervous circuits and endocrine network connecting the 5-HT3 receptor in the CeA with thymus.

As a third messenger, c-Fos is putatively accepted as a representation of cell activity and a tool commonly applied to illustrate nervous conductive pathways[22]. We studied the expression profiles of c-Fos in different brain regions at various intervals (1, 2, 4 and 8 h) after the administration of PBG in the CeA in order to demonstrate potential
nervous circuits. A substantial number of c-Fos-positive cells began to successively emerge and reached maximum at 1 h in the CeA, 1-2 h in the hippocampus, 2 h in the cortex, 4 h in the hypothalamus and 8 h in the PAG after the administration of PBG by i.c.a. Subsequently, the number of cells expressing c-Fos gradually reduced to the minimum at 4 h in the CeA, at 8 h in the hippocampus, cortex and hypothalamus. These increased levels of expression were inhibited when TRP was injected as a pretreatment. Similar to our previous study which demonstrated that c-Fos-positive cells are expressed in the hippocampus, cortex, hypothalamus and PAG after the administration of PBG in the hippocampus, the present study suggests that the signal originating from the activation of 5-HT3 receptors in the CeA can be transferred and/or transmitted by neurons lying in the CeA, hippocampus, cortex, hypothalamus and PAG through ascending and descending projections. It is known that the thymus is dominated by sympathetic nerves and might also be innervated by vagal nerves. Bhatt et al. have proposed that basal amygdaloid kindled seizures in rats can induce functional and pathological changes in the thymus which depend on the role of the sympathetic nervous system, showing a decreased ratio of CD4+ to CD8+ cells and reduced proliferative response of thymocytes to T-cell mitogens. Using fluorescent tracer and HRP in an investigation of efferent vagal neurons in the rat brainstem, Dovas et al. demonstrated that the thymus is innervated by collaterals of recurrent laryngeal nerve fibres. By means of an electrophysiological method, Niijima reported that the thymic branch of the vagus nerve plays a role in the modulation of thymic function, and Ter Horst et al. proposed that one of the major outputs from nucleus tractus solitarius is the ascending projections to forebrain sites including the amygdala and various parts of the hypothalamus (PVN, LHA, ARC, DMN). However, how 5-HT3 receptors in the CeA influence the thymus function through the nervous system remains to be clarified by further research.

According to Fig.3, it is clear that nervous pathways, which generally lead to a rapid and short-term effect, are not the only mechanism by which 5-HT3 receptors in the CeA may impact on the function of the thymus. When PBG was administered by i.c.a. for 1 d or consecutive 3, 5 and 7 d, the enhanced proliferation of thymocytes occurred on the 1st day, and then gradually increased, reaching its peak on the 5th day. Obviously such a delayed enhancement requires neuroendocrinological explanations. Stimulated by the signal originating from the activated 5-HT3 receptors in the CeA, functional changes of neurons in different brain regions may occur and lead to alterations in the release of hormones and peptides. This can then influence thymus function through modulation of the expression of involved genes. Numerous data have proven that thymus function can be modulated by the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. On the other hand, the activity of the central 5-HT system can impact on the function of the LHPA axis through direct (eg. hippocampus and hypothalamus) and indirect (eg. basolateral and central nuclei) circuits, so that it is of high importance to consider that the proliferation of thymocytes is affected, at least to a certain extent, by activated 5-HT3 receptors in the CeA via the LHPA axis, although the underlying regulatory mechanisms are still unclear.

The 5-HT3 receptors are involved in neuropsychiatric disorders via modulating the release of neurotransmitters such as dopamine (DA) in the nucleus acumbens and striatum. Several preclinical studies have shown that the 5-HT3 receptor antagonists are beneficial to alleviate nausea and emesis induced by chemotherapy or radiation therapy in cancer patients. In addition, these antagonists are potential therapeutic agents for the treatment of anxiety, schizophrenia, ethanol addiction and drug abuse. A lot of neuron-behavioral disorders are accompanied with immune compromise. Whether and how 5-HT3 receptors in the limbic system interact with immune compromise through crosstalk with the thymus, a pivotal organ involved in the immune regulation, remains to be further investigated.

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