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Protective effect of the orientin on noise-induced cognitive impairments in mice

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Abstract
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Abstract

There is increasing evidence that chronic noise stress impairs cognition and induces oxidative stress in the brain. Recently, orientin, a phenolic compound abundant in some fruits, millet, and herbs, has been shown to have antioxidant properties. This study investigated the potential effects of orientin against chronic noise-induced cognitive decline and its underlying mechanisms. A moderate-intensity noise exposure model was used to investigate the effects of orientin on behavior and biochemical alterations in mice. After 3 weeks of the noise exposure, the mice were treated with orientin (20 mg/kg and 40 mg/kg, oral gavage) for 3 weeks. The chronic noise exposure impaired the learning and memory in mice in the Morris water maze and step-through tests. The noise exposure also decreased exploration and interest in a novel environment in the open field test. The administration of orientin significantly reversed noise-induced alterations in these behavior tests. Moreover, the orientin treatment significantly improved the noise-induced alteration of serum corticosterone and catecholamine levels and oxidative stress in the hippocampus and prefrontal cortex. Furthermore, the orientin treatment ameliorated the noise-induced decrease in brain-derived neurotrophic factor and synapse-associated proteins (synaptophysin and postsynaptic density protein 95) in the hippocampus and prefrontal cortex. Thus orientin exerts protective effects on noise-induced cognitive decline in mice, specifically by improving central oxidative stress, neurotransmission, and increases synapse -associated proteins. Therefore, supplementation with orientin-enriched food or fruit could be beneficial as a preventive strategy for chronic noise-induced

cognitive decline.

Keywords: Orientin, Cognitive impairments, Oxidative stress, Synaptic-associated plasticity.

1. Introduction

Noise, defined as ‘unwanted sound’, is perceived as an environmental stressor and annoyance. With the development of urbanization and industrialization, noise pollution has become a risk factor for neurodegenerative diseases, depression and cognitive decline. Exposure to environmental noise can induce hearing deficit in the auditory system and sleep disturbance [1]. Recently, increasing animal and clinical evidence has shown that chronic noise exposure has obvious effects on cognitive impairment, such as declined memory and learning. For example, working memory was impaired after chronic noise stress in rats with reduced dentritic count in the hippocampus and prefrontal cortex [2]. Noise exposure during pregnancy impaired spatial learning ability and decreased neurogenesis in the hippocampus in rat pups [3]. In addition, a large cross-national and cross-sectional clinical study has demonstrated that chronic aircraft noise impaired cognitive development in children, specifically reading comprehension [4].

The physiological responses to chronic stress include the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenomedullary system (SAM), through which the levels of corticosterone and catecholamine are altered [5, 6]. In a continuously open auditory system, noise-induced stress can cause the significant release of hypothalamic hormones such as the corticotrophin-releasing hormone and the adrenocorticotropic hormone, which increase the activity of the HPA-axis and the release of corticosterone from the adrenal cortex [7]. It is known

that high levels of corticosterone, induced by the hyperactivity of the HPA axis, impair cognition, including learning, memory, and spatial recognition [8]. Furthermore, catecholamines such as norepinephrine (NE) and dopamine (DA) are essential monoamines for learning and memory through their ability to regulate synaptic plasticity [9, 10]. The increased level of serum catecholamines such as norepinephrine (NE) and dopamine (DA), is considered to be an immediate response in combating the stress induced by the activation of the SAM system [11]. Moreover, increasing evidence shows that the activation of the oxidative stress process by chronic stress may cause lipid peroxidation, reduced antioxidant enzyme activity, and increased monoamine catabolism, which are all related to cognitive decline [12]. Chronic exposure to low frequency noise at moderate levels increased the levels of oxidative stress in mice, while attenuating oxidative stress inhibited cognitive impairment [13, 14]. These findings strongly suggest that corticosterone, catecholamine and oxidative stress are closely associated with noise stress induced cognitive impairment.

The hippocampus and prefrontal cortex are important brain regions responsible for enhancing cognition. Studies in rodents have showed that chronic noise impairs the neurotransmitter signaling system in the hippocampus and prefrontal cortex [15], which in turn impairs cognitive function. Brain-derived neurotrophic factor (BDNF) in the hippocampus and prefrontal cortex plays a critical role in synaptic plasticity and cognition [16, 17]. For example, the effect of exercise enhancing cognitive function was blocked by inhibiting BDNF action in the hippocampus [17]. Recently,

synaptophysin (SYN) and post-synaptic density 95 (PSD-95) biomarkers in synaptic reconstruction have been reported to be regulated by BDNF to facilitate regeneration of neurons and axons [18-20]. Growing evidence suggests that neurotrophic factors and synaptic proteins in the hippocampus and prefrontal cortex may function as attractive therapeutic targets for neuropsychiatric diseases.

Orientin (luteolin-8-C-glucoside) is a phenolic compound found abundantly in millet and the juice and peel of passionfruit [21, 22]. It is also abundant in bamboo leaves, which have a long history of nutritional and medical applications in Asia [23]. Several recent studies have demonstrated that orientin exerts a variety of pharmacological effects, including antioxidant, anti-inflammatory, and neuroprotective effects [24, 25]. For example, orientin (20 mg/kg and 40 mg/kg, intragastric administration) has shown significant antioxidant properties in improving the neuronal ultrastructure in the hippocampus of D-galactose-aged mice [26]. Importantly, our previous study have shown orientin (20 mg/kg and 40 mg/kg, oral gavage) alleviated the chronic unpredictable mild stress (CUMS)-induced depression-like behavior and increased the brain-derived neurotrophic factor and synapse-associated proteins [27]. However, the protective effect of orientin on noise-induced cognitive impairments and the mechanisms of cognitive improvement are yet to be reported. This study aimed to evaluate the protective effect of orientin on noise-induced cognitive impairment in mice by examining behavior tests. In addition, this study also determined the neuroendocrine changes (corticosterone and catecholamine) and alterations in anti-oxidative status and synaptic plasticity in the

hippocampus and prefrontal cortex which are associated with noise.

2. Methods and materials

2.1 Animals

Adult male Kunming mice (18 to 22 g) were provided by Laboratory Animal Center, Xuzhou Medical College, Xuzhou, Jiangsu, China. The mice were housed with *ad libitum* access to food and water under controlled temperature (22±2°C) and humidity (50±10%) and maintained on a 12-hour light/dark cycle for 1 week of adaptation. All procedures were approved by the Animal Ethics Committee, Xuzhou Medical College, China, and complied with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2 Noise stress procedure

The continuous noise used in this study was generated by a noise generator (type DG1032Z, Beijing puyuan, China). The amplitude of the noise was 80 dB SPL, and the range of noise frequency was from 10 to 10,000 Hz [28]. The noise level was measured with a sound level meter (type WS-1361, Wensn, Dongwan, Guangdong province). The noise exposure was performed for 2 h continuously and randomly per day for six weeks.

2.3 Drug and treatments

The mice were randomized into five groups (n=14 per group): control group

(without noise stress procedure); noise group (exposure to noise stress procedure); noise + orientin-L group (exposure to noise stress procedure and low dosage of orientin 20 mg/kg); noise + orientin-H group (exposure to noise stress procedure and high dosage of orientin 40 mg/kg); orientin-H group (without noise stress procedure and high dosage of orientin 40 mg/kg). After three weeks of the noise stress procedure, the orientin was administered orally (gavage daily) for 3 weeks. Both the control group and the noise group received the same volume of normal saline. In this study the orientin (purity, 99.8%, WT, 464) used was purchased from Extrasynthese (Genay, France).

2.4 Behavioral tests

Morris water maze test: As previously described [29], the Morris water maze test was performed in a circular pool filled with water at room temperature (diameter, 120cm; height, 60cm; water temperature, 24 ± 1°C). An escape platform (10 cm in diameter) was hidden 1.5 cm below the surface of water in the center of one quadrant. The pool was virtually divided into four quadrants, i.e., NE, SE, SW, and NW. The mice received four consecutive daily training trials. At the beginning of each trial, the mice were released at one of the four possible starting points facing the wall, and allowed to swim freely until they reached the platform. The time it took them to reach the hidden platform (escape latency) was recorded. If a mouse did not find the escape platform within 90 s, it was given a latency score of 90 s.

On the probe trial performed on the fifth day. The mice were placed and released

opposite the site where the platform had been located. The probe trial consisted of a 90 s free swim in the pool without the platform. The percentage of time spent in the target quadrant indicates the degree of memory consolidation.

Open-field test: The locomotor and exploratory activities were assessed by the open-field test as previously described [30]. The apparatus consisted of a wooden box (40 × 40 cm), with the floor divided into 25 equal squares (8 × 8 cm) marked with black lines. In the test, mice were individually placed in the center of the arena and permitted for free explorations. The locomotor (number of crossings) and exploratory activities (number of rearings) were recorded during the 5-min test.

Step-through test: Immediately after the open field test, animals were tested for emotional cognitive impairment by the step-through avoidance test [11], which is considered to be a simple and rapid memory test. The test was conducted on 2 consecutive days at the same time of the day. The compartment (15 ×10 ×11 cm) was divided into a light chamber equipped with an illuminator and a dark chamber with an interconnecting semicircular door (3 cm in diameter). On the 1st day (training trial), each mouse was placed in the illuminated chamber with its back facing the door for 3 min for environmental adaptation. After the door opened, the mice move to the darkened adjacent chamber to avoid the light (mice prefer instinctively being in the dark than in the light) and received a mild 0.8 mA (36 V) footshock for one second. A training trial was performed for 5 min as mentioned above. On the 2nd day, retention trial was performed by the same procedure as training trial. During the retention trial,

the number of mistakes and the step-through latency (the time that the mouse initially entered the dark chamber) were recorded for 5 min.

2.5 Sample collection

Following the behavioral tests, the mice were sacrificed. The blood were collected and centrifuged at 4,000 rpm for 15 min at 4°C. The brains were carefully removed followed by the rapid dissection of the hippocampus and the prefrontal cortex. The brain and serum samples were stored at -80 °C until assay.

2.6 Measurement of corticosterone (CORT), norepinephrine (NE) and dopamine (DA) levels

CORT levels in the serum were determined with a commercial enzyme-linked immunosorbent assay (ELISA) kits (Adlitteram Diagnostic Laboratories, USA) according to the manufacturer's instructions. Similarly, the concentrations of NE and DA in the brain and serum were analyzed with ELISA kits (Adlitteram Diagnostic Laboratories, USA).

2.7 Measurement of oxidative stress

The hippocampus and prefrontal cortex were and homogenized in volumes (1:9 w/v) of ice-cold normal saline. The homogenates were centrifuged for 10 min at 4000 rpm at 4 ^{o}C and the supernatants were used to determination the oxidative markers.

Determination of lipid peroxidation: The malondialdehyde (MDA) content, an

index of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances [31]. The MDA level was determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of superoxide dismutase (SOD) activity: The level of SOD activity was measured as previously described [32]. Briefly, the measurement was based on the generation of superoxide radicals produced by xanthine and xantine oxidase, both of which react with nitro-blue tetrazolium (NTB) to form formazan dye. This was followed by the measurement of SOD activity at the wavelength of 550 nm by the degree of inhibition of this reaction. One unit of enzyme was defined as the amount of enzyme required at the inhibition rate of 50%.

Determination of catalase (CAT) activity: The level of CAT activity was measured as previously described [33]. Briefly, 0.1 ml of supernatant was added to a cuvette containing 1.91 ml of 50 mmol/L phosphate buffer (pH 7.0). The reaction started with the addition of 1 ml freshly prepared 30 mmol/L H_2O_2. The variations in the decomposition rate of H_2O_2 were determined by spectrophotometry at 240 nm.

Determination of glutathione (GSH): The GSH concentration was determined as previously described [34]. Briefly, 160 μl of supernatant was added to 2 ml of Ellman's reagent (5,5'-dithiobis [2-nitrobenzoic acid] 10 mM, $NaHCO_3$ 15 mM). The mixture was incubated at room temperature for 5 min and absorbance was read at 412 nm.

2.8 Western blot

For protein extraction, tissues were homogenized in an ice-cold extraction buffer (20 mM Tris-HCl buffer, pH 7.6, 150 mM NaCl, 2 mM EDTA·2Na, 50 mM sodium fluoride, 1 mM sodium vanadate, 1% Nonidet™ P-40, 1% sodium deoxycholate, 0.1% SDS, 1 mg/ml aprotinin, and 1 mg/ml leupeptin). Homogenates were centrifuged at 10,000×g for 10 min at 4°C. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (Sigma-Aldrich). Equal amounts of protein (20 μg) were isolated by 10% SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% skim milk powder in a washing buffer [Tris-buffered saline containing 0.05% (v/v) Tween 20] for 2 h at 25°C, and subsequently incubated overnight with the primary antibodies against SYN (1:1,000) and PSD-95 (1:2,000) (Cell Signaling Technology Inc, Danvers, MA, USA) were employed. Each membrane was thrice rinsed for 15 min each and incubated with the secondary antibodies. Bands corresponding to the proteins of interest were scanned and band density was analyzed using the Quantity One automatic imaging analysis system (Bio-Rad Laboratories, Hercules, CA, USA). All quantitative analyses were normalised to β-actin, based on our previous studies [35].

2.9 Reverse transcriptase-PCR (RT-PCR)

The total RNA was extracted using TRI reagent (Sigma-Aldrich, MO, USA). The cDNA was synthesized using a High Capacity RNA-to-cDNA kit (Sigma-Aldrich) according to the manufacturer's protocol. The sequences of the forward and reverse primers are shown in Table 1. The housekeeping gene β-actin was used as an internal

control for the normalization parallel with each gene examined. Amplified products were separated by electrophoresis on a 1% agarose gel, followed by visualization under a UV transilluminator and photography. Each brain sample was analyzed in duplicate in two independent experiments for each gene. The values obtained for the BDNF mRNA expression were normalized to β-actin and quantified relative to the expression in the control samples. The products were analyzed with densitometry using the Quantity One 1-D analysis software (BioRad Laboratories, Hercules, CA, USA).

2.10 Histological analysis

After the behavioral test, mice were perfused with ice-cold normal saline followed by 4% paraformaldehyde via the left ventricle of the heart under anesthesia. The whole brain was removed and post fixed in 4% paraformaldehyde for 48 h, followed by dehydration and embedding in the paraffin. The brain sections in paraffin (5 μm) were prepared and stained with Nissl's staining. The population of intact cells in the cerebral cortex and the hippocampal CA1 subfield were counted by an investigator blinded to sample identity, and the average value from adjacent two sections was used for each animal.

2.11 Statistical analysis

Values are expressed as mean±SEM (standard error of the mean). Data were analyzed by one-way ANOVA followed by Dunnett's *post hoc* test. $P < 0.05$ was

considered to be statistically significant. Statistical analysis was conducted using SPSS 16.0 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1 Orientin enhances the spatial learning and memory in the noise-exposed mice

To examine whether orientin could attenuate noise-induced cognitive impairments, we tested the spatial learning and memory using the Morris water maze test (Fig. 1). The noise-exposed mice showed a significant increase in escape latency compared with the control mice on day 3 and 4 during training ($P < 0.001$, Fig. 1A) suggesting chronic noise exposure impaired spatial learning. However, treatment with orientin at doses of the 20 mg/kg and 40 mg/kg significantly decreased the escape latency as compared to the noise-exposed mice ($P < 0.05$, $P < 0.01$) on day 3 and ($P < 0.001$, $P < 0.01$) on day 4, respectively.

In the probe trial on day 5, the noise-exposed mice had an increased escape latency ($P < 0.001$, Fig. 1A) and spent significantly less time in the target quadrant ($P < 0.001$, Fig. 1B) compared with the control group, indicating that they had a decreased ability to locate the position of the hidden platform on the basis of spatial memory of the visual cues. Both the low and high dose orientin treatments (20 mg/kg and 40 mg/kg) showed a significantly longer stay in the target quadrant compared with the noise group ($P < 0.05$, $P < 0.01$, Fig. 1B). The mice in orientin-H group without noise exposure performed similarly to the control group, indicating that

orientin *per se* had no effect on the spatial learning and memory of mice in the control group.

3.2 Orientin improves the exploratory and locomotor activities in the noise-exposed mice

Exploration and locomotion are two instinctive activities of normal animals exposed to a novel environment [36]. Effects of orientin on the exploratory and locomotor activities were measured in the open field test (Fig. 2). Compared with the control group, the number of crossings was significantly decreased in the noise-exposed mice ($P < 0.01$), while this decrease was only improved in the high dose orientin treatment group (40 mg/kg, $P < 0.01$), but not the low dose orientin treatment group (20 mg/kg) (Fig. 2A). Furthermore, the number of rearings was significantly lower in the noise-exposed group compared with the control group ($P < 0.01$, Fig. 2B). Both the low and high dose orientin treatments increased the number of rearings in the noise-exposed mice ($P < 0.05$, $P < 0.01$) (Fig. 2B). However, there was no significant difference in the number of crossings and rearings between the mice treated with orientin (40 mg/kg) without noise exposure and the control mice.

3.3 Orientin improves memory acquisition of the passive avoidance response in the noise-exposed mice

The noise-exposed group significantly decreased the step-through latency during the retention trial ($P < 0.001$, Fig. 3A) compared with the control group. However, the

low and high dose (20 mg/kg and 40 mg/kg) orientin treatments evidently increased the latency in the noise-exposed mice ($P < 0.01$, $P < 0.001$). In addition, the number of mistakes in the noise-exposure group significantly increased compared to the control group ($P < 0.001$, Fig. 3B). However, the number of mistakes was significantly decreased in the noise + orientin-H group ($P < 0.001$), but not in the noise + L group (Fig. 3B). There was no significant difference in the step-through latency and the number of mistakes between the mice treated with orientin-H (40 mg/kg) without noise exposure and the control mice.

3.4 Orientin decreases serum CORT concentration in the noise-exposed mice

The CORT levels in serum were significantly higher in the noise-exposed group than in the control group ($P < 0.01$, Fig. 4), while serum CORT levels significantly decreased in the orientin treatment groups (20 mg/kg and 40 mg/kg, $P < 0.05$, $P < 0.01$).

3.5 Orientin inhibits biogenic catecholamine reduction in the brain and serum in the noise-exposed mice

As shown in Fig. 5, chronic noise exposure significantly decreased the levels of NE and DA in the brain and serum compared to the control group (all $P < 0.05$). High dose orientin treatment (40 mg/kg) resulted in a significantly elevated in NE level in the in the hippocampus and prefrontal cortex as compared to the noise-exposure mice, respectively ($P < 0.01$, $P < 0.01$), while the low dose orientin treatment (20 mg/kg)

only increased NE in the hippocampus ($P < 0.05$) (Fig. 5A). Both the low and high dose orientin treatments (20 mg/kg and 40 mg/kg) significantly inhibited the depletion of DA levels in the hippocampus and prefrontal cortex compared to the noise-exposed mice, respectively ($P < 0.05$, $P < 0.01$) (Fig. 5B). Moreover, the high dose orientin treatment (40 mg/kg) resulted in elevated serum NE and DA levels compared to the noise-exposed mice, respectively ($P < 0.05$, $P < 0.01$) (Fig. 5C, D). The low dose orientin treatment (20 mg/kg) only inhibited the reduction of serum DA in noise-exposed mice ($P < 0.05$, Fig. 5D).

3.6 Orientin attenuates oxidative stress in the brain of noise-exposed mice

The effects of orientin on the cerebral antioxidant system were also assessed (Fig. 6.) The noise-exposed mice were observed to elicit significantly elevated MDA levels in the hippocampus and the prefrontal cortex ($P < 0.001$, $P < 0.001$), and a significant decline in GSH ($P < 0.001$, $P < 0.001$), SOD ($P < 0.01$, $P < 0.01$) and CAT ($P < 0.001$, $P < 0.001$) activity when compared to the control group, respectively (Fig. 6). Treatment with high dose orientin (40 mg/kg) significantly decreased the MDA levels and increased the GSH, SOD and CAT activity in the hippocampus and the prefrontal cortex in noise-exposed mice (all $P < 0.05$). The low dose orientin treatment only decreased the MDA level in the prefrontal cortex ($P < 0.01$), increased the GSH level in the hippocampus and prefrontal cortex ($P < 0.01$, $P < 0.05$) and increased CAT activity in the prefrontal cortex ($P < 0.05$) in the noise-exposed mice.

3.7 Orientin enhances the level of synaptic proteins in the brain of the noise-exposed

mice

In the noise-exposed mice, pre-synaptic, SYN levels significantly decreased as compared with control mice both in the hippocampus and prefrontal cortex ($P < 0.01$, $P < 0.01$, Fig. 7A); however, high dose orientin treatment (40 mg/kg) increased the SYN levels in the hippocampus and prefrontal cortex ($P < 0.01$，$P < 0.05$). The low dose orientin treatment (20 mg/kg) only increased the SYN level in the prefrontal cortex ($P < 0.05$). We also observed a significant reduction in the post-synaptic PSD-95 protein levels in the hippocampus and prefrontal cortex of noise-exposed mice ($P < 0.01$, $P < 0.01$, Fig. 7B). High dose orientin treatment (40 mg/kg) significantly increased in the PSD-95 levels in the hippocampus and prefrontal cortex ($P < 0.01$，$P < 0.05$). The low dose orientin treatment (20 mg/kg) only increased the PSD level in the hippocampus ($P < 0.05$).

3.8 Orientin increases BDNF mRNA and protein expression in the brain of the noise-exposed mice

Increasing evidence has indicated that BDNF can affect synaptic plasticity by regulating the expression of synaptic proteins in the brain [29]. We examined if BDNF also changed with increased synaptic proteins after orientin treatment. The BDNF mRNA levels were significantly decreased in the hippocampus and prefrontal cortex in noise-exposed mice as compared to the control mice (all $P < 0.01$, Fig. 8A). Similarly, BDNF protein expressions in the hippocampus and prefrontal cortex in noise-exposed mice were markedly decreased compared with the control mice (all $P <$

0.01, Fig. 8B). Importantly, both the low and high dose orientin treatments significantly increased the BDNF mRNA and protein expression in the hippocampus and prefrontal cortex compared with the noise-exposed mice (all $P < 0.05$).

3.9 Histological analysis in hippocampus and prefrontal cortex

Microphotographies of the hippocampal CA1 and the cerebral cortex subfield in each group were shown in Fig. 9A and B. In the hippocampal CA1 subfield and cerebral cortex of noise-exposed mice, the neurons were significantly shrunken, irregularly arranged, and weakly stained, which indicates that neurons were diffusely deteriorated or dead. The number of surviving neurons in the hippocampal CA1 subfield and cerebral cortex in noise-exposed mice was significantly lower than that in the control mice (all $P < 0.01$; Fig. 9C). The orientin treatment (20 and 40 mg/kg) significantly increased the number of surviving neurons compared to the noise-exposed mice (all $P < 0.05$).

3.10 Correlation analyses between the Morris water maze test and synaptic proteins

The previous findings indicate that neuronal plasticity may be involved in the cognitive impairment induced by noise exposure [37]. We examined the correlations between the Morris water maze test and the levels of SYN and PSD-95 in the hippocampus and prefrontal cortex. Pearson correlation analysis showed that the escape latency in the test was negatively correlated with levels of SYN in the hippocampus and prefrontal cortex ($r = -0.560$, $P < 0.01$; $r = -0.497$, $P < 0.05$; Fig.

10A, B). The escape latency in the test was negatively correlated with levels of PSD-95 in the hippocampus and prefrontal cortex (r = -0.650, $P < 0.001$; r = -0.584, $P < 0.01$; Fig. 10C, D). The time spent in target quadrant (%) in the test was positively correlated with levels of SYN only in the hippocampus (r = 0.588, $P < 0.01$, Fig. 10E) but not in the prefrontal cortex (r = 0.226, $p > 0.05$; Fig. 10F). The time spent in target quadrant (%) was also positively correlated with levels of PSD-95 in the hippocampus and prefrontal cortex (r = 0.497, $P < 0.05$; r = 0.646, $P < 0.001$; Fig. 10G, H).

4. Discussion

Noise pollution, as an acoustic stressor, is recognized as a serious human health problem in modern society. In the present study, we demonstrated that orientin, a natural polyphenolic compound abundant in millet, passionfruit and bamboo leaves, can function as a potential agent that improve cognition behavior by correcting alterations of corticosterone and catecholamine. Of particular significance was our finding that orientin treatment attenuated oxidative stress and enhanced synaptic proteins and neurotrophic factor within the brain regions associated with cognition.

In clinical studies, it has been reported that chronic aircraft noise is positively correlated with cognitive impairment in children [4, 38]. In this animal study, we found that moderate noise exposure increased escape latency and decreased the time spent in target quadrant in Morris water maze test (reflecting a damage of spatial learning and memory); and decreased step-through latency and increased the number of mistakes in step-through test (reflecting an injury of memory acquisition of the

passive avoidance response, consistent with Cheng's study [28]). Furthermore, we found that in open-field test, the mice with moderate noise exposure had the lower number of crossings and rearings, suggesting decreased exploration and interest in a novel environment. Importantly, long term treatment with orientin both in low and high dose significantly reversed these behavior changes, indicating that orientin exhibits protective potential to noise-induced cognition impairment.

Stress, a physiological response to an environmental demand, usually activates a specific neuroendocrine pathway known as the hypothalamic–pituitary–adrenal (HPA) axis and increases corticosterone [5, 39]. Many studies have reported that elevated corticosterone concentrations contributed to stress-induced impairments in cognitive and learning abilities [8]. In our study, the serum corticosterone level was markedly increased after noise exposure, while treatment with orientin markedly decreased the serum corticosterone level. Furthermore, catecholamines, such as NE and DA, are important monoamines widely distributed in the brain and their functional role during stressful conditions is well-established [11]. It has also been considered that stress-induced cognitive dysfunction is associated with the depletion of monoamines by sustained stress [40]. In the current study, orientin treatment markedly reversed the reductions of NE and DA induced by chronic noise exposure. The NE and DA neurotransmitter systems are involved in learning and memory processes, and a substantial part of learning and memory impairment is due to changes in neurotransmission [41]. Therefore, the reversal of the noise-induced alteration of the levels of NE and DA may contribute to orientin's effect in improving the cognitive

impairments induced by chronic noise exposure.

The central nervous system (CNS), compared with peripheral tissues, is most vulnerable to free radical damage due to oxidative stress, including enhanced oxygen consumption, abundant lipid content, and the relative paucity of antioxidant enzymes [42]. Chronic stress can stimulate oxidative stress and the increased production of free radicals, which may contribute to cognition impairment [12, 13]. In the present study, chronic noise resulted in increased lipid peroxidation and decreased endogenous antioxidant activity in the hippocampus and prefrontal cortex of the mice with cognition impairment. Some flavonoids have been demonstrated to have strong antioxidant properties and enhance memory and learning [43]. Furthermore, the supplementation with bamboo leaf extract (rich in orientin) increased the total glutathione content in blood in high-fat diet-fed mice [44]. Orientin treatment significantly decreased the MDA level in the hippocampus of the aging mouse model [26]. In the present study, the chronic orientin treatment significantly inhibited the MDA level and increased the glutathione level and SOD and CAT activity in the hippocampus and prefrontal cortex of our noise exposed mice. Therefore, the present results support the hypothesis that orientin could inhibit the chronic noise-induced pro-oxidant–antioxidant disequilibrium contributing to its effect on improving cognition.

Studies previously found that prolonged elevations in corticosterone secretion is mediated by reduction of hippocampal BDNF expression in young rats with chronic

stress [45], suggesting that chronic stress is inter-mediated by corticosterone and BDNF. To further study the signalling pathways linked to effects of orientin in improving cognition in chronic noise-induced stress, we examined the expression of BDNF mRNA, an important neurotrophin in modulating synaptic plasticity [46]. The role of BDNF in regulating synaptic plasticity is essential to the cellular processes underlying learning, memory, and other cognitive behavior [17, 47]. Previously, it has been reported that BDNF protein levels are reduced in the hippocampus of rat exposed to the chronic mild stress [48]. Here, we demonstrated that chronic moderate noise exposure significantly reduced BDNF mRNA expressions and protein level in the hippocampus and prefrontal cortex of mice. Recent studies support the notion that decreased plasma BDNF is a biomarker for memory impairment and decreased general cognitive function in aged women and schizophrenia patients [49, 50]. Furthermore, inhibiting BDNF action in the hippocampus blocked the benefit of exercise on cognitive function, including the learning and recall abilities in rats [17]. In our study, the potential of oriention in improving cognition may be associated with its ability to increase BDNF mRNA expression and protein levels, and synaptic plasticity in the hippocampus and prefrontal cortex.

Mounting evidence has suggested that cognition is usually coordinated with an array of synapse-associated proteins [51]. SYN is the major pre-synaptic vesicle protein marker which regulates synaptic function, vesicle fusion, and neurotransmitter release [52]. It has been demonstrated that impairments in learning and memory, most notably reduced object novelty recognition and reduced spatial learning are observed

in synaptophysin knockout mice [53]. PSD-95, a major post-synaptic component of synaptic plasticity, is an important regulator of synaptic strength and plasticity [54]. The level of PSD-95 is significantly decreased in the hippocampus of subjects with mild cognitive impairment [55]. Thus, in this study, the reduction in synaptic proteins in the noise-exposure model may contribute to cognitive impairment. More importantly, we found that chronic treatment with orientin significantly reversed this noise-induced reduction in both SYN and PSD-95 levels. Both SYN and PSD-95 are regulated by BDNF and its subsequent signaling GSK-3β [18-20]. Thus, these neurochemical results indicate that the neuroprotect effect of orientin may be due to its ability to increase both BDNF and the synaptic plasticity-associated structural proteins (SYN and PSD-95) and thus improving cognitive behaviors in noise-exposed mice.

In conclusion, the present study indicated that orientin exhibited protective effects on the cognition decline induced by chronic noise exposure in mice. The orientin treatment also corrected the neuroendocrine changes (corticosterone and catecholamine), decreased oxidative stress and increased neurotrophin BDNF and the synapse-associated proteins in the hippocampus and prefrontal cortex of noise exposed mice. These behavioral and neurochemical improvements suggest that supplementation with orientin-enriched food or fruit could be a promising novel treatment to improve chronic noise-induced cognition decline.

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Figure captions

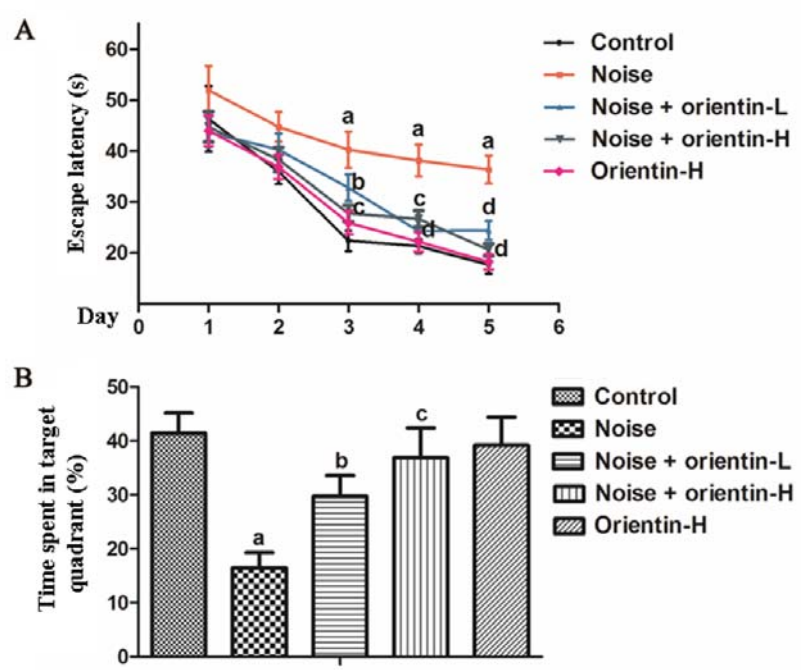

Fig. 1. Orientin enhances the spatial learning and memory in the noise-exposed mice. Escape latency appeared during the training and the probe sessions (A). The percentage of time spent in the target quadrant during the probe trial (B). Data are reported as mean±SEM (n=14). $^{a}P < 0.001$ vs. the control group; $^{b}P < 0.05$, $^{c}P < 0.01$, $^{d}P < 0.001$ vs. the noise group.

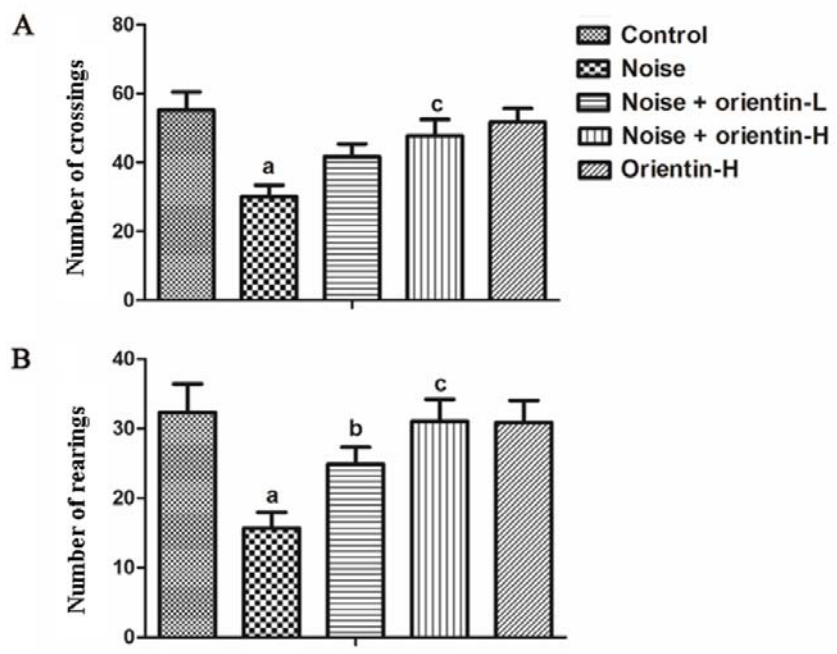

Fig. 2. Orientin improves the exploratory and locomotor activities in the noise-exposed mice by examining number of crossings (A) and number of rearings (B). Data are reported as mean±SEM (n=14). $^{a}P < 0.01$ vs. the control group; $^{b}P < 0.05$, $^{c}P < 0.01$ vs. the noise group.

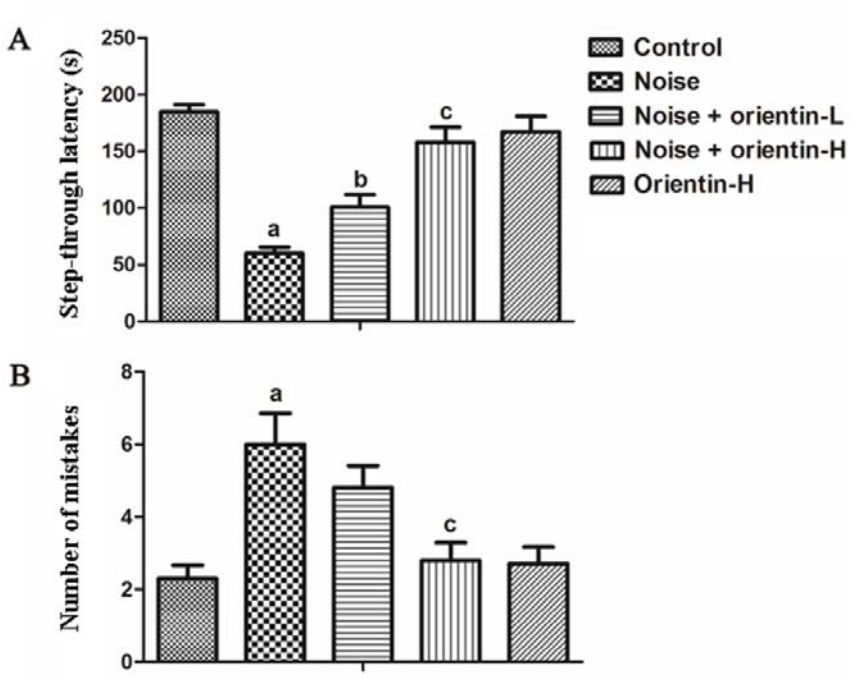

Fig. 3. Orientin improves memory acquisition of the passive avoidance response in the noise-exposed mice by examining step-through latency is shown (A) and number of mistakes (B). Data are reported as mean±SEM (n=14). $^{a}P < 0.001$ vs. the control group; $^{b}P < 0.01$, $^{c}P < 0.001$ vs. the noise group.

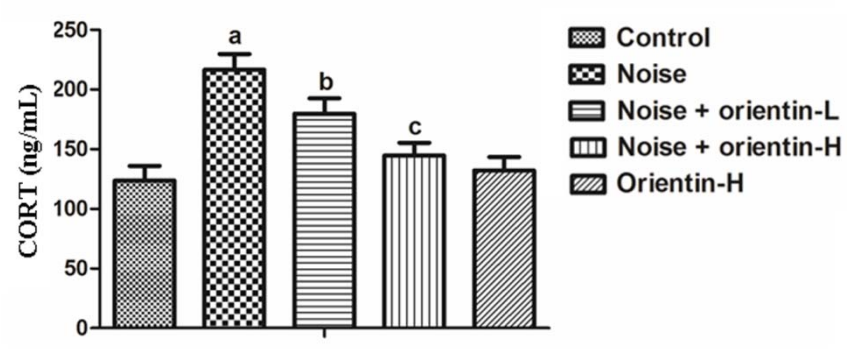

Fig. 4. Orientin decreases serum CORT concentration in the noise-exposed mice. Data are reported as mean±SEM (n=6). $^{a}P < 0.01$ vs. the control group; $^{b}P < 0.05$, $^{c}P < 0.01$ vs. the noise group.

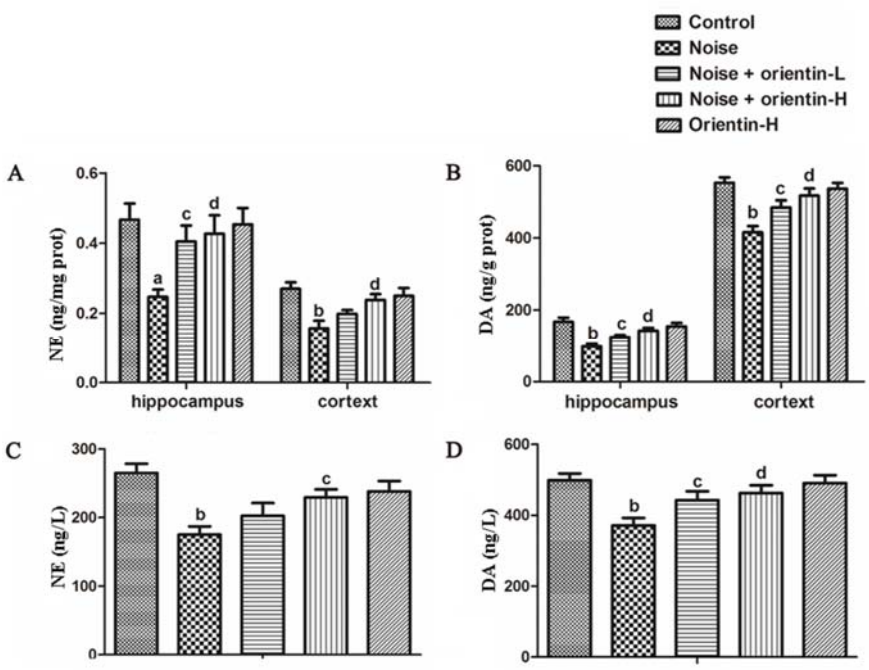

Fig. 5. Orientin inhibits biogenic catecholamine reduction in the brain (A and B) and serum (C and D). Data are showed as mean±SEM (n=6). $^{a}P < 0.05$, $^{b}P < 0.01$ vs. the control group; $^{c}P < 0.05$, $^{d}P < 0.01$ vs. the noise group.

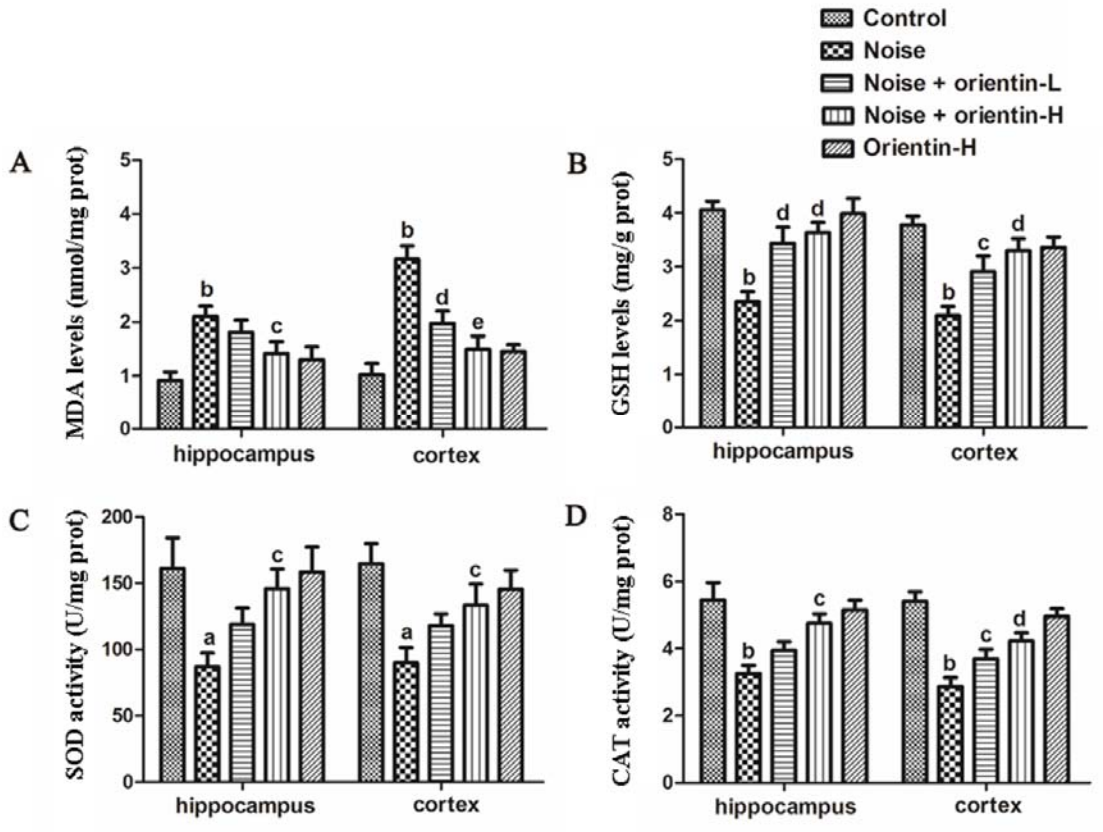

Fig. 6. Orientin attenuates oxidative stress by decreasing the levels of MDA (A), and increasing the

GSH level (B), and SOD (C) and CAT (D) activity in the hippocampus and prefrontal cortex of the noise-exposed mice. Data are reported as mean±SEM (n=6). [a]$P < 0.01$, [b]$P < 0.001$ vs. control group; [c]$P < 0.05$, [d]$P < 0.01$, [e]$P < 0.001$ vs. the noise group.

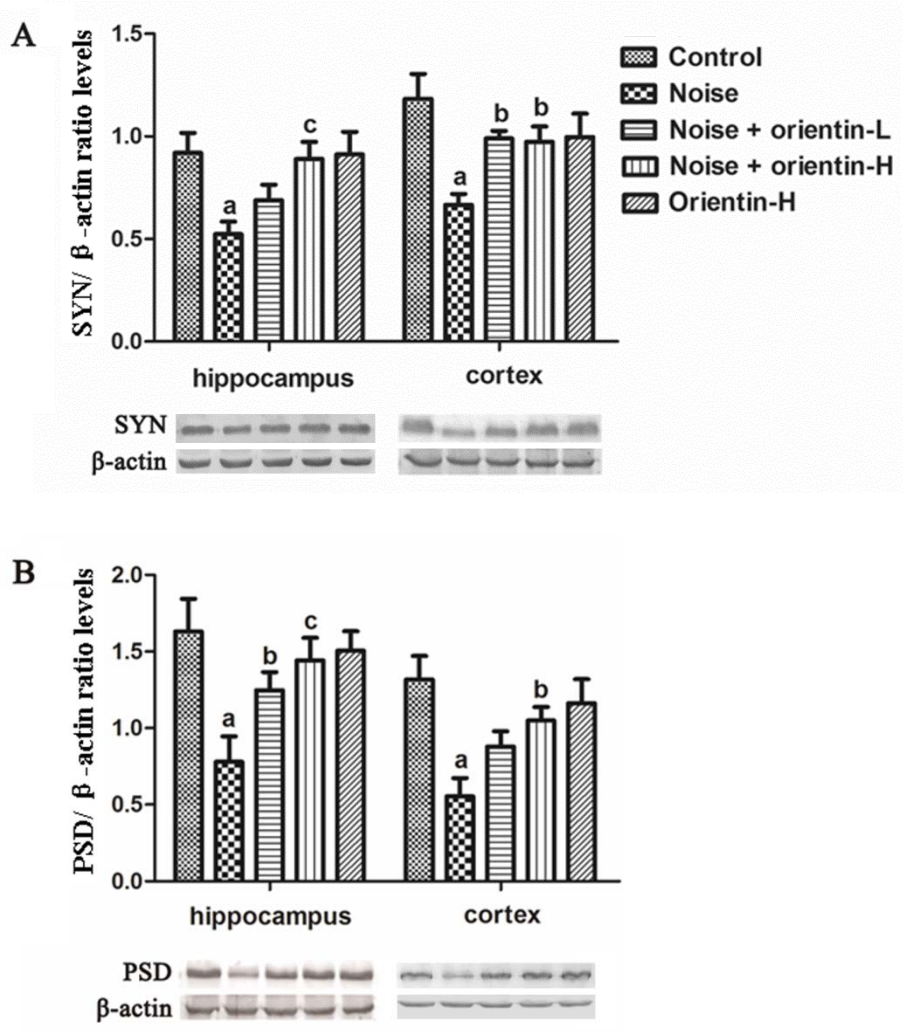

Fig. 7. Orientin enhanced synaptic proteins levels in the brain of noise-exposed mice. The protein bands of SYN (A) and PSD-95 (B) in cerebral cortex and hippocampus were detected by western blotting. β-actin was used as the internal standard in each sample. Data are reported as mean±SEM (n=5). [a]$P < 0.01$ vs. the control group; [b]$P < 0.05$, [c]$P < 0.01$ vs. the noise group.

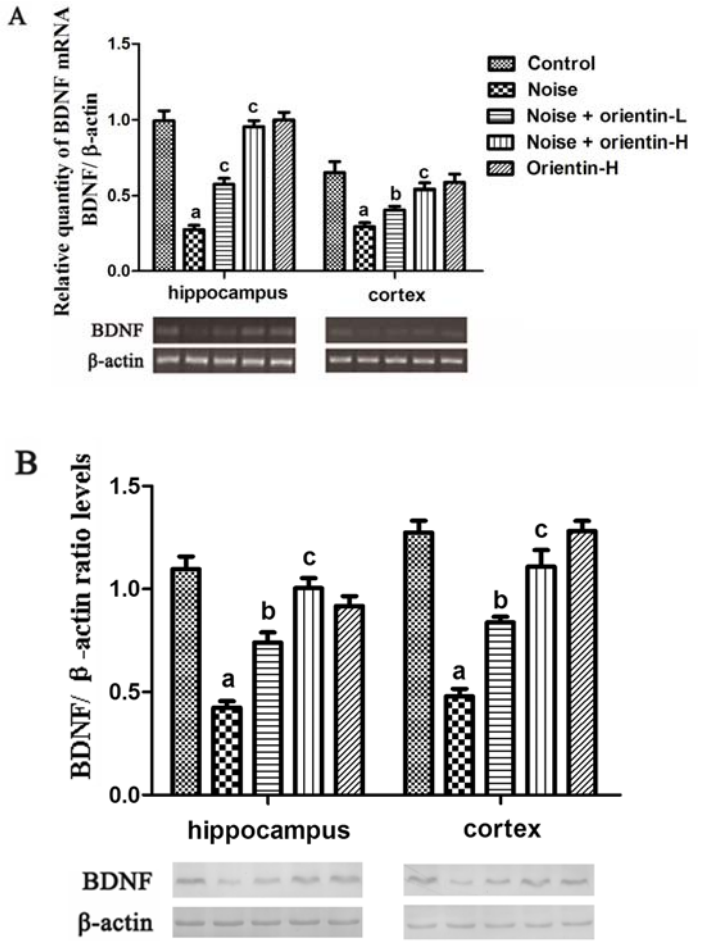

Fig.8. Orientin increases BDNF mRNA levels (A) and BDNF protein expressions (B) in the hippocampus and prefrontal cortex of noise-exposed mice. Data are reported as mean±SEM (n=5). $^{a}P <$ 0.01 vs. the control group; $^{b}P < 0.05$, $^{c}P < 0.01$ vs. the noise group.

Fig.9

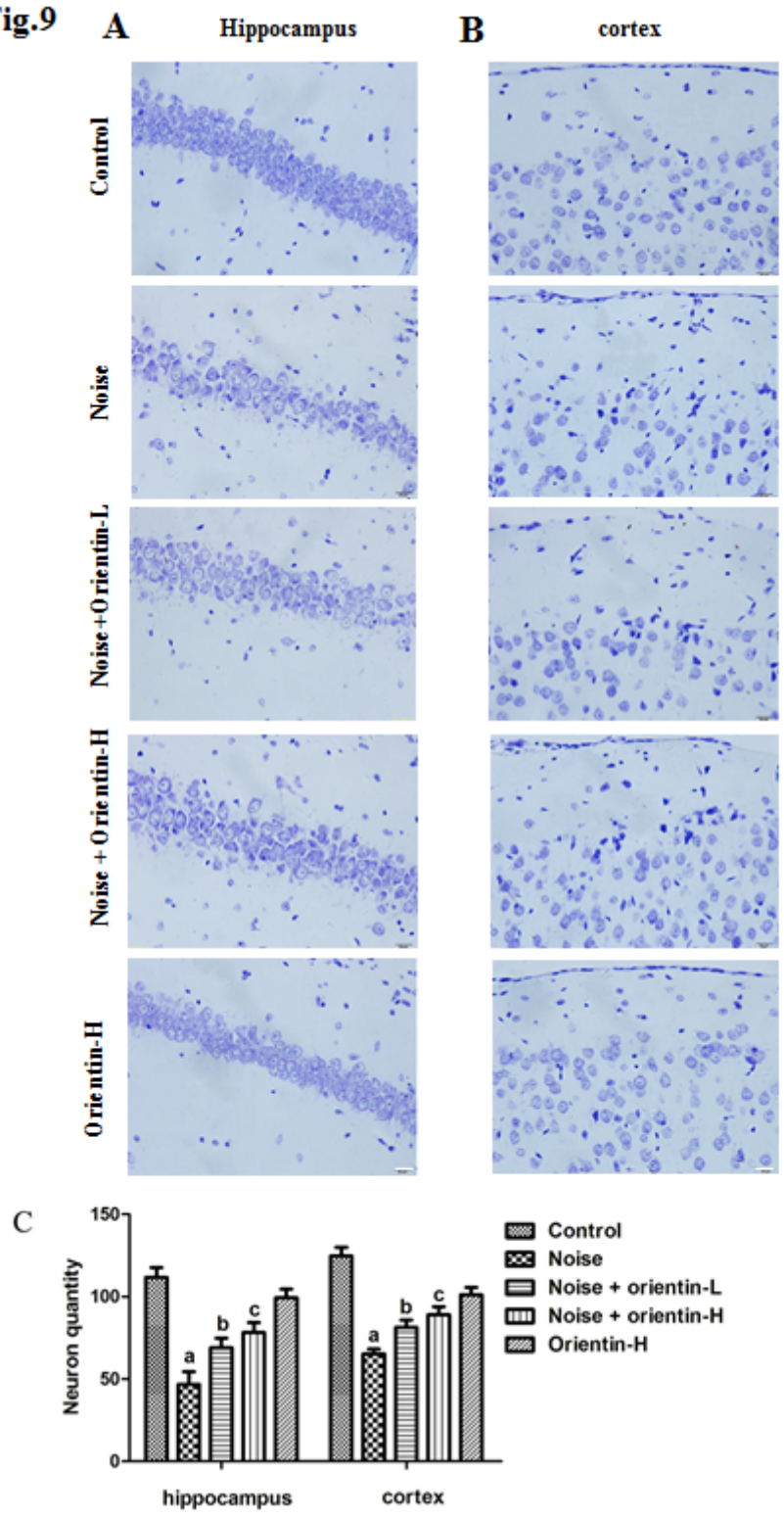

Fig.9 Nissl stained neurons in the hippocampal CA1 subfield (A) and cortex (B). The population of intact cells in the hippocampal CA1 subfield and cortex were counted as shown in (C). Data are reported as mean±SEM (n=3). $^{a}P < 0.01$, vs. the control group; $^{b}P < 0.05$, $^{c}P < 0.01$ vs. the noise group. Bar=20 μm.

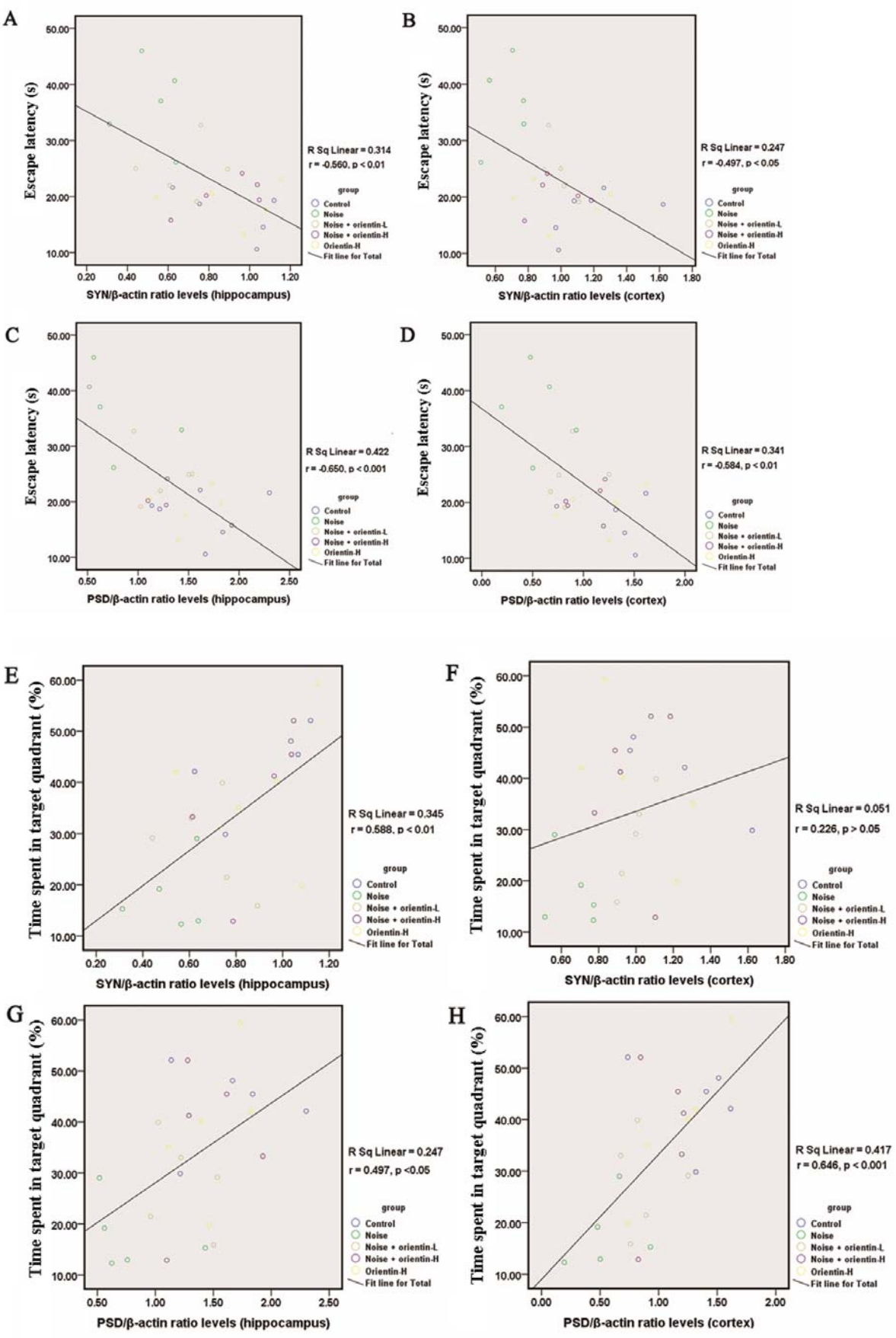

Fig. 10. Correlations between escape latency in the Morris water maze test and the level of SYN (A

and B) and PSD-95 (C, D) in hippocampus and prefrontal cortex. Correlations between the time spent in target quadrant (%) and the levels of SYN (E, F) and PSD-95 (G, H) in the hippocampus and prefrontal cortex.

Table 1. Sequences and annealing temperatures of the oligo primers used in this study

Target mRNA sequences	Primer sequence	Annealing Tm
BDNF	5’ GATGCCGCAAACATGTCTATGA 3’	59
	5’ TAATACTGTCACACACGCTCAGCTC 3’	
β-actin	5’ ATGGTCACGCACGATTTCCC 3’	59
	5’ GAGACCTTCAACACCCCAGC 3’	