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Vanillin-induced amelioration of depression-like behaviors in rats by modulating monoamine neurotransmitters in the brain

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ABSTRACT

Olfaction plays an important role in emotions in our daily life. Pleasant odors are known to evoke positive emotions, inducing relaxation and calmness. The beneficial effects of vanillin on depressive model rats were investigated using a combination of behavioral assessments and neurotransmitter measurements. Before and after chronic stress condition (or olfactory bulbectomy), and at the end of vanillin or fluoxetine treatment, body weight, immobility time on the forced swimming test and sucrose consumption in the sucrose consumption test were measured. Changes in these assessments revealed the characteristic phenotypes of depression in rats. Neurotransmitters were measured using ultrahigh-performance liquid chromatography. Our results indicated that vanillin could alleviate depressive symptoms in the rat model of chronic depression via the olfactory pathway. Preliminary analysis of the monoamine neurotransmitters revealed that vanillin elevated both serotonin and dopamine levels in brain tissue. These results provide important mechanistic insights into the protective effect of vanillin against chronic depressive disorder via olfactory pathway. This suggests that vanillin may be a potential pharmacological agent for the treatment of major depressive disorder.

1. Introduction

Major depressive disorder (MDD) is a neuropsychiatric disorder characterized by persistent despondent state accompanied by low self-esteem and a loss of interest or pleasure in normally enjoyable activities (anhedonia). It is a highly prevalent, multifactorial disorder with high disability and mortality rates (Zalsman et al., 2006). The psychosocial difficulties lead to serious physical, mental and socioeconomic consequences, such as impairments in social communication and vocational function. Many sufferers do not live independently. Half of patients attempt suicide at least once and up to 20% ultimately commit suicide (Pompili et al., 2009). Almost all of them need long-term medication and so research into pharmacotherapy of MDD has evolved over the centuries. Some powerful antidepressants have made indelible contributions, such as selective serotonin reuptake inhibitor (SSRIs), tricyclic antidepressant (TCA), and monoamine oxidase (MAO) inhibitors. However, some of them are of variable effectiveness and some exert undesirable side effects (Xu et al., 2005). Therefore, the development of alternative antidepressants is still the ideal aim pursued by researchers. Olfaction is an important function for animals, playing roles in food hunting, sexual behavior, aggression, territorial defense, identification, and among others (Thiessen and Rice, 1976). The olfactory centers include the prepyriform cortex, amygdala, hypothalamus, hippocampus and other limbic system structures (Benignus and Prah, 1982). Interestingly, a number of these regions also play important roles in emotion processing and this overlap explains the high level of functional connectivity between odor and emotions. This has made olfactory stimulation a promising method for mood induction (Zald and Pardo, 1997; Rolls, 2004; Royet et al., 2003). Vanillin, a single molecule, extracted from vanilla beans, is a popular odor used widely in perfume, food and medicine (Ho et al., 2009, 2011). It has even been used by injection, at an effective dose amount, to calm or sedate patients (Abraham et al., 1997). As an odorant, vanillin is generally rated as pleasant and correspondingly evokes

Abbreviations: MDD, major depressive disorder; CUMS, chronic unpredictable mild stress; FST, forced swimming test; SCT, sucrose consumption test; ANOVA, analysis of variance; UPLC, ultrahigh-performance liquid chromatographic technique; 5-HT, serotonin; DA, dopamine; NE, noradrenaline; HPA, hypothalamic–pituitary–adrenal axis

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positive moods (Seubert et al., 2009). This makes vanillin a promising aroma for modulating emotions.

On the basis of the close anatomic and physiological link between olfaction and emotions, we hypothesized that odorants with strong hedonic qualities, such as vanillin, could evoke happiness and modulate emotions in MDD. The aim of the present study was to assess the influence of vanillin on depression-like behaviors in rats. Additionally, to elucidate the possible underlying mechanisms, a quantitative analysis of monoamine neurotransmitters in the brain was performed using ultrahigh-performance liquid chromatography (UPLC).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g) were obtained from the Department of Laboratory Animal Science, Anhui Medical University (Hefei, China). The animals were housed individually under standard colony conditions, with a 12 h light/dark cycle and ad libitum food and water. They were allowed to acclimatize to the colony for at least 7 days before any experimentation. All experimental manipulations were carried out during the light phase of the light/dark cycle.

All experimental procedures were performed in compliance with the Animal Scientific Procedures Act of revised directive of 2010/63/EU on the protection of animals used for scientific purpose and received local ethics committee approval (number: LLSC2013006). All efforts were made to minimize the number of animals used and their suffering.

2.2. Animal model

Two models of depressive disorder were used to assess the effects of vanillin in rats. The chronic depressive disorder model was induced by chronic unpredictable mild stress (CUMS), and the acute depressive disorder model was induced by olfactory bulbectomy which disrupted the olfactory pathway (Jesberger and Richardson, 1988). The experimental protocol is shown in Fig. 1. Here, pre-model is defined as the parameters measured before modeling (CUMS or olfactory bulbectomy) and post-model is defined as the parameters observed after modeling. By analogy, the principle is also applied to pre-CUMS, post-CUMS, pre-operation and post-operation.

2.2.1. CUMS procedure

Stressors were administered once daily between 08:30 and 10:30 h, with the exception of the 24 h duration stressors. The CUMS procedure was revised from the reference (Wu et al., 2007). Stressors consisted of (1) 5 min warm swim at 42 °C; (2) 24 h wet litter;(3) 24 h food deprivation; (4) 90 s tail pinch; (5) 24 h water deprivation; (6) 5 min cold swim at 4 °C, after which they were toweled dry; (7) 24 h cage tilt (cages were tilted to 45° from the horizontal). The stressors were distributed randomly with intervals of at least 7 days. All stressors were administered four times within 4 weeks.

After the procedure, the animals were divided into three groups with 8–10 rats per group: the stress+fluoxetine group; the stress+vanillin aromatherapy group and the stress (untreated) group.

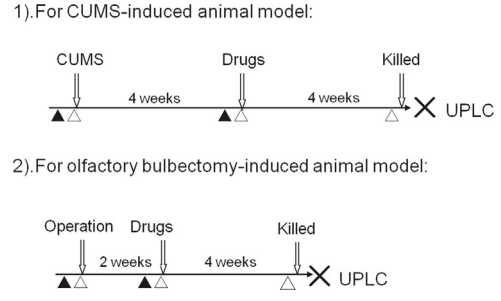

Fig. 1. Overall flow of methodology used for studying the effects of vanillin on: (1) CUMS induced animal model; and (2) olfactory bulbectomy-induced animal model. ▲: Determination of serum corticosterone from tail blood, 1 day ahead of behavioral assessments; △: Assessments of depression-like behaviors at different time points.

2.2.2. Olfactory bulbectomy

In order to investigate the pathway by which vanillin worked, another two groups (10 rats per group) were added: the bulbectomy+vanillin group and the control group (sham). Olfactory bulbectomy was performed as described previously (Jaako-Movits et al., 2006). Animals were deeply anesthetized with 10% chloral hydrate (330 μl/100 g body weight, i.p.). The head hair was shaved and swabbed with antiseptic, after which the animal was placed under a stereotaxic instrument and a midline frontal incision was made in the scalp, with the skin being retracted bilaterally. The surgical procedure involved drilling two burr holes on either side 1 mm from the midline of the frontal bone coving the olfactory bulbs. The bulbs were aspirated. The cavity was packed with surgical foam and the skin was closed with surgical sutures. The animals were allowed to recover by warming to maintain body temperature. Rats in the sham surgery condition received burr holes only. The completeness of olfactory bulb removal was verified upon sacrifice. After surgery the animals were housed individually for 2 weeks of recovery. Before operation and after recovery, the animals underwent stress hormone determination and behavioral assessments to confirm the validity of olfactory bulbectomy-induced depressive disorder model. The bulbectomy+vanillin group was treated as the stress+vanillin group and the control group was treated as the stress group.

2.3. Pharmacological treatment

For the stress+fluoxetine group, the animals were administered a daily oral dose (10 mg/kg/d, diluted in distilled water) of the SSRI fluoxetine (Eli Lilly &Co., Souzhou, China) each morning. For the stress+vanillin group and the bulbectomy+vanillin group, vanillin (Sangon Biotech, Shanghai co., Ltd, China) was administrated in a Plexiglas cylinder 50 cm tall and 35 cm diameter with two layers separated by a porous Plexiglas board. The rat still in its cage was gently placed on the upper layer, and 5 ml of 600 mg/l vanillin (in distilled water) sprayed on to the floor of the lower layer (Atanasova et al., 2012). The odor of vanillin would pervade to the upper layer through the porous board where the rat received vanillin aromatherapy for 1/2h at 8 hourly intervals. Rats in the stress and the control groups received similar handling to the stress+vanillin group, but without any odor administrated.

2.4. Body weight

The effect of the stress and treatment on body weight was measured by electronic balance and recorded at fixed time points. This is a diagnostic criterion for depressive disorder with accompanying weight loss or weight gain (Nestler et al., 2002).

2.5. Tail Blood corticosterone determination

Corticosterone is an important stress hormone in animals and has significant activities as glucocorticoid involved in MDD. When elevated it reveals chronic stress and so provides an endocrinological diagnostic criterion for depressive disorder in animal models. For CUMS-induced depressive disorder model, serum corticosterone was determined before the CUMS procedure and next day after the CUMS procedure. For olfactory bulbectomy-induced depressive disorder model, serum corticosterone was determined before the operation and 14 days after the operation. In both models, blood was drawn from the tail vein of anesthetized rats at 9:30 -11:00 a.m. of the day, which was 1 day ahead of other behavioral assessments. The blood was centrifuged at 3000 rpm for 10 min at 4 °C (Eppendorf, 5810R).The clear serum was extracted(about 0.5 ml) and sent to the endocrinology center of the First Affiliated Hospital of Anhui Medical University where serum corticosterone concentration was determined by chemiluminescence detection (ADVIA centaur immunoassay system, Siemens, German).

2.6. Behavioral assessments

Before and after CUMS (or operation), and at the end of administration, immobility time in the forced swimming test (FST) and the sucrose consumption in the sucrose consumption test (SCT) were measured. Changes in these parameters reflected the behavioral characteristic phenotypes of depression in rats.

2.6.1. FST

The FST is a procedure in which rats are forced to swim for 6 min. This parameter was performed as described previously (Porsolt et al., 1978). The rat was placed individually in a Plexiglas cylinder 50 cm tall, 20 cm diameter filled to 40 ± 1.5 cm with 24 ± 0.5 °C water from which escape was impossible. Fifteen minutes later, the rat was removed and dried before being returned to its home cage. Fresh water was used for each rat and the cylinder was also cleaned between trials. After 24 h, the animals were returned to the cylinders and the procedure was repeated for another 6 min. Immobility time was recorded for 2–6 min by video camera through the side of the cylinder, which was illuminated from the opposite side. An experimenter monitored the video camera unaware of the treatment

received by the animals. Immobility was defined as the lack of motion of the whole body, except for small movements necessary to keep the animal's head above the water. This indicates learned helpless and it is one of the core diagnostic criteria, increasing with the severity of the condition.

2.6.2. SCT

The SCT was described by Papp et al. (1991) as a measure of hedonic behavior. It is one of the core diagnostic criteria for depressive disorder with a decrease in the volume of sucrose consumed indicating a depressed state. Sucrose intake was introduced at weekly intervals during the CUMS procedure. After a 24 h period of water deprivation, the SCT was performed in the home cage. Animals were given a 1% sucrose solution and after 24 h, the consumption of sucrose was measured by weighing the bottles.

2.7. Neurotransmitter analyses

At the end of the experiment, the rats were deeply anesthetized with chloral hydrate and decapitated. Their brains were rapidly removed from the skull. The blood vessels and the soft cerebral covering were removed carefully from the brain before being frozen on dry ice. They were then weighed, homogenized with an Ultrasonic Turax T25 (Bio-block Scientific, France) in 1 × PBS (10 mlPBS/g brain tissue). They were then centrifuged at 10,000 rmp for10 min at 4 °C. The supernatants were kept at −80 °C until quantification of monoamine neurotransmitter levels and the total soluble protein were performed.

The dopamine (DA), norepinephrine (NE) and serotonin (5-HT) levels were determined by UPLC with fluorescence detection (Acquity UPLC BEH C18 1.7 μm, Waters, USA) according to a previously described HPLC procedure for measuring monoamine neurotransmitters (Sa et al., 2012; Zhao et al., 2011)(see Fig. S1 and S2). Unlike the HPLC procedure, the flow rate was set at 0.3 ml/min and the injection volume was 5 μl. The detailed information about the UPLC procedure was well documented in the supplemental data. The linear equations, linear ranges and detection limits of monoamine neurotransmitters were given as Table S1.

The total soluble protein in supernatants was used as the internal reference standard for the quantification of monoamine neurotransmitters. It was detected by improved concentration BCA protein assay kits (Sangon Biotech, Shanghai Co., Ltd, China) (see Table S2).

2.8. Data analysis

Data were expressed as the mean ± S.E.M (standard error of the mean). Data were analyzed using Spss11.5 software using repeated measures of analysis of variance (ANOVA) by post-hoc test for the parameters within the same group and one-way ANOVA followed by least significant difference test for the parameters among the different groups (two-tailed). In all instance, $P<0.05$ was considered statistically significant.

3. Results

3.1. Body weight and serum corticosterone

After stress condition, body weight of the CUMS-induced model groups was decreased without significant difference among groups [$F(2,24)=3.448$, n.s.]. However, body weight of olfactory bulbectomy-induced model groups was still increased slightly after the operation. After 4 weeks of chronic treatment, body weight of all the groups was increased. Among them, body weight of the stress+vanillin, stress+fluoxetine and control groups was increased significantly compared with those of post-model within the group [$F(4,41)=11.15$, $P<0.01$; $F(4,41)=8.514$, $P=0.022$; $F(4,46)=26.783$, $P<0.01$, respectively](see Table 1).

After the CUMS procedure, the serum corticosterone was significantly higher than before [$F(3,89)=14.16$, $P<0.01$]. No similar result was found in the bulbectomy+vanillin and control groups when compared with the pre-model data (see Fig.2).

3.2. Vanillin decreased immobility time in the FST

Immobility time was homogeneous among pre-model animals. After the CUMS procedure (or operation), immobility time was noticeably increased except for the control group. No significant difference was found among the groups after CUMS procedure [$F(2,24)=3.486$, n.s.]. Following antidepressant treatment for 4 weeks, immobility time in the stress+vanillin and stress+fluoxetine groups was significantly decreased [$F(4,42)=34.73$, $P<0.01$; $F(4,42)=13.55$, $P<0.01$, respectively]. However, in the bulbectomy+vanillin group, no similar effect was found (see Table 2).

3.3. Vanillin increased sucrose consumption

Before modeling, sucrose consumption was similar among the groups. However, CUMS procedure significantly decreased sucrose consumption. The bulbectomy+vanillin group was similarly affected. No remarkable difference was found among CUMS-induced model groups [$F(2,24)=2.168$, n.s.]. Treatment with vanillin or fluoxetine remarkably alleviated the decrease in sucrose consumption in CUMS model animals [$F(4,42)=12.32$, $P<0.01$; $F(4,42)=5.65$, $P<0.01$, respectively]. However, vanillin aromatherapy had no effect on sucrose consumption in the bulbectomy+vanillin group with the disrupted olfactory pathway (see Table 3).

3.4. Monoamine neurotransmitters in the rat brain

In this study, monoamine neurotransmitter levels were presented as relative values of the monoamine neurotransmitters and the total protein. In CUMS model rats, 5-HT level in the stress+vanillin and stress+fluoxetine groups was significantly increased when compared with the stress group [$F(4,42)=4.846$, $P=0.030$; $F(4,42)=4.846$, $P=0.036$, respectively], whereas NE in the two groups was elevated but not significantly [$F(4,42)=6.977$, n.s.]. DA was significantly increased in the stress+vanillin group compared with the stress group [$F(4.42)=6.174$, $P=0.041$]. However, for the stress+fluoxetine group, DA was increased but not significantly [$F(4.42)=6.174$, n.s.]. In the bulbectomy+vanillin group, after vanillin aromatherapy for 4 weeks, 5-HT, NE, DA were still dramatically lower than the control group [$F(4,42)=4.846$, $P=0.037$; $F(4,42)=6.977$,$P<0.01$; $F(4,42)=6.174$, $P<0.01$, respectively] (see Table 4).

4. Discussion

The present study was designed to assess the effect of a positive odor (vanillin) on mood in rodents since they share similar olfactory perception with humans (Mandairon et al., 2009). Pleasant olfactory stimuli, such as rose, orange, citrus, lemon and lavender odors, can be experienced as extremely relaxing and calming (Komori et al., 1995a, 1995b; Umezu et al., 2002; Zald, 2003; Almeida et al., 2004; Lehrner et al., 2005; Bradley et al., 2007; Matsukawa et al., 2011). In many countries, aromatherapy is increasingly becoming a beneficial supplement to mainstream treatments for postpartum depression, especially when considering safety of drug treatment (Weier and Beal, 2004; Cavanagh and Wilkinson, 2002). Some researches into complementary therapies for the treatment of depression have revealed that odorant aromatherapy can act on the central nervous system, relieving depression and anxiety, relaxing and restoring both physical and emotional well-being (Motomura et al., 2001; Edge, 2003). It has been verified by fMRI that the limbic structures are involved in both olfactory and emotional processing (Royet et al., 2003; Stöcker et al., 2006). The close relationship between olfaction and emotions is a logical consequence of how both processes share several limbic regions (Zald and Pardo, 1997). In this study, we aimed to validate the hypothesis that vanillin, a pleasant odor, would combat depressive symptoms and modulate emotions in MDD via olfactory pathway.

The CUMS procedure is a classic method for inducing depressive disorder in animals and mimics the stressful events common in human society. A prominent mechanism by which the brain reacts to stress is by activation of the hypothalamic–pituitary–adrenal (HPA) axis. It is also possible that a sign of hyper function of the HPA is peripheral

Table 1
Measurement of body weight at different time points in the groups (unit: g).

Groups	Pre-model	Post-model	2w	3w	4w
Stress+vanillin	226.43 ± 6.06	221.14 ± 13.90	245.86 ± 11.36	270.86 ± 19.57**	293.71 ± 21.07**
Stress+fluoxetine	235.37 ± 5.83	223.63 ± 5.38	262.50 ± 21.37	284.87 ± 23.09*	287.87 ± 28.03*
Stress	235.63 ± 4.04	210.13 ± 6.63	224.37 ± 19.94	239.75 ± 16.73	240.62 ± 16.53
Bulbectomy+vanillin	234.76 ± 10.59	249.50 ± 9.48	257.77 ± 9.98	262.06 ± 18.93	268.19 ± 8.07
control	225.75 ± 5.19	240.18 ± 7.44	262.67 ± 9.04**	266.61 ± 11.18**	275.01 ± 9.61**

* < 0.05 Compared with the post-model within the group.
** < 0.01 Compared with the post-model within the group.

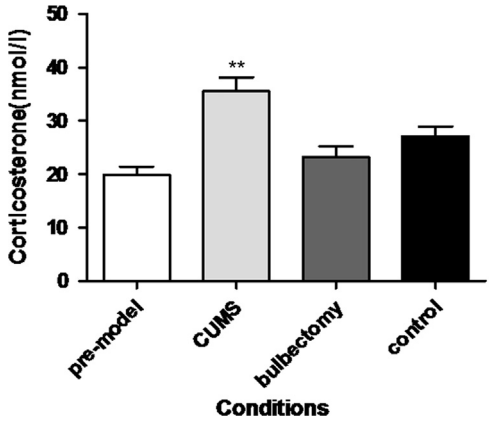

Fig. 2. Changes of serum corticosterone before and after model (unit: nmol/l). Note: The presented data are mean+S.E.M. Statistical analysis was carried out by one way ANOVA. $^{**}P < 0.01$ vs pre-model.

Table 2
Changes of immobility time in the FST at different time points (unit: s).

Groups	Pre-model	Post-model	4w
Stress+vanillin	30.99 ± 6.11	67.07 ± 5.15**	19.56 ± 3.59△△
Stress+fluoxetine	24.57 ± 5.31	59.30 ± 12.30*	18.92 ± 4.30△△
Stress	27.32 ± 5.03	68.11 ± 7.64**	51.00 ± 3.51
Bulbectomy+vanillin	24.70 ± 1.83	98.80 ± 17.78**	94.30 ± 5.33
Control	19.16 ± 1.02	30.85 ± 4.72	17.71 ± 2.38

* < 0.05 Compared with the pre-model within the group;
** < 0.01 Compared with the pre-model within the group;
△△ < 0.01 Compared with the post-model within the group.

glucocorticoid (corticosterone in animals) elevation, which mediates damage to the brain and subsequent behavioral changes (Strome et al., 2002; Nestler,et al., 2002). In this study, serum corticosterone as well as other behavioral parameters was used to assess the animal model. After the CUMS procedure, FST, SCT and the serum corticosterone in particular were significantly different compared with those of pre-CUMS states (Tables 2 and 3; Fig. 2). Body weight was also decreased as expected after CUMS (Table 1). Although there was slight difference among body weight after CUMS procedure, neither significant difference among groups nor obvious influence on other behaviors was found. It was clear that the animal model of depression was established successfully by this procedure. Another documented method for establishing depressive disorder model is olfactory bulbectomy. In our study, the procedure was used to destroy the olfactory pathway and served as a verification to demonstrate that vanillin would be ineffective on the depression-like behaviors of animals without an intact olfactory pathway. Given that the rats would develop more serious syndromes when the olfactory bulbectomy operation was practiced on CUMS model animals, we used normal animals instead. In the bulbectomy+vanillin group and the control group, no significant corticosterone change was found. Similar result was also reported in a previous study (Jaako-Movits,et al.,2006). By comparing the behavioral parameters within groups (post-operation vs pre-operation) and between groups (the bulbectomy+vanillin group vs the control group), we confirmed that the olfactory bulbectomy-induced depressive model was established (Tables 2 and 3). This was also confirmed by the subsequent neurotransmitter analyses (Table 4). In this study, the two methods were supplementary to each other.

Table 3
Change of sucrose consumption in SCT at different time points (unit: g).

Groups	Pre-model	Post-model	4w
Stress+vanillin	88.87 ± 7.89	55.00 ± 7.82*	93.90 ± 4.84△△
Stress+fluoxetine	83.13 ± 7.28	59.72 ± 6.22*	88.50 ± 6.12△△
Stress	91.75 ± 6.26	49.17 ± 6.32*	60.50 ± 4.12
Bulbectomy+vanillin	93.25 ± 5.71	46.63 ± 7.95**	45.00 ± 5.05
Control	94.50 ± 5.09	78.33 ± 10.27	75.37 ± 8.36

* < 0.05 Compared with the pre-model within the group
** < 0.01 Compared with the pre-model within the group.
△△ < 0.01 Compared with the post-model within the group.

Our data show that the body weight of all groups was increased during chronic treatment with the increase in the stress+vanillin group being the largest (Table 1). Even so, we need to be cautious in interpreting this as a sign of improvement, because vanillin is a kind of milky odorant that may stimulate the appetite of rats.

Immobility time in the FST is an index of behavioral despair and antidepressant drugs are able to reduce the time spent in this posture (Dalvi and Lucki, 1999; Cryan et al., 2002). Its predictive validity is so high that if a treatment reduces immobility time, it suggests that it has an antidepressive effect (Cryan et al., 2002). Another essential parameter is consumption of sucrose in the SCT. A lower level of sucrose consumption or sucrose preference in stressed animals has been interpreted as a marker of anhedonia, which is a core symptom of depression when interest in pleasurable and rewarding experiences is lost (Willner et al., 1992; Muscat et al., 1990). After chronic treatment, both immobility time and sucrose consumption were reversed to normal in the stress+vanillin group and stress+fluoxetine group. However, no obvious improvement was found in the bulbectomy+vanillin group. Previous research has reported that depression-like behaviors of bulbectomized rats not exposed to active treatment during the 4–6 weeks after surgery remained unimproved (Megan et al., 2007). It seems that an intact olfactory pathway is necessary for the anti-depressant effect of vanillin.

The mechanisms of behavioral variation induced by odors involves the expression of cholecystokinin mRNA in the mesocorticolimbic pathway and amygdala activation (Hebb et al., 2002; Zald and Pardo, 1997). In this study, monoamine neurotransmitters were analyzed preliminarily since they may be the main pathological mechanisms in the occurrence and development of MDD. Depletion of monoamine neurotransmitters leads to depressive disorder, whereas almost every compound synthesized for the

Table 4
Relative values between monoamine neurotransmitters (ng/ml) and the total soluble protein (ug/ml) in the brain.

Neuro-transmitters	Stress+vanillin	Stress+fluoxetine	Stress	Bulbectomy+vanillin	Control
5-H($\times 10^{-4}$)	0.138 ± 0.013*	0.138 ± 0.011*	0.102 ± 0.007	$0.070 \pm 0.006^{\triangle}$	0.122 ± 0.021
NE($\times 10^{-3}$)	2.08 ± 0.23	2.07 ± 0.22	1.77 ± 0.16	$0.58 \pm 0.10^{\triangle\triangle}$	2.02 ± 0.27
DA($\times 10^{-3}$)	0.81 ± 0.05*	0.77 ± 0.05	0.66 ± 0.04	$0.50 \pm 0.10^{\triangle\triangle}$	1.00 ± 0.12

* <0.05 Compared with the stress group.
$^{\triangle}$ <0.05 Compared with the control group.
$^{\triangle\triangle}$ <0.01 Compared with the control group.

purpose of increasing monoamine neurotransmitter levels has proven to be a clinically effective antidepressant (Belmaker and Agam, 2008). In this study, 5-HT and DA in brain tissue homogenate were found elevated in the stress+vanillin group (Table 4). This result was in line with the anti-stress effect observed with lemon oil modulating 5-HT and DA activity (Komiya et al., 2006). However, no similar result was found in the bulbectomy+vanillin group after 4 weeks of vanillin aromatherapy.

The data from the present study have revealed that depression-like behaviors can be reversed by chronic vanillin aromatherapy. One possible assumption is that the volatile compound can act pharmacologically by entering in the blood stream by way of the nasal or lung mucosa. This effect depends on the concentration of the compound (Herz, 2009). However, in our study, it is difficult to meet the requirement with current intensity of vanillin. The other possibility is by the olfactory pathway. Buck and Axel (1991) have interpreted the basic operating principles of olfaction. They demonstrated that an odorant would bind to the receptor of an olfactory sensory neuron (OSN) and induce an action potential. It is the nerve impulse evoked rather than the odorant itself that is transmitted to the olfactory projection centers. Moreover, odorant signaling cascade is produced during the transduction of neural signal so that odorant can be detected at minute concentrations (Bozza et al., 2002). After considering comprehensively, we would like to prefer the later.

The olfactory nerve is the only cranial nerve connected to the tenecephanon. The projection centers are extensive and most of them is shared with emotional centers. So, the olfaction may be a convenient, safe pathway for treating MDD. As far as odor concentration is concerned, the medium intensity of vanillin used is appropriate since it can be perceived by depressive patients for whom the threshold concentration of pleasant odors is elevated while hedonic valence remains unchanged (Pause et al., 2001). Olfactory perception has another phenomenon known as the adaptation effect. Here, to avoid olfactory adaptation during vanillin aromatherapy, a protocol of repeated exposes was adopted.

What these findings seem to highlight is that odorants with positive hedonic qualities may serve as potential antidepressants via olfactory pathway. The underlying mechanisms may be related to the modulation of monoamine neurotransmitters in brain. Certainly, there were some limitations in determination of monoamine neurotransmitters in whole brain. In further studies, monoamines levels will be examined in some brain regions of interest: the amygdala, the hippocampus, the prefrontal cortex and others involved in emotional processing as well as olfaction function.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2014.11.056.

References

Abraham, D.J., Harris, L.S., Meade, B.J., Munson, A.E., Swerdlow, P.S., Patrick, G.A., 1997. US patent 5668182—method of calming or sedating an animal with a hydroxyl benzaldehyde compound. ⟨http://www.patentstorm.us/patents/5668182/description.html⟩ (accessed on 27.03.2010).
Almeida, R.N., Motta, S.C., Brito, F.C., Catallani, B., Leite, J.R, 2004. Anxiolytic like effects of rose oil inhalation on the elevated plus maze test in rats. Pharmacol. Biochem. Behav. 77, 361–364.
Belmaker, R.H., Agam, G., 2008. Major depressive disorder. New Engl. J. Med. 358, 55–68.
Benignus, V.A., Prah, J.D., 1982. Olfaction: anatomy, physiology and behavior. Environ. Health Perspect. 44, 15–21.
Bozza, T., Feinstein, P., Zheng, C., Mombaerts, P., 2002. Odorant receptor expression defines functional units in the mouse olfactory system. J. Neurosci. 22, 3033–3043.
Bradley, B.F., Starkey, N.J., Brown, S.L., Lea, R.W., 2007. Anxiolytic effects of Lavandula angustifolia odour on the Mongolian gerbil elevated plus maze. J. Ethnopharmacol. 111, 517–525.
Buck, L., Axel, R., 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65, 175–187.
Cavanagh, H.M.A., Wilkinson, J.M., 2002. Biological activities of lavender essential oil. Phytother. Res. 16, 301–308.
Cryan, J.F., Markou, A., Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol. Sci. 23, 238–245.
Dalvi, A., Lucki, I., 1999. Murine models of depression. Psychopharmacology 147, 14–16.
Edge, J., 2003. A pilot study addressing the effect of aromatherapy massage on mood, anxiety and relaxation in adult mental health. Complement. Ther. Nurs. Midwifery 9, 90–97.
Hebb, A.L.O., Zacharko, R.M., Dominguez, H., Trudel, F., Laforest, S., Drolet, G., 2002. Odor-induced variation in anxiety-like behavior in mice is associated with discrete and differential effects on mesocorticolimbic cholecystokinin mRNA expression. Neuropsychopharmacology 27, 744–755.
Herz, R., 2009. Aromatherapy facts and fictions: a scientific analysis of olfactory effects on mood, physiology and behaviour. Int. J. Neurosci. 119, 263–290.
Ho, K., Yazan, L.S., Ismail, N., Ismail, M., 2009. Apoptosis and cell cycle arrest of human colorectal cancer cell line HT-29 induced by vanillin. Cancer Epidemiol. 33, 155–160.
Ho, K., Yazan, L.S., Ismail, N., Ismail, M., 2011. Toxicology study of vanillin on rats via oral and intra-peritoneal administration. Food Chem. Toxicol. 49, 25–30.
Jaako-Movits, K., Zharkovsky, T., Pedersen, M., Zharkovsky, A., 2006. Decreased hippocampal neurogenesis following olfactory bulbectomy is reversed by repeated citalopram administration. Cell. Mol. Neurobiol. 26, 1557–1568.
Jesberger, J.A., Richardson, J.S., 1988. Brain output dysregulation induced by olfactory bulbectomy: an approximation in the rat of major depressive disorder in humans? Int. J. Neurosci. 38, 241–265.
Komiya, M., Takachi, T., Harada, E., 2006. Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. Behav. Brain Res. 172, 240–249.
Komori, T., Fujiwara, R., Tanida, M., Nomura, J., 1995a. Effects of citrus fragrance on immune function and depressive state. Neuroimunomodulat 2, 174–180.

Komori, T., Fujiwara, R., Tanida, M., Nomura, J., 1995b. Potential antidepressant effects of lemon odor in rats. Eur. Neuropsychopharmacol. 5, 477–480.
Lehrner, J., Marwinski, G., Lehr, S., Johren, P., Deecke, L., 2005. Ambient odor of orange and lavender reduce anxiety and improve mood in a dental office. Physiol. Behav. 86, 92–95.
Mandairon, N., Poncelet, J., Bensafi, M., Didier, A., 2009. Humans and mice express similar olfactory preferences. PLoS One 4, e4209.
Matsukawa, M., Imada, M., Murakami, T., Aizawa, S., Sato, T., 2011. Rose odor can innately counteract predator odor. Brain Res. 1381, 117–123.
Megan, E.B., Lucianne, G., Ronald, S.O., Herman, G.M.W., Berend, O., 2007. Long-term behavioral changes after cessation of chronic antidepressant treatment in olfactory bulbectomized rats. Biol. Psychiatry 61, 990–995.
Motomura, N., Sakurai, A., Yotsuya, Y., 2001. Reduction of mental stress with lavender odorant. Percep. Motor Skills 93, 713–718.
Muscat, R., Sampson, D., Willner, P., 1990. Dopaminergic mechanism of imipramine action in an animal model of depression. Biol. Psychiatry 28, 223–230.
Nestler, E.J., Barrot, M., Dileone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M., 2002. Neurobiology of depression. Neuron 34, 13–25.
Papp, M., Willner, P., Muscat, R., 1991. An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology 104, 255–259.
Pause, B.M., Miranda, A., Göder, R., Aldenhoff, J.B., Ferstl, R., 2001. Reduced olfactory performance in patients with major depression. J. Psychiatr. Res. 35, 271–277.
Pompili, M., Rihmer, Z., Innamorati, M., Lester, D., Girardi, P., Tatarelli, R., 2009. Assessment and treatment of suicide risk in bipolar disorders. Expert Rev. Neurother. 9, 109–136.
Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1978. Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47, 379–391.
Rolls, E.T., 2004. Convergence of sensory systems in the orbitofrontal cortex in primates and brain design for emotion. Anat. Rec. A: Discov. Mol. Cell. Evol. Biol. 281, 1212–1225.
Royet, J.P., Plailly, J., Delon-Martin, C., Kareken, D.A., Segebarth, C., 2003. fMRI of emotional responses to odors: influence of hedonic valence and judgment, handedness, and gender. Neuroimage 20, 713–728.
Sa, M., Ying, L., Tang, A.G., Xiao, L.D., Ren, Y.P., 2012. Simultaneous determination of tyrosine, tryptophan and 5-hydroxytryptamine in serum of MDD patients by high performance liquid chromatography with fluorescence detection. Clin. Chim. Acta 413, 973–977.
Seubert, J., Rea, A.F., Loughead, J., Habel, U., 2009. Mood induction with olfactory stimuli reveals differential affective responses in males and females. Chem. Senses 34, 77–84.
Stöcker, T., Kellermann, T., Schneider, F., Habel, U., Amunts, K., Pieperhoff, P., Zilles, K., Shah, N.J., 2006. Dependence of amygdala activation on echo time: results from olfactory fMRI experiments. Neuroimage 30, 151–159.
Strome, E.M., Wheler, G.H., Higley, J.D., Loriaux, D.L., Suomi, S.J., Doudet, D.J., 2002. Intracerebroventricular corticotropin-releasing factor increases limbic glucose metabolism and has social context-dependent behavioral effects in nonhuman primates. Proc. Natl. Acad. Sci. 99, 15749–15754.
Thiessen, D., Rice, M., 1976. Mammalian scent gland marking and social behavior. Psychol. Bull. 83, 505–539.
Umezu, T., Ito, H., Nagano, K., Yamakoshi, M., Oouchi, H., Sakaniwa, M., Morita, M., 2002. Anticonflict effects of rose oil and identification of its active constituents. Life Sci. 72, 91–102.
Weier, K.M., Beal, M.W., 2004. Complementary therapies as adjuncts in the treatment of postpartum depression. J. Midwifery Women's Health 49, 96–104.
Willner, P., Muscat, R., Papp, M., 1992. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci. Biobehav. Re.v 16, 525–534.
Wu, L.M., Han, H., Wang, Q.N., Hou, H.L., Tong, H., Yan, X.B., Zhou, J.N., 2007. Mifepristone repairs region-dependent alteration of synapsin I in hippocampus in rat model of depression. Neuropsychopharmacology 32, 2500–2510.
Xu, Y., Ku, B.S., Yao, H.Y., Lin, Y.H., Ma, X., Zhang, Y.H., Li, X.J., 2005. Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. Pharmacol. Biochem. Behav. 82, 200–206.
Zald, D.H., Pardo, J.V., 1997. Emotion, olfaction, and the human amygdala: amygdale activation during aversive olfactory stimulation. Proc. Natl. Acad. Sci. USA 94, 4119–4124.
Zald, D.H., 2003. The human amygdala and the emotional evaluation of sensory stimuli. Brain Res. Rev. 41, 88–123.
Zalsman, G., Braun, M., Arendt, M., Grunebaum, M.F., Sher, L., Burke, A.K., Brent, D. A., Chaudhury, S.R., Mann, J.J., Oquendo, M.A., 2006. A comparison of the medical lethality of suicide attempts in bipolar and major depressive disorders. Bipolar Disord. 8, 558–565.
Zhao, Y.Y., Liu, L.Y., Han, Y.Y., Bai, J., Du, G.L., Gao, Q., 2011. Simultaneous detection of 8 monoamine neurotransmitters in the different sections of mouse brains by high performance liquid chromatography with fluorescence detection. Chin J Chromatogr. 29, 146–151.