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Venlafaxine plus melatonin ameliorate reserpine-induced depression-like behavior in zebrafish

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ABSTRACT

Venlafaxine (VEN) is one of the first clinical drugs for the treatment of depression. Long-term use may cause a potentially life-threatening serotonin syndrome. Melatonin (MT) could ameliorate depression behavior. Therefore, the aim of this study was to investigate the antidepressant effects of venlafaxine in combination with melatonin on zebrafish. Reserpine was used to induce depression-like behavioral zebrafish. To explore the effects of combined use of venlafaxine and melatonin on depression-like zebrafish induced by reserpine. We tested the depressive behavior of adult zebrafish through a novel tank test, and evaluated the levels of serotonin (5-HT), dopamine (DA) and noradrenaline (NA) in zebrafish brain using enzyme-linked immunosorbent assay (ELISA), besides that the gene expression of serotonin transporters a (*serta*), dopamine transporters (*dat*) and norepinephrine transporters (*net*), vesicular monoamine transporter2 (*vmat2*) and monoamine oxidase (*mao*) were evaluated by qRT-PCR. The results showed that, compared with reserpine-only group, venlafaxine (VEN, 0.025 mg/L) and melatonin (MT, 1 μM) increased the parameters of exploration in the top of the tank and decreased freezing behavior significantly. Compared with reserpine-only group, the use of VEN combined with MT increased serotonin and norepinephrine levels significantly, while there was no obvious difference in dopamine content. The results of qRT-PCR showed that the use of VEN combined with MT significantly reduced the expression of **serta** and promoted the expression of *vmat2*, but had no significant effect on the expression of *net*, *dat* and *mao*. The results indicated that venlafaxine combined with melatonin showed more effective role to remedy the depressive symptoms in zebrafish, providing a reference for the clinical application of antidepressants.

1. Introduction

Depression is a common psychological disorder characterized by low mood, slow thinking activity, and reduced language action. Pathological changes mainly involve central nervous system function, immune function and neuroendocrine function (Haenisch and Bonisch, 2011; Hirschfeld, 2014). The exact pathogenesis of depression is still unclear. At present, there are mainly neurobiological controversies such as the monoamine hypothesis, the neurotransmitter receptor hypothesis, the post-receptor hypothesis, the neuroendocrine function change hypothesis, and the immune system abnormal hypothesis. It is now widely accepted that serotonin, norepinephrine, dopamine, GABA, etc. may be involved in neurobiochemical mechanisms (Torres et al., 2003). More studies have supported the reduction of monoamine neurotransmitter function in the central nervous system as its main pathological changes, so in order to achieve the purpose of treatment, various antidepressants have increased the concentration of monoamine neurotransmitters in the synaptic interstitial space of neurons through different pathways. Venlafaxine (Effexor) is a selective serotonin and norepinephrine reuptake inhibitor (SNRI) for the treatment of human depression(Ilyas and Moncrieff, 2012).The mechanism of SNRIs is similar to that of selective serotonin reuptake inhibitors (SSRIs), the main difference being that SNRI not only inhibits norepinephrine reuptake presynaptic receptors but also inhibits serotonin reuptake presynaptic receptors(Mariappan et al., 2007; Rampono et al., 2009). However, when treated with venlafaxine hydrochloride sustained-release tablets, a potentially life-threatening serotonin syndrome may occur, manifested as changes in mental status (e.g., agonism,

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hallucinations, coma), autonomic nervous system disorders (e.g., tachycardia, changes in blood pressure, overheating), neuromuscular disorders (e.g., hyperreflexia, dysmotility) and/or gastrointestinal symptoms (eg, nausea, vomiting, diarrhea) (Boyer and Shannon, 2005; Evans, 2007).

Melatonin is mainly produced in the pineal organs and retina, and is a key signal of the circadian rhythm clock, which is related to many functions(Falcon et al., 2007). In addition, due to its ability to detoxify free radicals and related oxygen derivatives, melatonin affects the molecular physiology of cells and helps to improve cells and physiology (Reiter et al., 2010). Preclinical studies have shown that melatonin has antidepressant-like properties. Administration of chronic melatonin to rats improved dendritic stability and increased the number of hippocampal pyramidal cells, suggesting that melatonin has a positive protective effect on the hippocampus (Cheng et al., 2008; Prieto-Gomez et al., 2008). The hippocampus plays an important role in the response to stress, especially in the adjustment of the repeated stress experience. (Esteban et al., 2010) found that melatonin exerts long-term effects on serotonin, dopamine and norepinephrine neurotransmission by enhancing monoamine synthesis in the hippocampus of aged rats, which may improve cognitive and the age-dependent motor coordination defect. (Hill et al., 2003) found that melatonin caused an antidepressant-like effect in forced swimming tests by reducing immobility.

Monoamine (DA, NA, 5-HT) content depends on monoamine transporter (DAT, NET, SERTa) reuptake, synaptic release, monoamine oxidase (MAO) and catechol-*O*-methyl transfer Enzyme (COMT) degradation and vesicular monoamine transporter (VMAT)-mediated vesicle transport. Studies have found (Stefanovic et al., 2016) that daily administration of melatonin significantly reduced the adverse effects of chronic unpredictable mild stress (CUMS) on norepinephrine storage, expression of VMAT protein, MAO-A protein and COMT mRNA in the hippocampus. The mode of SNRI antidepressants involves the inhibition of serotonin and norepinephrine reuptake. In this SNRI mechanism, in addition to the 5-HT receptor and the NE receptor, there are other factors such as monoamine oxidase (MAO) or vesicular monoamine transporter (VMAT). SNRI also has affinity for dopamine, such as high doses of venlafaxine may have an effect on dopamine reuptake (Shang et al., 2007). MAO is a monoamine responsible for the oxidative deamination of large amounts of amines, including serotonin, dopamine and norepinephrine (Brunner et al., 1993). VMAT is a transporter whose function is to take up different monoamines stored in vesicles and subsequently releases them in the central nervous system (CNS) (Lohr et al., 2014).

The zebrafish (*Danio rerio*) is a vertebrate model widely used in biomedical research, including neurotoxicology studies (Lee and Freeman, 2014). Zebrafish exhibit a holistic nervous system and neurotransmitter system similar to humans, including glutamatergic, cholinergic, serotonergic, dopaminergic, adrenergic, GABA, and histamine (Babin et al., 2014). The mature zebrafish central nervous system (CNS) develops well and is more complex and can be used to simulate complex brain diseases, including anxiety and depression (Faria et al., 2018). Furthermore, reserpine has the effect of lowering blood pressure and slowing heart rate, initially as one kind of good antihypertensive and sedative. However the previous research showed that acute reserpine exposure can cause depression (Zhang et al., 2018). Therefore, in this study, we detected reserpine-induced depression-like behavior. And we observed the concentration of three monoamine neurotransmitters and evaluated *serta*, *net*, *dat*, *vmat2* and *mao* mRNA levels. This study was to investigate the effects of venlafaxine and melatonin on depressive behavior and related biochemical markers in zebrafish induced by reserpine.

2. Methods and materials

2.1. Experiment ethics and animal housing

Adult zebrafish (AB strains) were raised in in a recirculating aquatic system of the State Key Laboratory of Medicinal Chemistry Biology, Nankai University. Under constant photoperiod (14 h bright/10 h dark), zebrafish were housed in a recirculating aquatic system (KCl 0.05 g/L, $NaHCO_3$ 0.025 g/L, NaCl 3.5 g/L and $CaCl_2$ 0.1 g/L) at 28 °C, feeding fresh shrimp twice a day. All fish used in the study were naive. All experimental procedures were approved by the Committee for Animal Experimentation of the College of Life Science at Nankai University (no. 2008) and performed in accordance with the National Institutes of Health's Guide to Care and Use of Laboratory Animal (No. 8023, Revised in 1996).

2.2. Chemical reagents and experimental design

Reserpine (purity ≥98.0%) and melatonin (purity 98%) were purchased from Shanghai Macklin Biomedical Co., Ltd. Venlafaxine hydrochloride (purity > 98%) was purchased from TCI Co., Ltd. (Shanghai, China). The experimental concentration of venlafaxine was selected to be 0.025 μg/mL by preliminary experiment. In this study, adult zebrafish (six months old, male to female ratio 1:1) were used to expose to 40 μg/mL reserpine for 20 min (Zhang et al., 2018). Then the four groups were exposed to system water (reserpine-only), venlafaxine (0.025 μg/mL, VEN), melatonin (1 μM, MT) and venlafaxine (0.025 μg/mL) combined with melatonin (1 μM) treatment group (VEN + MT). At the same time, the untreated zebrafish was used as a control group (control), and there were five groups. The chemical exposures were updated daily, and all groups were fed twice with fresh brine shrimp for seven consecutive days.

2.3. Behavioral test

The novel tank test was used to assess the depressive status of zebrafish. The experimental device was a 5-liter rectangular box made of transparent plexiglass (23 cm length * 15 cm width * depth 15 cm). The water tank is filled to the maximum extent with system water at 28 °C. The tank is divided into two parts on average. The upper and lower parts represent the “top half (top)” of the novel tank and the “lower half (bottom)” of the novel tank respectively. Behavioral testing was conducted between 10:00 and 16:00. After placing the zebrafish in the test tank, two trained observers recorded the zebrafish swimming behavior for 5 min. Record the following endpoints: latency to the top (s), time spent in top (s), distance travelled in the top (m), total distance travelled (m), freezing bouts and freezing duration (s). In general, a decrease in exploratory behavior is associated with depression/anxiety (Faria et al., 2018; Li et al., 2017).

2.4. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of dopamine, noradrenaline, 5-hydroxytryptamine were measured by ELISA Kits of DA, NA and 5-HT (CUSABIO, Wuhan, China). Zebrafish brain tissue was harvested and rinsed with 1 × PBS after treatment. Brain tissues of 100 mg were homogenized in 1 ml of 1× PBS and stored at −20 °C. After twice freezing and thawing, homogenates were centrifuged at 5000*g* for 5 min at 4 °C. The supernatant was used to assay immediately according to the manufacturer's instruction.

2.5. Quantitative RT-PCR

Total RNA was extracted from brain tissue of each group using Trizol according to the manufacturer's protocol (Invitrogen, USA). Then RNA was reverse transcribed by MMLV reverse transcriptase (Promega,

Madison, WI). The Quantitative RT-PCR was performed using the SYBR Green Labeling System (BioRad, CA, USA). The procedure of the Quantitative RT-PCR included a denaturing step at 95 °C for 2 min, 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for real time plate read, and a final extension at 72 °C for 5 min. Beta-actin was used for data normalization. Primer sequences for *serta*, *net*, *dat*, *vmat2*, *mao* and actin are listed in Supplementary Table 1.

2.6. UPLC and Q-TOF-MS

The powdered venlafaxine and melatonin was dissolved in system water to obtain a solution respectively. Next, we used the mixed solution for ultra-performance liquid chromatography (UPLC) and quadrupole time-of-flight mass spectrometry (Q-TOF-MS) analyses as described before (Li et al., 2019).

2.7. Data analysis

The histogram is expressed as the mean ± SEM (standard error of the mean), generated by GraphPad Prism 6. $P < 0.05$ was considered to represent a significant difference. The 3D swimming trajectory reconstruction is drawn by MATLAB based on the parameter data using the Watson–William test. 3D reconstruction and 3D spatiotemporal reconstruction of the swim path were performed in accordance with the method described before (Li et al., 2017). Java Tree View (University of Glasgow, UK) was utilized to visualize the clustering result. In the clustering map, each small square represents the average relative value and standard deviation, green indicates lower than control group, red is higher than control group, and black is similar to control group. We used a package based on R language (Ade4 package) to analyze the spontaneous motion behavior of zebrafish. Eight characteristic parameters were selected as variables of the whole process. Two behavior variables were related to spatial position and six behaviors were related to motor activity behavior. All characteristic parameters will be mapped to two orthogonal axes. Each axis represented a major component. The transverse axis (x axis) represented the first principal component and the longitudinal axis (y axis) represented the second principal component. The meaning of x axis and y axis was divided according to the contribution of each characteristic parameter in the coordinate axis.

3. Results

3.1. UPLC and Q-TOF-MS analysis of venlafaxine and melatonin

UPLC and Q-TOF-MS analysis were performed for melatonin and venlafaxine respectively. Protonated [M + H] + ions were obtained with as much feature fragment information as possible to infer the molecular and elemental composition of the drugs being tested. The electrospray ionization-mass spectrometry (ESI-MS) spectra of the two drugs were obtained in positive ion voltage mode. The chromatogram showed the retention time of venlafaxine was at 3.66, and the component was identified by the mass spectrum (Fig. 1c). The retention time of melatonin was at 2.97, and the component of the main peak was confirmed by the mass spectrum (Fig. 1d).

3.2. The combination of venlafaxine and melatonin reverses the effect of reserpine on the behavior of zebrafish

The effects of venlafaxine and melatonin on the motility of zebrafish were studied by three-dimensional swimming path reconstruction and spatiotemporal swimming trajectory reconstruction. The wild-type zebrafish has a normal pattern of movement: the wild-type zebrafish has a wide spatial range and a dense trajectory. Before this experiment, there were treatments not pre-exposed to reserpine for 20 mins, but exposed to MT, VEN and VEN + MT. The experimental results showed that the motility behavior of zebrafish exposed only to MT, VEN or VEN + MT did not significantly change compared with the control group (Supplementary Fig. 3a-g). Therefore, we have only conducted the following experiments and discussions. After the addition of reserpine, the swimming path pattern of the zebrafish was disturbed, resulting in sparse trajectory and reduced activity, especially in the reserpine-only group (Fig. 2a; Supplementary Fig. 1a–b). Among them, reserpine inhibited the zebrafish's exploration behavior in the novel tank, reduced its swimming distance at the top, and increased its freezing behavior (Fig. 2b–c). To explore the precise effect of venlafaxine and melatonin on the instantaneous activity of each position of the zebrafish within 5 min, the activity in the test cylinder was color coded. Interestingly, VEN + MT group zebrafish showed a similar trajectory of change in the swimming path pattern similar to Control group (Fig. 2a–c; Supplementary Fig. 1a–b).

The antidepressant effect was expressed by measuring the zebrafish's motor behavior (total distance travelled, etc.) and the exploration behavior (time spent in top, distance travelled in top, latency to the top, etc.) and the freezing behavior (freezing bouts, freezing duration).

Compared with control group, the total distance travelled of reserpine-only group did not change significantly (Fig. 2d), and time spent in top (Fig. 2f) and distance travelled in top (Fig. 2g) decreased significantly. Latency to enter the top (Fig. 2e) and freezing behavior (Fig. 2h–i) increased significantly. After 7 days of treatment with venlafaxine or/and melatonin, there was no significant change in total distance travelled compared with reserpine-only group. In addition, VEN + MT group significantly rescued the reduced reserpine-induced exploring behavior, which can be manifested by time spent in top, distance travelled in top and latency to enter the top. Compared with reserpine-only group, freezing bouts (Fig. 2h) and freezing duration (Fig. 2i) of each treatment group were significantly reduced, and freezing behavior of zebrafish was alleviated. Compared with venlafaxine and melatonin alone, total distance travelled was significantly greater in the VEN + MT group than in the VEN group, but there were no significant differences in other indicators.

In order to find out the relationship between behavioral parameters and drug differences, principal component analysis (Fig. 3a-d) was performed on the spontaneous movement behavior of zebrafish. Eight behavior parameters were selected as the characteristic variables of the whole process, and the cluster analysis (Fig. 3a) of these eight behavior parameters was carried out. The results showed that the eight behavioral parameters were divided into three categories, two were related to spatial position retention, three were related to motor activity and three were related to static behavior. The positive and negative direction results of the x and y axis were composed of the projection results of the eigenvalue vector on the corresponding axis in the coordinate system. In Fig. 3b, there were five eigenvalues in the positive direction of the x axis (right side) and three eigenvalues in the negative direction. The results of contribution analysis (Supplementary Fig. 2a-c) showed that two eigenvalues had no significant effect on the x axis or the first principal component (*Total distance travelled* and *Distance travelled in the bottom*). After excluding it, we found that the anxiety related variables were concentrated on the positive half axis of x, and the anti-anxiety indexes were concentrated on the negative half axis at the same time. Therefore, we defined the x axis or the first principal component to represent the anxiety level of zebrafish. In addition, the meaning and direction of x axis and y axis were the same in each principal component analysis (Fig. 3b–d). So that the behavior characteristic value and the comparison among the groups were connected together. Importantly, the biological difference image among the groups was distinguished by the spatial arrangement in the coordinate system. In the comparison between groups (Fig. 3c), we found that the reserpine-only group increased the anxiety level of zebrafish, while the addition of VEN relieved this state. In MT group and VEN + MT group, anxiety-like state was returned to a level similar to that in Control group. This result is consistent with the geometric distribution map results of various

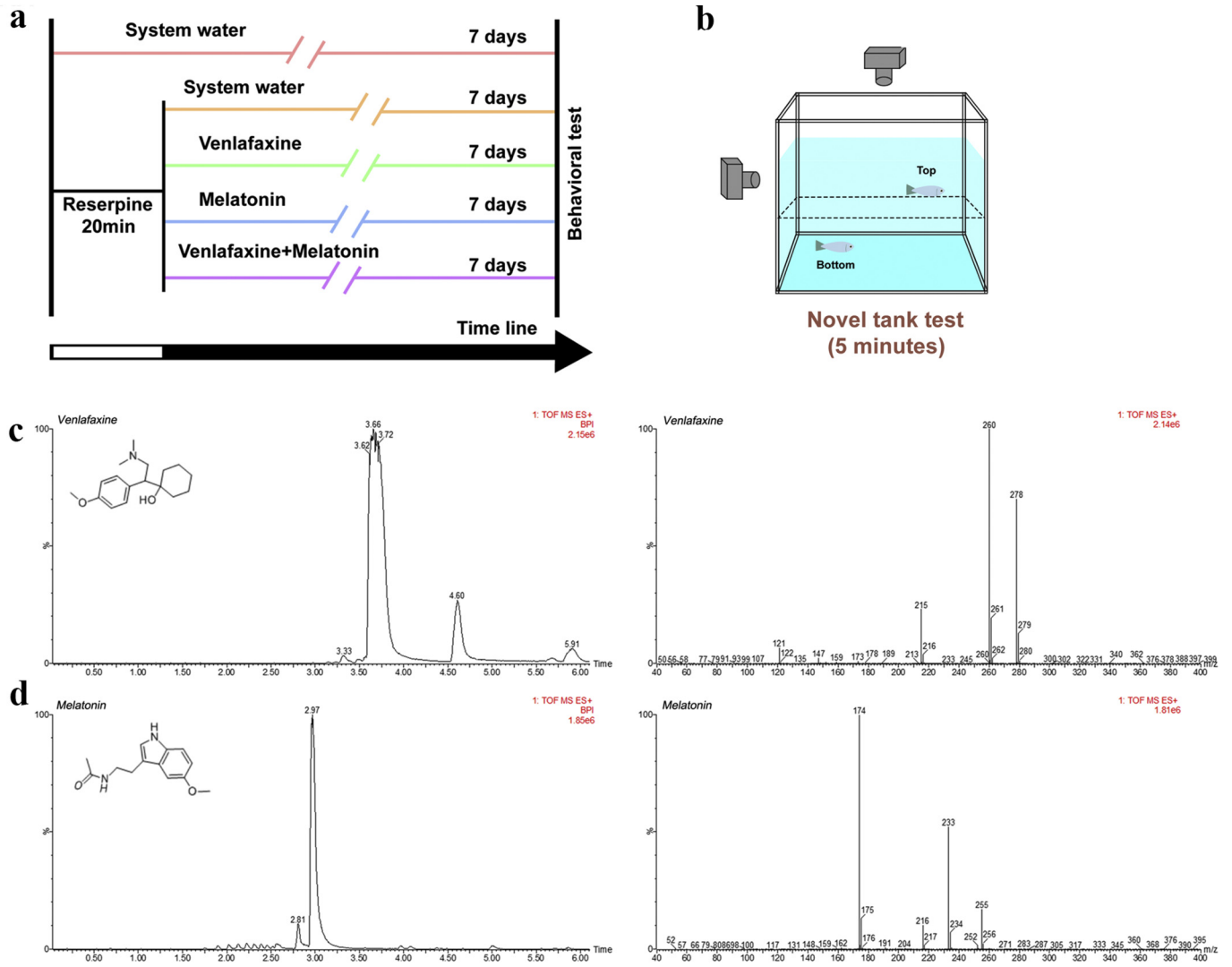

Fig. 1. Experimental paradigm and ESI–MS spectra of melatonin and venlafaxine. (a) Timeline of the procedure for drug delivery and schematic diagram of the apparatus used for behavioral phenotyping in the novel tank (b). Analyze the chemical constituents from venlafaxine (c) and melatonin (d) respectively.

coordinates (Fig. 3d).

3.3. VEN and MT rescued 5-HT and NA levels in depression-like zebrafish

After different treatments, 5-HT, NA and DA levels were detected in zebrafish brain tissue. Compared with control group, the level of 5-HT in reserpine-only group was significantly reduced. Both melatonin and venlafaxine increased the 5-HT level, compared with reserpine-only group, there were no significant difference between VEN + MT group and VEN group or MT group (Fig. 4b). The level of NA in the reserpine-only group was significantly lower than control group. However, compared with the reserpine-only group, the VEN and VEN + MT groups showed a significant increase in NA concentration (Fig. 4f). There was no difference of DA level in the reserpine-only, VEN, MT, and VEN + MT groups compared with control. (Fig. 4d).

3.4. Gene expression level

To further elucidate the effects of melatonin and venlafaxine on monoamine transport process, the expression of relevant genes *serta*, *net*, *dat*, *vmat2* and *mao* were investigated. The results showed that *serta* was up-regulated by reserpine, while venlafaxine significantly reduced reserpine-induced *serta* upregulation, and melatonin down-regulated *serta* expression, but not significant (Fig. 5a). In this experiment, there was no significant change in the expression of zebrafish *net* after reserpine treatment (Fig. 5b). In addition, compared with the control group, the expression of *dat* in the reserpine-only group was up-regulated after treatment with venlafaxine and melatonin, but it was not significant (Fig. 5c). Melatonin reduces *mao* expression, while venlafaxine has no effect on its expression (Fig. 5d).Reserpine significantly down-regulated the expression of *vmat2*, and its expression was significantly up-regulated after treatment with venlafaxine. In particular, the VEN + MT group significantly up-regulated the expression of *vmat2* to the control level compared to treatment with venlafaxine or melatonin alone (Fig. 5e).

4. Discussion

Depression is a kind of mental disorder with the main clinical manifestations of apathy and loss of pleasure. More and more attention has been paid to its characteristics such as high prevalence and suicide rate. Venlafaxine and its active metabolite *O*-desmethyl venlafaxine (ODV) can effectively antagonize the reuptake of 5-HT and NA, and also have a certain effect on the reuptake of DA. And it is a first-line clinical treatment drugs for depression. However, clinical studies have shown (Vanderkooy et al., 2002) that when venlafaxine is used to treat

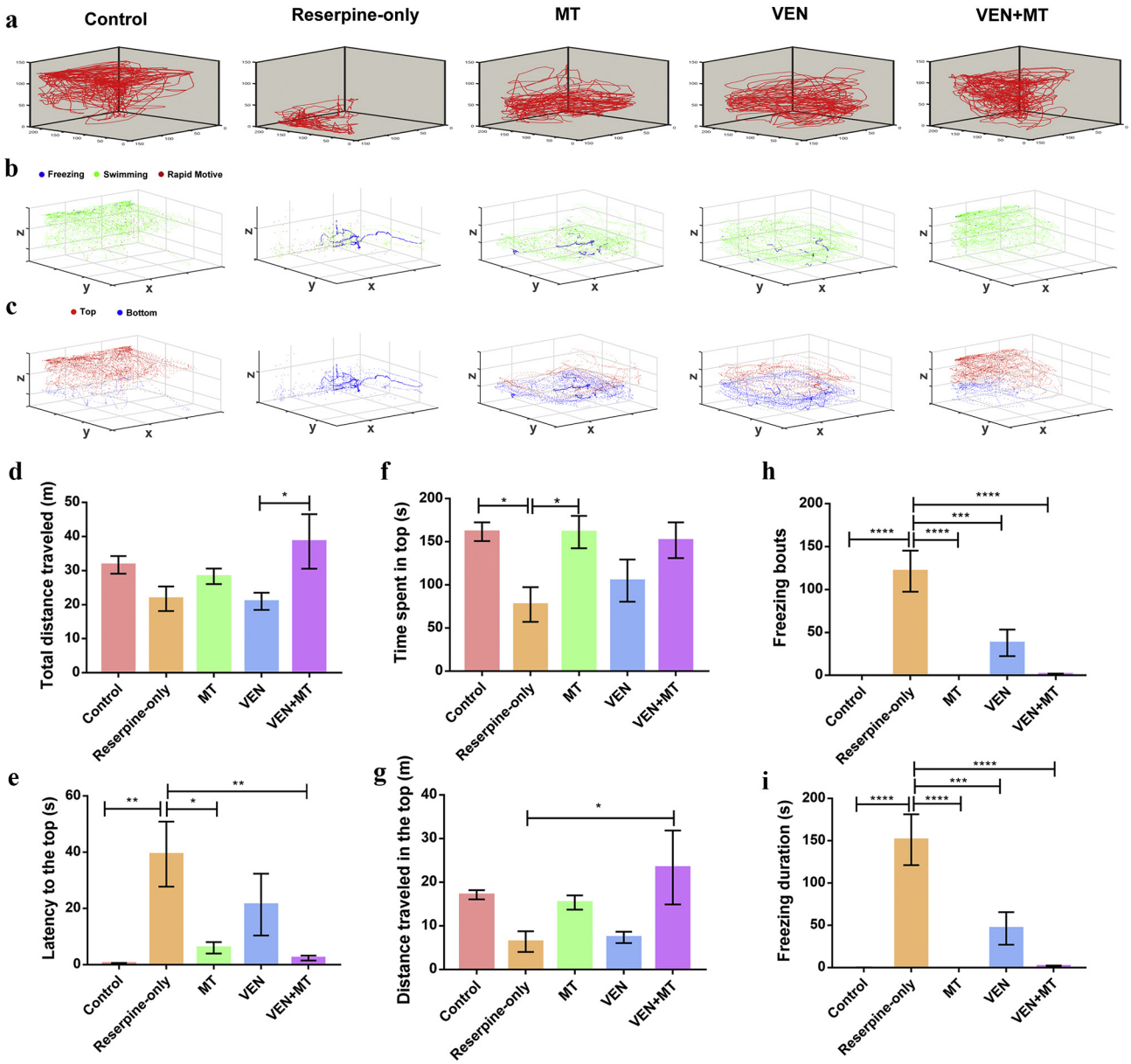

Fig. 2. Swim trajectories and locomotion profiles of adult zebrafish exposed to different regents. (a) "3D reconstruction" indicates the spatial swimming path changes in the whole novel tank. (b) "Mobility" represents instantaneous locomotion at every point in the trajectories, blue indicating freezing, green indicating swimming, red indicating rapid motive; (c) "Which half" represents the spatiotemporal swimming path in red indicating trajectories in the top part and in blue indicating trajectories in the bottom part. Histogram revealing the locomotion behavior and freezing behavior of adult zebrafish by the (d) total distance travelled, (e) latency to the top, (f) time spent in top, (g) distance travelled in the top, (h) freezing bouts and (i) freezing duration. Control: untreated AB strain zebrafish. Reserpine-only: after acute treatment with reserpine, zebrafish were exposed to system water for 7 days to generate the depression model. MT: after acute treatment with reserpine, zebrafish were exposed to melatonin for 7 days. VEN: after acute treatment with reserpine, zebrafish were exposed to venlafaxine for 7 days. VEN + MT: after acute treatment with reserpine, zebrafish were exposed to venlafaxine and melatonin for 7 days. The data are expressed as the mean ± S.E.M. and were analysed by one-way ANOVA followed by the Tukey post hoc test. Significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

depression, it is usually accompanied by adverse reactions such as nausea and vomiting. Melatonin has the functions of regulating biological rhythm, improving sleep, regulating neuroendocrine and immunity, and may have certain antidepressant effects (Crupi et al., 2010). To this end, this paper studied the anti-depression effect of venlafaxine combined with melatonin by constructing a zebrafish depression model induced by reserpine.

We used a 3D video tracking system to monitor changes in zebrafish swimming behavior in novel tank. The depressive symptoms of zebrafish usually manifest as the reduction of motion behavior (total distance travelled, etc.) and the exploration behavior (time spent in top, distance travelled in top, latency to the top, etc.) and the increase of freezing behavior (freezing bouts, freezing duration). As previously described (Kyzar et al., 2013), reserpine treatment of zebrafish for 20 min did not immediately show significant depressive symptoms, but after 7 days, its motor activity was significantly reduced. Compared with the zebrafish in reserpine-only group, venlafaxine and melatonin showed different behaviors. In order to compare the behavioral differences among groups, we carried out principal component analysis (PCA).The differences between different groups can be compared by comparing the characteristic variables and different groups in the first and second groups. In the comparison between groups, we found that reserpine-

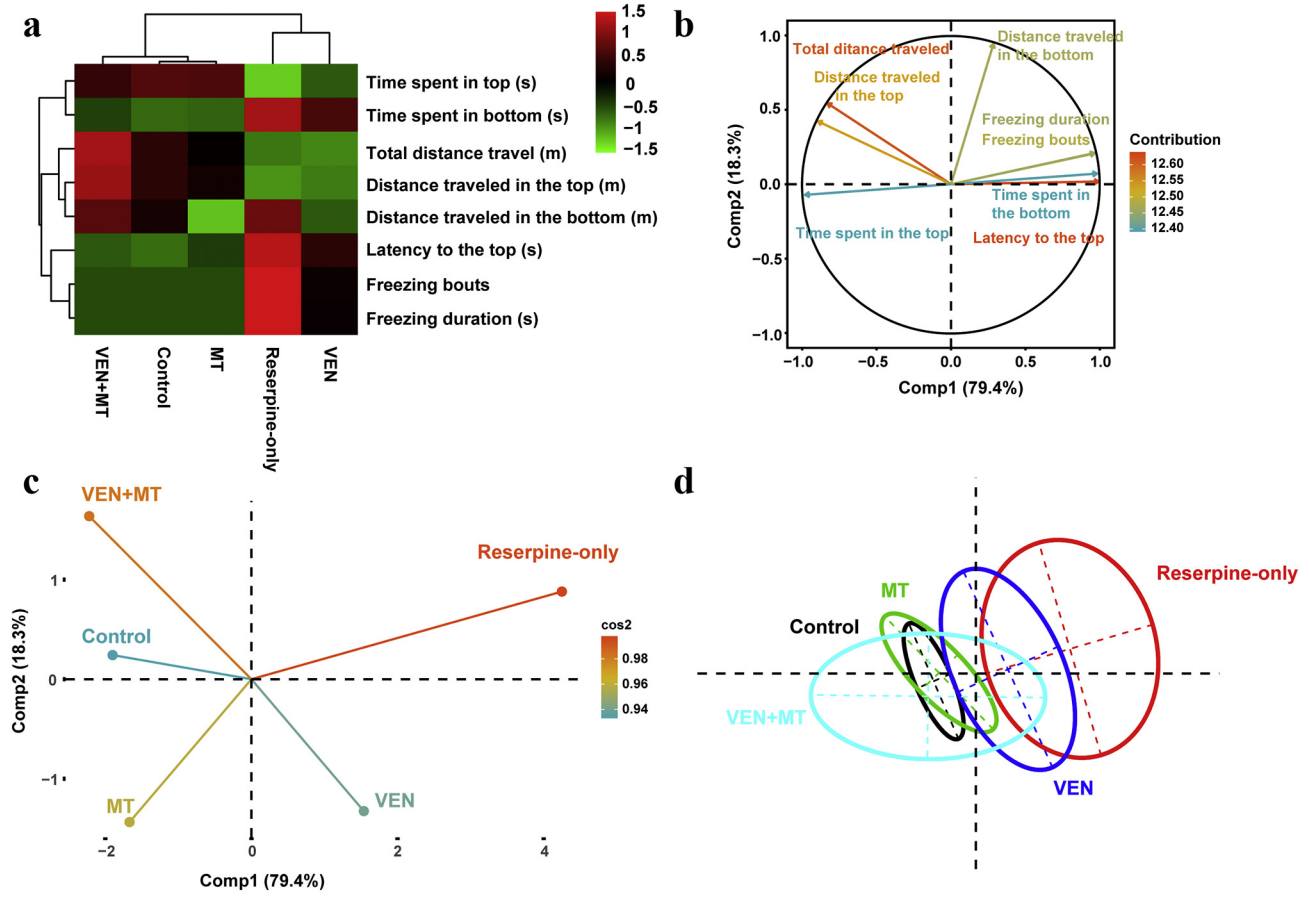

Fig. 3. Principal component analysis of zebrafish behavior. (a) The cluster analysis of eight behavior parameters. (b) Contribution of the eight variables to the variances of the first and second principal component. (c) Projection of barycenters in the control, reserpine-only, MT, VEN, and VEN + MT groups into the first and second principal components. (d) The envelopes of the five groups from the same principal components analysis were showed. Each envelope represented the geometric distribution range of individual values in each group on the two-dimensional axis.

only group increased the anxiety level of zebrafish, and the addition of VEN alleviated this state. In MT group and VEN + MT group, the anxiety state returned to a level similar to that of control group.

Monoamine neurotransmitters such as dopamine (DA), noradrenaline (NA), and serotonin (5-HT) have broad biological activities and participate in many physiological reactions of the central nervous system, such as emotional reactions, mental activities, body temperature regulation, and sleep, etc. (Delgado and Moreno, 2000; Dunlop and Nemeroff, 2007; Wang et al., 2006).. Consistent with our anticipation, we observed that venlafaxine elevated 5-HT and NA levels in depression-like zebrafish induced by reserpine, by selectively inhibiting the reuptake of 5-HT and NA. As previously reported, our study has showed that melatonin regulated the levels of 5-HT and NA in the synapse and had a certain antidepressant effect (Park et al., 2018; Ramírez-Rodríguez, 2016). In our study, the combined use of venlafaxine and melatonin therapy alleviated the reduction in 5-HT and NE levels caused by reserpine, and ameliorate the depressive symptoms of zebrafish. Therefore, the increased monoamine degree in depression-like zebrafish brain and the recovery behaviors revealed the potential association between monoamine modulatory system and exploring abilities.

Reserpine has the effect of lowering blood pressure and slowing heart rate, initially as an antihypertensive and sedative. However, clinical phenomena suggest that long-term use of such drugs can cause depression. Reserpine is a vesicular monoamine transporter (VMAT) inhibitor that produces a pathological effect by blocking VMAT (Leao et al., 2015). Our experimental results showed that the expression of VMAT mRNA in the brain of zebrafish decreased after reserpine treatment. After seven days of treatment with venlafaxine and melatonin, the expression of VMAT mRNA was up-regulated to normal levels. VMAT acts as a neurotransmitter carrier that transports monoamines stored in vesicles to the surface of the cell membrane, and then monoamines are released into the synaptic cleft by exocytosis (Eiden and Weihe, 2011). Monoamines produced by presynaptic nerve endings, such as dopamine, serotonin, and norepinephrine, are Oxidized by monoamine oxidase (MAO) and catechol ortho-methyltransferase (COMT) without VMAT protection (Guillot and Miller, 2009; Shih et al., 1999). Our results suggest that melatonin treatment down-regulates MAO mRNA expression, whereas venlafaxine alone does not. Stefanovic B's research showed that (Stefanovic et al., 2016), melatonin can regulate depression by affecting the expression of MAO and VMAT2.

Dopamine, noradrenaline, and serotonin were important neurotransmitters, often associated with depressive symptoms, and their activity was dependent on their receptors and transporters. Previous research has shown that serotonin transporter (SERT) and serotonin receptors play an important role in synaptic 5-HT neurotransmission: SERT acts as both a signal transporter and an anti-transporter in the presynaptic membrane, responsible for synapses removal and reuptake of serotonin (Gershon and Tack, 2007; Theodoridi et al., 2017). Consistent with our experimental results, venlafaxine regulates 5-HT levels in the synaptic cleft by inhibiting the expression of SERTa mRNA. However, there was no significant effect on the expression of NET and DAT mRNA. Therefore, it needs more future experiment to confirm this phenomenon.

In our study, the combined use of venlafaxine and melatonin could alleviate the reduction of VMAT2 induced by reserpine (Fig. 6).The

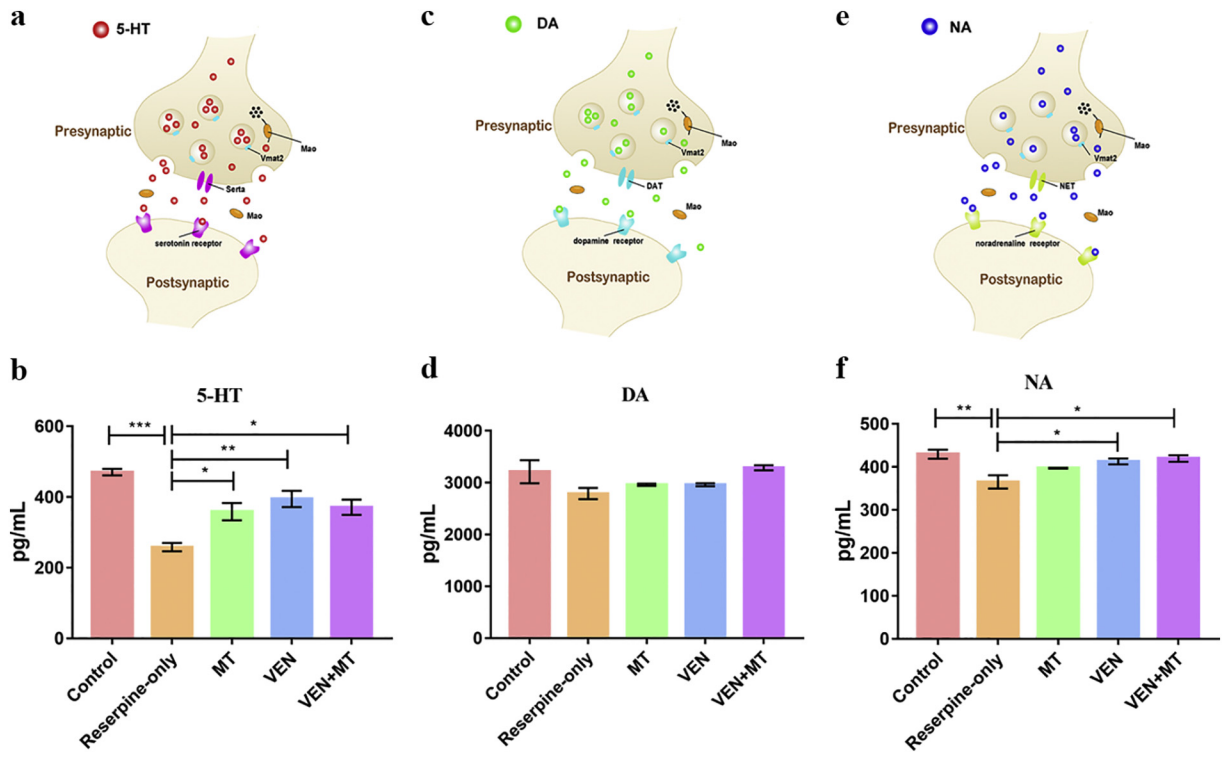

Fig. 4. The schematic diagram of monoamine transport between synapses and monoamine levels in zebrafish brain. The schematic diagram of 5-HT(a), DA (c), and NA(e) transport. The levels of 5-HT(b), DA (d), and NA(f) in zebrafish after different treatments. The data are expressed as the mean ± S.E.M. and were analysed by one-way ANOVA followed by the Tukey post hoc test. Significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

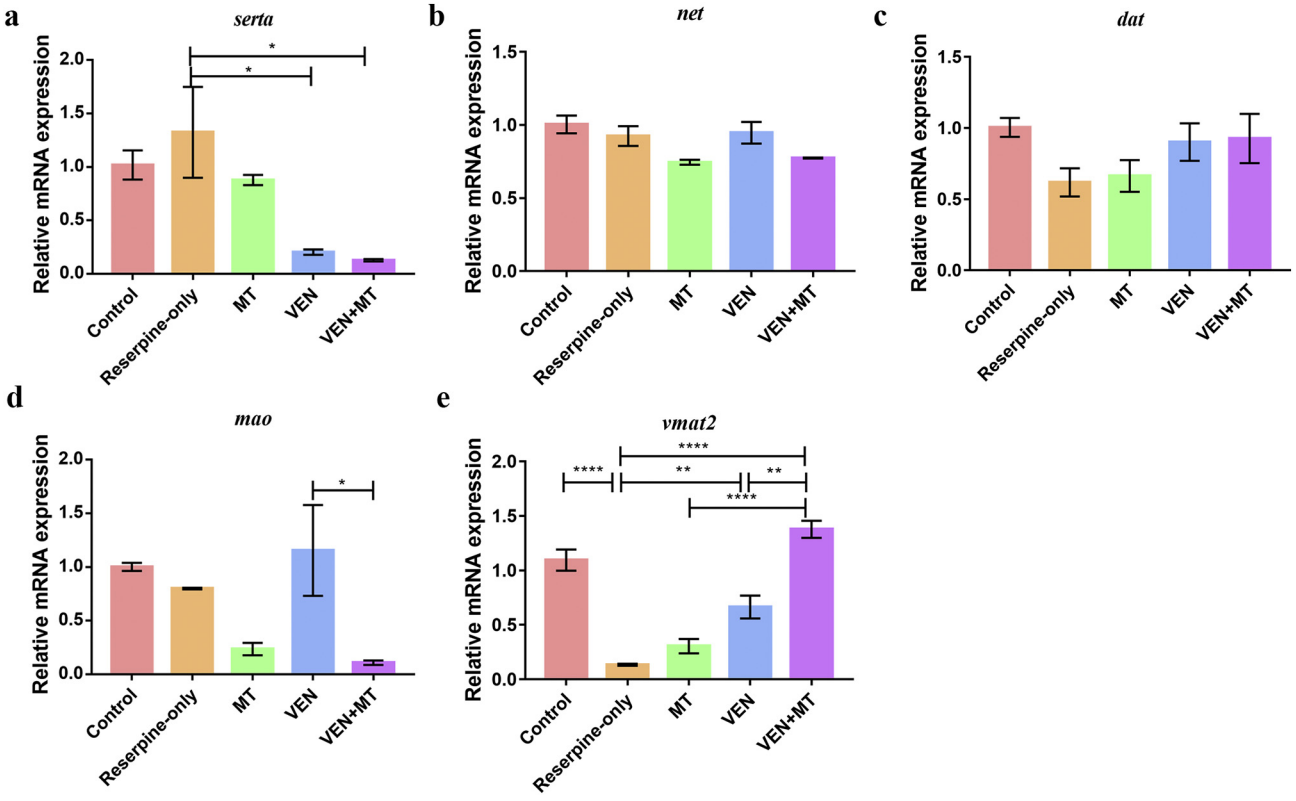

Fig. 5. The effects of venlafaxine or/and melatonin treatment on related genes expression in zebrafish. The level of *serta* (a), *ne t*(b), *dat* (c), *mao* (d) and *vmat2* (e) in zebrafish after different treatments. The data are expressed as the mean ± S.E.M. and were analysed by one-way ANOVA followed by the Tukey post hoc test. Significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$.

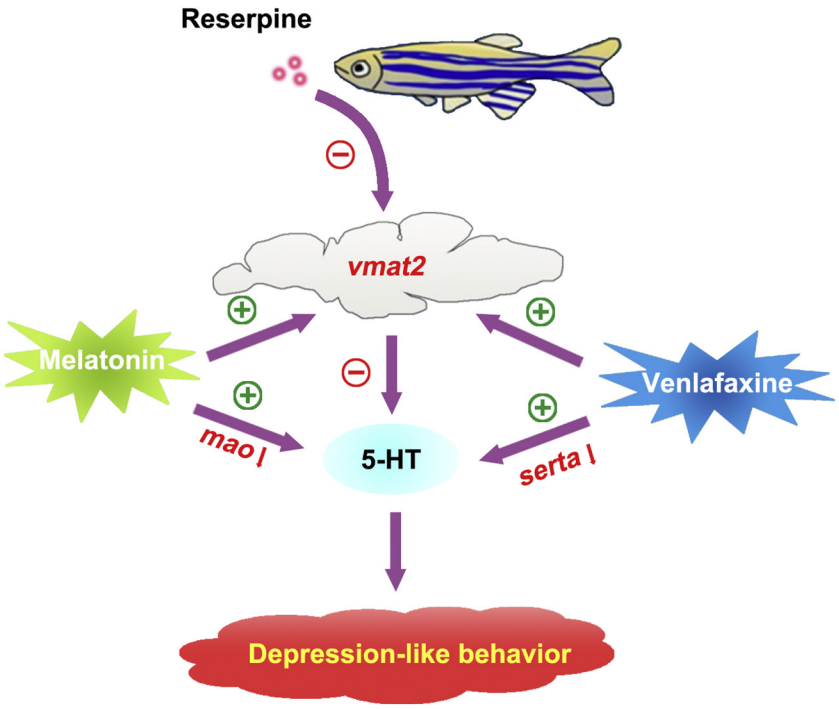

Fig. 6. Diagram illustrating the protective effects of venlafaxine and melatonin on depression-like behavior induced by reserpine. Reserpine induces the depression-related gene expression. Venlafaxine and melatonin could inhibit the effects of reserpine.

function of most monoamine neurotransmitters depended on the vesicle release that may be produced by the vesicular monoamine transporter (VMAT). Serotonin and dopamine are loaded into secretory vesicles by VMAT. If VMAT is inhibited, monoamine neurotransmitters would be metabolized by enzyme monoamine oxidase (MAO), resulting in rapid consumption of these neurotransmitters; since norepinephrine was secreted dopamine synthesized in vesicles, so VMAT inhibition also reduced norepinephrine levels (Bottalico et al., 2004; Moret and Briley, 2011; Rommelfanger and Weinshenker, 2007). Concerning the widely beneficial effects of venlafaxine and melatonin on mental illness, we believe that monoamine signaling regulation mechanisms might contribute to modulate and improve many neurological functions in a wide range of depressive diseases.

5. Conclusion

In this experiment, the novel tank test revealed that the combination of melatonin and venlafaxine has an antidepressant effect on zebrafish after reserpine exposure. After exposure to reserpine, zebrafish exhibited reduced exploration behavior suggestive of a depression-like phenotype. Three-dimensional reconstruction showed that reserpine reduced the swimming path of zebrafish by limiting its movement area to the bottom of the novel tank, while the 3D-spatiotemporal reconstructions revealed that reserpine produced similar effects on the instantaneous movement of zebrafish at each position of the swimming path. VEN + MT treatment reverses depression-like behavior by increasing exercise, reducing irregular movements, increasing exploration behavior and ameliorating the phenotype of depression. Although from the behavioral parametric analysis, there was no significant difference in VEN + MT treatment compared with venlafaxine treatment alone. However, through the three-dimensional reconstruction model, the motion trajectory after MT + VEN treatment was similar to that of the control group. Depression-like behavior in zebrafish is mainly caused by changes in hormones and monoamine neurotransmitters in the brain. A significant decrease in 5-HT and NA levels was observed in the zebrafish depression model, suggesting that reserpine-induced anxiety behavior is associated with monoamine levels. VEN + MT treatment reversed the decrease in monoamine levels and thus played an anxiolytic role. Overall, these results indicate that venlafaxine and melatonin relieve zebrafish depressive symptoms by down-regulating serotonin and norepinephrine levels. After exposure to reserpine, *vmat2* mRNA expression was down-regulated and *serta* mRNA expression was up-regulated in zebrafish. This phenomenon was reversed after VEN + MT treatment, and *mao* mRNA expression was down-regulated. Our study shows that venlafaxine combined with melatonin in the treatment of reserpine-induced zebrafish depression may be achieved through the influence of related regulatory genes, providing a reference for the clinical application of antidepressants.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

X.Z.F. and Y.Q.T. conceived and designed the experiments. Behavior experiments were conducted by Y.Q.T., and the video analysis for behavior was conducted by Y.Q.T.. Enzyme-linked immunosorbent assay (ELISA) and qPCR was conducted by Z. R. L. and D. Y. C.. Data analysis and paper writing were conducted by Y. Q. T.. Principal component analysis was conducted by S. Z. Z.. P. M. made important contributions to the discussion.

All authors discussed the results and reviewed the manuscript at all

stages.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ntt.2019.106835.

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