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HIPPOCAMPAL UCP2 IS ESSENTIAL FOR COGNITION AND RESISTANCE TO ANXIETY BUT NOT REQUIRED FOR THE BENEFITS OF EXERCISE

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Abstract—Uncoupling protein-2 (UCP2) reduces oxidative stress by facilitating the influx of protons into mitochondrial matrix, thus dissociating mitochondrial oxidation from ATP synthesis. UCP2 is expressed abundantly in brain areas and plays a key role in neuroprotection. Here, we sought to determine if UCP2 deficiency produces cognitive impairment and anxiety in young mice, and to determine if hippocampal UCP2 is essential for the beneficial effects of voluntary exercise. Antisense oligonucleotide (ASO) was used to produce UCP2 knockdown in mice. Our results firstly showed that UCP2-targeted ASO significantly reduced UCP2 mRNA and protein expression in the hippocampus. ASO treatment impaired learning and memory of the mice in Y-maze, T-maze, and object recognition tests (ORT). ASO-treated mice exhibited more anxiously in OPT, light/dark box test, and elevated plus maze (EPM) than the control mice. We also found that wheel running ameliorated cognitive dysfunction and anxiety-like behaviors in ASO-treated mice. Furthermore, voluntary exercise reversed ASO-induced changes in hippocampal levels of serotonin (5-HT), dopamine (DA), and norepinephrine (NE). However, UCP2 protein in the hippocampus was not correlated with cognitive and anxiolytic benefits of exercise. These findings suggest that hippocampal UCP2 is essential for cognitive function and the resistance to anxiety of mice, but not required for the beneficial effects of exercise.

Key words: hippocampus, uncoupling protein-2, voluntary exercise, cognition, anxiety, antisense oligonucleotide.

INTRODUCTION

Uncoupling proteins (UCPs) can dissociate mitochondrial oxidation from phosphorylation, so they reduce mitochondrial energy production and increase cell thermogenesis (Ricquier and Bouillaud, 2000; Azzu and Brand, 2010). Of these proteins, uncoupling protein-2 (UCP2) is expressed abundantly in several tissues. In the rodent brain, expression of UCP2 mRNA is abundant in the ventral septal region, hypothalamus, medulla, ventricular regions and cerebellum (Richard et al., 1999). In the primate brain, UCP2 protein is evolutionary conserved and expressed abundantly in the pituitary gland and the hypothalamus. Throughout the rodent and the primate, UCP2 is involved in central autonomic, endocrine and metabolic regulation (Diano et al., 2000). Therefore, UCP2 deficiency may disorder the endocrine, mitochondrial bioenergetics and thermoregulation of the neurons, which are associated with cognition, mood and behavior. In this study, roles of UCP2 in cognition and anxiety have been investigated using UCP2-deficient mice.

The hippocampus is a key brain structure to regulate both cognition and emotion (Cornwell et al., 2012). Recently, UCP2 has been shown to play a critical role in the development of hippocampal neurons and hippocampus-related behaviors. Inhibition of UCP2 diminished neuronal number and size, dendritic growth and synaptogenesis in hippocampi and further impaired locomotor activity and spatial memory in the adult mice (Simon-Areces et al., 2012). Peripheral ghrelin promoted learning and memory and reward-seeking behavior via UCP2-dependent mitochondrial respiration, ROS production, and mitochondrial biogenesis (Andrews et al., 2009). Similarly, it was through AMPK/UCP2 pathway that chronic dietary curcumin improved cerebrovascular dysfunction and cognitive decline in aging mice (Pu et al., 2013). In an anxiety-like behavioral study, UCP2-knockout mice were significantly more anxious in the elevated plus-maze than wild-type mice (Gimsa et al., 2011). Paradoxically, UCP2-knockout mice exhibited increased locomotion

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Abbreviations: 5-HT, serotonin; ANOVA, analysis of variance; ASO, antisense oligonucleotides; BSA, bovine serum albumin; DA, dopamine; EDTA, ethylenediaminetetraacetic acid; EPM, elevated plus maze; EX, exercise control; HPLC, high-performance liquid chromatography; NE, norepinephrine; OFT, open field test; ORT, object recognition test; PVDF, polyvinylidene fluoride; SDS, sodium dodecyl sulfate; SED, sedentary control; UCP2, uncoupling protein-2.

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activity in another relative behavioral study (Alves-Guerra et al., 2012). Most of these studies appeared to implicate a necessary role of UCP2 in the maintenance of cognitive ability and the resistance to anxiety.

Physical activity and exercise has been shown to improve cognitive function and anxiety disorders in rodents and human, while the effects of exercise are contingent depending on exercise protocol in some case (Trejo et al., 2008; Pietrelli et al., 2012; Jayakody et al., 2014). Significant data indicated that the beneficial effects of exercise, including improved cognition and reduced anxiety, depended on the level of serum insulin-like growth factor I and related to increased hippocampal neurogenesis (Trejo et al., 2008). Also, the cognitive benefits of exercise depended on allelic polymorphism of brain-derived neurotrophic factor (BDNF). Hopkins et al. (2012) suggested that separate neural mechanisms mediate cognition and anxiety mood, because the cognitive benefits of exercise were not correlated with mood changes. Importantly, in the animal studies of cognition and behavior, forced and voluntary exercises are inherently different. Forced but not voluntary exercise was found to increase anxiety-like behaviors in the open field test (OFT), although forced exercise ultimately produced more neurons than voluntary exercise (Leasure and Jones, 2008). Thus, increased neurogenesis may not be required for the benefits of exercise. Assuming that UCP2 is required for rodent cognitive ability and mood control, we are very much interested to examine whether the behavioral effects of voluntary exercise are related to UCP2 expression in the hippocampus.

EXPERIMENTAL PROCEDURES

Animal care

Male C57BL/6J mice at 6 weeks of age were housed in standard cages under controlled conditions of temperature (23 ± 2 °C), relative humidity (50 ± 5%), and lighting (lights on from 7:00 to 19:00). They were given free access to water and food. After one-week habituation, the animals were randomly assigned to four groups: (1) Sedentary control (SED), (2) Sedentary ASO-treated (SED + ASO), (3) Exercise control (EX), and (4) Exercise ASO-treated (EX + ASO). UCP2-targeted antisense oligonucleotides (ASO, 5′-TGAGATCT GCAATGCA-3′) were produced according to the Mus musculus UCP2 sequence as described previously (De Souza et al., 2007). ISIS 141923 (5′-CCTTCCCTGAAGGTTCCTCC-3′), which has the same chemical composition as the UCP2 ASO but no complementarity to any known gene sequence, was used as a control ASO. The ASOs were diluted to a final concentration of 50 nmol/ml in dilution buffer (10 mmol/L Tris–HCl and 1.0 mmol/L EDTA). UCP2 ASO and control ASO solutions were injected i.p. twice a week (50 mg/kg/week) for 8 weeks. During this period, the mice assigned into the exercise-treated group were allowed 8 weeks of continuous voluntary access to a rodent wheel running. Running distance was monitored using magnetic counters that totaled the number of laps each mouse ran per day. All behavioral tests were carried out at 13–15 weeks of age (Fig. 1A). All animal experiments were conducted in accordance with the guidelines for the use of laboratory animals published by China Ministry of Health and a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at our university.

Behavioral tests

Y-maze test was used to investigate hippocampal-dependent reference memory. We used the ‘‘paddling’’ Y-maze visuo-spatial task as previously described (Cunningham et al., 2009; Murray et al., 2013). A clear perspex Y-maze was filled with 2 cm of water at 20–22 °C, sufficient to motivate mice to leave the maze by paddling to an exit tube at the distal end of one arm, 2 cm above the floor. The mouse exits to a tube in which it is returned to its home cage. Mice were placed in one of two possible start arms in a pseudorandomized sequence for 10 trials and the groups were counter-balanced with respect to the location of the exit and start arm. For any individual mouse the exit arm was fixed. The task was conducted for 20 trials. An arm entry was defined as entry of the whole body, excluding the tail. A correct trial was defined as entry to the exit arm without entering other arms.

T-maze test was used to assess hippocampal-dependent working memory. We used the paddling T-maze task as previously described (Murray et al., 2012, 2013). Each mouse was placed in the start arm of the maze with one arm blocked such that they were forced to make a left (or right) turn, selected in a pseudorandomized sequence (equal numbers of left and right turns, no more than two consecutive runs to the same arm). On taking the turn the mouse could escape from the shallow water and was held in a holding cage for 25 s (intra trial interval) during which time the guillotine door was removed and the exit tube was switched to the alternate arm. The mouse was then replaced in the start arm and could choose either arm. The mouse must alternate from its original arm to escape. Correct trials were recorded when the mouse alternated from its original turn and exited the maze. On choosing correctly mice escape from the maze and are returned to their home cage. On choosing incorrectly, the mice were allowed to self-correct to find the correct exit arm. Mice were trained for blocks of ten trials for 12 days (20 min inter-trial interval).

Object recognition test (*ORT*) of working memory, which is based on the natural and robust tendency of rodents to preferentially explore novel objects, was performed as described elsewhere (Lee et al., 2013). After the mice were transferred to a cage for the ORT and acclimated for 24 h, they were exposed to two differently shaped objects for 10 min. The number of actions of exploring and/or sniffing two objects was counted for the initial 5-min period (Training). The next day, to examine memory retention, one of the original objects was replaced with a novel one with a different shape, and then the number of actions of exploring and/or sniffing the novel object was counted for 5 min (Retention). The recognition index was calculated by dividing the number of actions of exploring and/or sniffing the novel object by

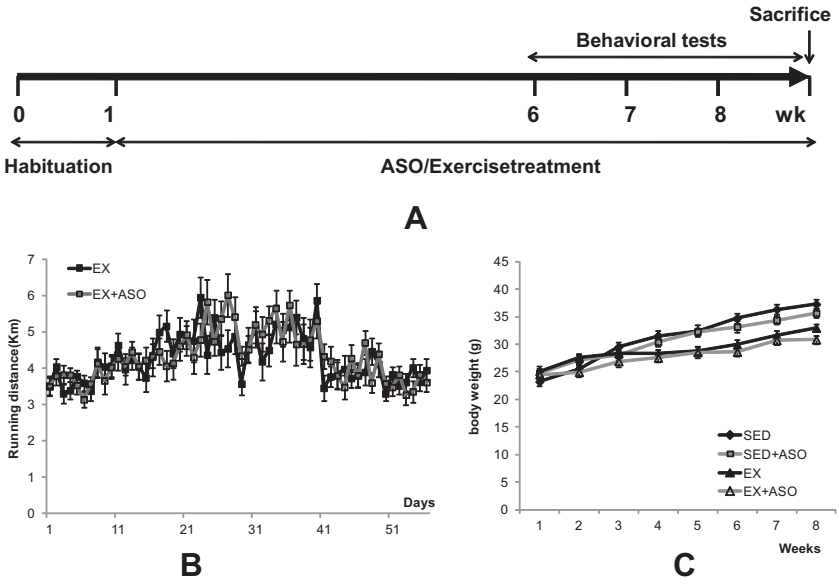

Fig. 1. Experimental procedures (A), running distance (B) and body weight (C). Habituation: habituation to cage, food and rodent wheel running (if any); ASO: antisense oligonucleotides; Behavioral tests order: Y-maze test, T-maze test, object recognition test (ORT), open field test (OFT), light–dark exploration test (LET), and elevated plus maze (EPM). Running distance (km) per day by exercised mice over eight weeks of running wheel access and body weight (g) per week are expressed as mean ± SEM (n = 12). Significance is not shown in the figure.

the total number of actions of exploring and/or sniffing (novel object + familiar object).

OFT was used to measure spontaneous locomotor activity. According to previous methods (Ma et al., 2011), the apparatus consisted of a square box with dimensions, 45 cm × 45 cm × 45 cm. Mice were placed at the center of the open box under a dark light and allowed to explore the arena for 60 min. A video-computerized tracking system was used to record the distance traveled as a measure of locomotor activity.

Light–dark exploration test (*LET*) was used to assess the anxiety-like behavior as described elsewhere (Fukui et al., 2007). The apparatus consisted of a mouse shuttle box where the chambers (20 × 16 × 20 cm/chamber) were separated by an automated sliding door. One chamber was illuminated with a high-intensity light, whereas the other was enclosed by a black cloth. Mice were placed on the lighted side; 10 s later the door to the adjoining chamber was opened and mice were given free access to the entire apparatus for 5 min. The time spent on each side and head pokes into light box were used to assess the anxiety-like behavior.

Elevated plus maze (*EPM*) was used to test for anxiety-like behavior in mice as previously described (Murray et al., 2013). The maze consists of four arms (two open without walls denoted North/South and two enclosed by high walls denoted East/West) 35 cm long and 5 cm wide. The maze was elevated 45 cm above the surface it was placed on. The mouse was placed in a start arm which was a closed arm. The groups were counterbalanced with respect to start arms. The time spent in the open and closed arms, latency to first emerge from a closed arm and the number of open and closed arm entries were recorded for five minutes. The number of entries and the time spent in the junction were also observed. When at the junction the mouse was regarded as being neither in an open arm nor in a closed arm.

Neurotransmitter analyses

Mice were sacrificed by decapitation after completing all of the behavioral tests. Hippocampi was immediately dissected out and kept at −80 °C for all measurements. Assay for serotonin (5-HT), dopamine (DA) as well as norepinephrine (NE) was performed by high-performance liquid chromatography (HPLC), according to Georgy et al. (2013). For the determination of these neurotransmitters, a 10% (w/v) homogenate was prepared in a 75% methanol for HPLC. Each homogenate was centrifuged at 1000g (4 °C) for 10 min. The supernatant was filtered (0.45 μm; Millipore), and then 20 μl were injected into an ODS-reversed phase column. The mobile phase consisted of potassium phosphate buffer:methanol [97:3 (v)] at a flow rate of 1.5 ml/min, and the corresponding peaks were detected at 270 nm.

Quantitative real-time PCR

Total RNA was extracted from snap frozen tissues with RNeasy Mini Prep (Qiagen) kits according to the manufacturer's instructions. cDNA generated by Superscript II enzyme (Qiagen) was analyzed by quantitative RT-PCR using SYBR Green based on real-time detection of accumulated fluorescence (ABI Prism 7700, Perkin-Elmer Inc., USA). Primer sequences used for UCP2 (NM_011671) mRNA quantification were: Forward ACTTCA CTTCTGCCTTCGGG and Reverse AGGAGGTGGGTTGCTATGTG, and Primer sequences used for β-actin (NM_007393) were: Forward GCGGAC TGTTACTGAGCTGCGT and Reverse GAAGCAAT GCTGTCACCTTCCC.

Western blots

Tissues were homogenized in RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA) supplemented with 1 mM PMSF, 1 mM Na3V02, 10 mM NaF, Phosphatases inhibitor cocktails (Beyotime; Shanghai, China). Homogenates were centrifuged at 10,000*g*/10 min at 4 °C. Supernatant protein was assayed by bicinchonininc acid (BCA) (BioRad; Hercules, California, USA) using bovine serum albumin (BSA) as a standard. For Western blots, 20 μg of total protein in sodium dodecyl sulfate (SDS) buffer was electrophoresed on SDS–polyacrylamide gels, followed by transfer to polyvinylidene fluoride (PVDF) membranes; PVDF membranes were blocked with 5% BSA for 1 h and probed with primary antibodies overnight at 4 °C, followed by secondary antibodies (1:10,000). Protein bands were quantified by Odyssey V3.0 software. α-Tubulin (1:10,000, Sigma; St. Louis, MO, USA) were loading controls.

Statistics

Data were expressed by mean ± S.E. Effects of factors were analyzed by using ANOVAs (analyses of variance) (two-way ANOVA with post hoc tests and General Linear Model). Differences were defined as statistically significant when $P < 0.05$.

RESULTS

Running distance and body weight

Overall there was no significant main effect of UCP2 ASO for running distance (km) per day ($F(1,22) = 1.95$, $p > 0.05$; Fig. 1B). EX mice ran an average of 4.28 km/day and EX + ASO mice ran an average of 4.32 km/day. Additionally, there was a significant main effect of Day for running distance per day ($F(56,1320) = 5.68$; $p < 0.01$; Fig. 1B). Mouse weights were recorded weekly as shown in Fig. 1C. Wheel running and ASO produced main effect (Exercise, $F(1,44) = 42.05$, $p < 0.01$; ASO, $F(1,44) = 7.17$, $p < 0.05$), which indicated that both exercise and UCP2 ASO reduced the body weight of mice (Fig. 1C). No significant interaction between exercise and ASO was found ($F(1,44) = 0.13$, $p > 0.05$; Fig. 1C).

Learning and memory

We assessed the performance of all mice in a visuo-spatial reference memory Y-maze task across 20 trials (Fig. 2A). A two-way ANOVA indicated that wheel running and ASO produced the main effect (Exercise, $F(1,44) = 19.33$, $p < 0.01$; ASO, $F(1,44) = 9.36$, $p < 0.01$), whereas we found no significant interaction between exercise and ASO ($F(1,44) = 0.58$, $p > 0.05$; Fig. 2A). UCP2 ASO impaired a visuo-spatial reference memory of mice ($P < 0.05$), while exercise elevated the performance on this task ($P < 0.05$). Significantly, having access to wheel running reversed ASO-induced cognitive impairment on learning of the task ($P < 0.01$; Fig. 2A). In the T-maze test, the percentage of alternations positively correlates with the cognitive ability of mice. UCP2 ASO produced a significant reduction in the percentage of alternations ($F(1,44) = 120.74$, $P < 0.01$; Fig. 2B). Exercise prevented the reduction in the percentage of alternations induced by ASO ($F(1,44) = 39.75$, $P < 0.01$; Fig. 2B). There was a significant main effect of Exercise × ASO interaction for the percentage of alternations ($F(1,44) = 31.18$, $P < 0.01$; Fig. 2B).

To examine the effect of ASO and exercise on learning and memory, all mice were subjected to ORT in our experiment (Fig. 2C). The control mice showed a strong preference for a novel object, whereas ASO treatment induced a significant decrease in the preference for a novel object ($F(1,44) = 4.61$, $P < 0.05$; Fig. 2C). In addition, wheel running induced a significantly stronger preference for a novel object ($F(1,44) = 7.62$, $P < 0.01$; Fig. 2C). Exercise × ASO interaction was also significant in ORT test ($F(1,44) = 5.29$, $P < 0.05$; Fig. 2C). Our findings suggested that UCP2 ASO produced the impairment of cognitive ability of mice, and that voluntary exercise prevented the impairment of learning and memory.

Anxiety-like behaviors

In the OFT, an ANOVA revealed that ASO produced main effect on number of entries into the central zone ($F(1,44) = 51.72$, $P < 0.01$) and retention time in the central zone ($F(1,44) = 4.34$, $P < 0.05$; Fig. 3B, C). The mice treated with UCP2 ASO showed significantly decreased total distance ($P < 0.05$; Fig. 3A), number of entries into the central zone ($P < 0.01$; Fig. 3B), and retention time in the central zone ($P < 0.01$; Fig. 3C), compared with the SED mice. Also, wheel running produced differential effect on total distance ($F(1,44) = 7.35$, $P < 0.01$), number of entries into the central zone ($F(1,44) = 23.09$, $P < 0.01$), and retention time in the central zone ($F(1,44) = 17.86$, $P < 0.01$; Fig. 3A–C). A significant interaction between wheel running and ASO was returned on number of entries into the central zone ($F(1,44) = 5.85$, $P < 0.05$). Compared to SED + ASO mice, wheel running had a reversal effect on ASO-induced anxiety-like behaviors ($P < 0.01$; Fig. 3A–C).

In the light/dark box test, an ANOVA revealed that ASO produced main effect on the number of entries into light box ($F(1,44) = 11.51$, $P < 0.01$) and the retention time spent in the light box ($F(1,44) = 7.29$, $P < 0.05$; Fig. 4A, B). UCP2 ASO significantly decreased the number of entries into light box and the retention time spent in the light box, compared with the SED mice ($P < 0.01$; Fig. 4A, B). Also, exercise produced differential effect on the retention time spent in the light box ($F(1,44) = 4.84$, $P < 0.05$; Fig. 4B). We found a significant interaction between exercise and ASO on the number of entries into light box ($F(1,44) = 8.89$, $P < 0.01$). Compared to SED + ASO mice, the number of entries into light box and the retention time spent in the light box were both increased in EX + ASO mice ($P < 0.05$, Fig. 4A, B).

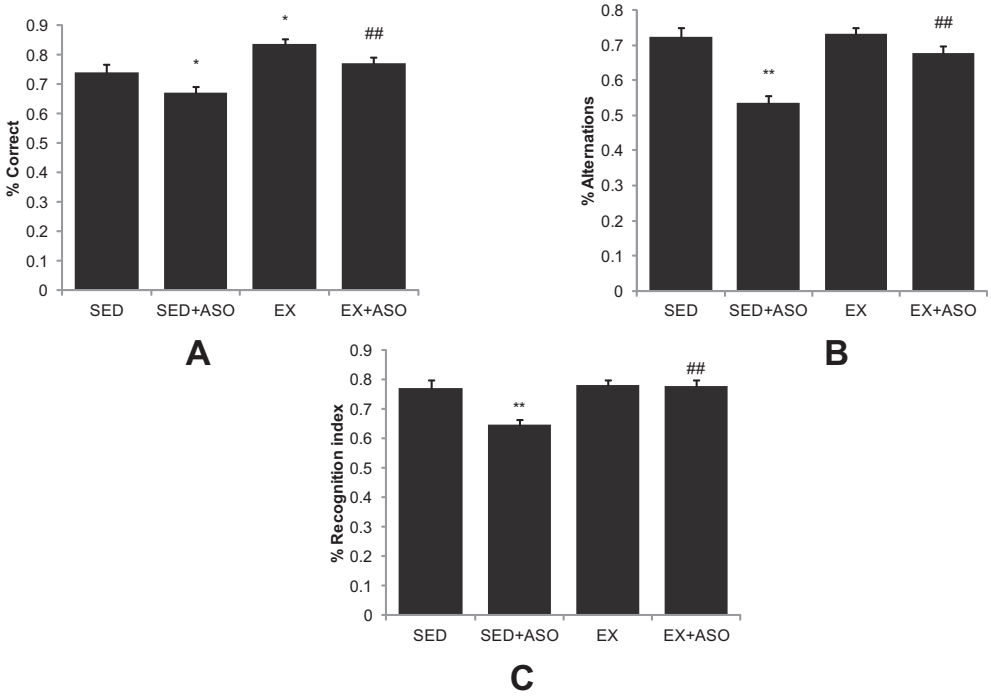

Fig. 2. Exercise reverses ASO-induced cognitive deficits in Y-maze test (A), T-maze test (B), and Object recognition test (C). Data are expressed as mean ± SEM (n = 12). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. SED; $^{\#\#}p < 0.01$ vs. SED + ASO.

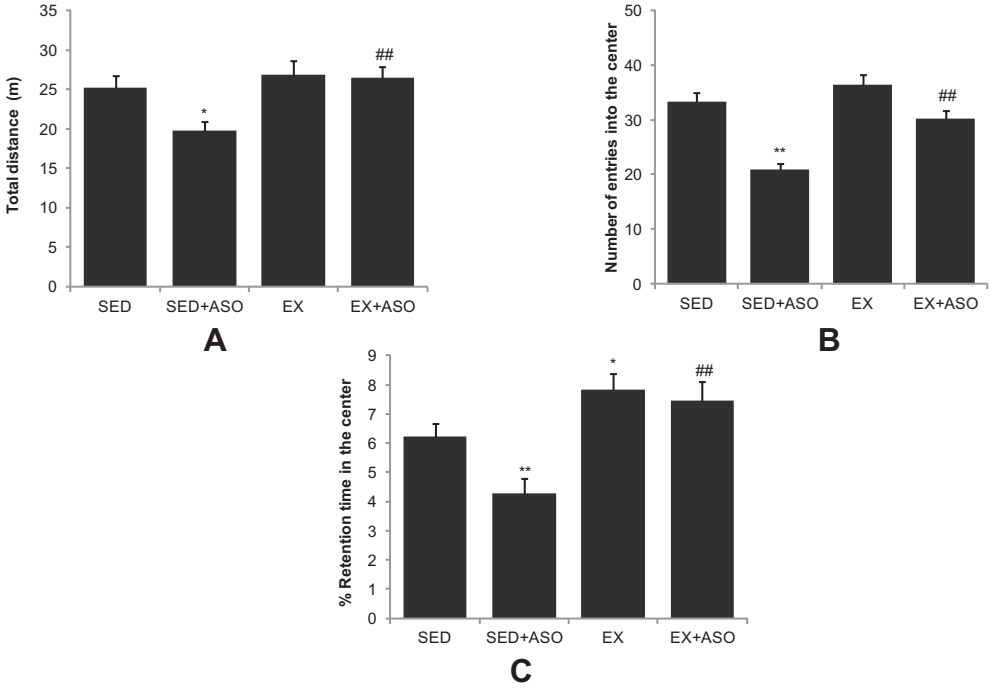

Fig. 3. Exercise reverses ASO-induced anxiety-like behaviors in the open field test. Data are expressed as mean ± SEM (n = 12). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. SED; $^{\#\#}p < 0.01$ vs. SED + ASO.

In EPM test, an ANOVA revealed that ASO produced main effect on the time spent in the open arms ($F(1,44) = 12.54$, $P < 0.01$; Fig. 5A). UCP2 ASO significantly decreased the retention time in the open arms ($P < 0.01$; Fig. 5A) and the number of entries into the open arms ($P < 0.05$; Fig. 5B). Also, exercise produced differential effect on the number of entries into the open arms ($F(1,44) = 10.55$, $P < 0.01$; Fig. 5B). There was a significant interaction between exercise and ASO on the number of entries into the open arms

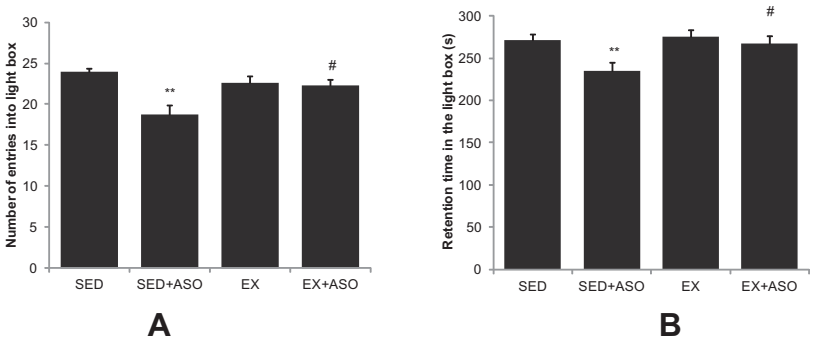

Fig. 4. Exercise reverses ASO-induced anxiety-like behaviors in the light–dark exploration test. Data are expressed as mean ± SEM (n = 12). $^{**}p < 0.01$ vs. SED; $^{\#}p < 0.05$, vs. SED + ASO.

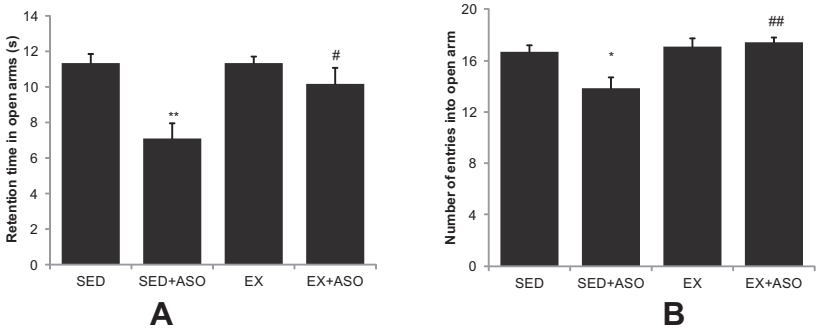

Fig. 5. Exercise reverses ASO-induced anxiety-like behaviors in the elevated plus maze test. Data are expressed as mean ± SEM (n = 12). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. SED; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. SED + ASO.

($F(1,44) = 6.61$, $P < 0.05$; Fig. 5B). Compared to SED + ASO mice, the time spent in the open arms ($P < 0.05$) and the number of entries into the open arms ($P < 0.01$) were both increased significantly in EX + ASO mice (Fig. 5A, B). Taken together, these data in the behavioral tests suggested a more anxious phenotype in the UCP2 ASO-treated mice. Further, our results showed that wheel running improved UCP2 deficiency-induced anxiety-like behaviors.

Hippocampal monoamine and UCP2 expression

As shown in Fig. 6, ASO administration significantly reduced hippocampal levels of 5-HT ($F(1,28) = 14.84$, $P < 0.01$; Fig. 6A) and DA ($F(1,28) = 22.60$, $P < 0.01$; Fig. 6B), compared to the SED mice. UCP2 ASO significantly enhanced hippocampal NE levels ($F(1,28) = 22.92$, $P < 0.01$; Fig. 6C). Also, wheel running produced differential effect on the hippocampal levels of DA ($F(1,28) = 16.43$, $P < 0.01$; Fig. 6B). There was a significant interaction between exercise and ASO on hippocampal levels of 5-HT ($F(1,28) = 5.02$, $P < 0.05$) and NE ($F(1,28) = 7.41$, $P < 0.05$). Importantly, wheel running significantly restored hippocampal levels of those monoamines to the control level (Fig. 6A–C).

To understand how well our model approximated true physiology, we measured UCP2 mRNA and protein expression in the hippocampus, liver and skeletal muscle. UCP2 ASO decreased UCP2 mRNA levels in the hippocampus ($F(1,14) = 14.51$, $P < 0.01$; Fig. 7A), and the drop in mRNA corresponded with a significant decrease in UCP2 protein (Fig. 7B). Additionally, UCP2 ASO decreased UCP2 mRNA levels in the liver ($F(1,14) = 31.56$, $P < 0.01$; Fig. 7A). However, we found no difference in UCP2 mRNA in the skeletal muscle (Fig. 7A). We tested protein content of UCP2 in the hippocampus as a possible explanation for the improved cognitive ability and anxiety-like behaviors. UCP2 ASO depressed UCP2 protein level in the hippocampus (Fig. 7B). However, we found no significant difference between SED + ASO and EX + ASO mice (Fig. 7B). Our findings showed that UCP2 knockdown in the hippocampus induced cognitive deficit and anxiety-like behaviors in mice. However, hippocampal UCP2 was not required for the beneficial effects of wheel running on cognition and anxiety.

DISCUSSION

The aims of the present study were to determine if UCP2 deficiency can produce rodent cognitive impairment and anxiety, and to determine if the beneficial effects of long-term exposure to voluntary exercise are UCP2 dependent. Herein, ASO was used to produce UCP2 knockdown in mice. As described above, UCP2-targeted ASO produced significant abnormalities in several behavioral tests of cognitive ability and anxiety. Specifically, ASO treatment impaired learning and memory of the mice as shown in Y-maze, T-maze, and

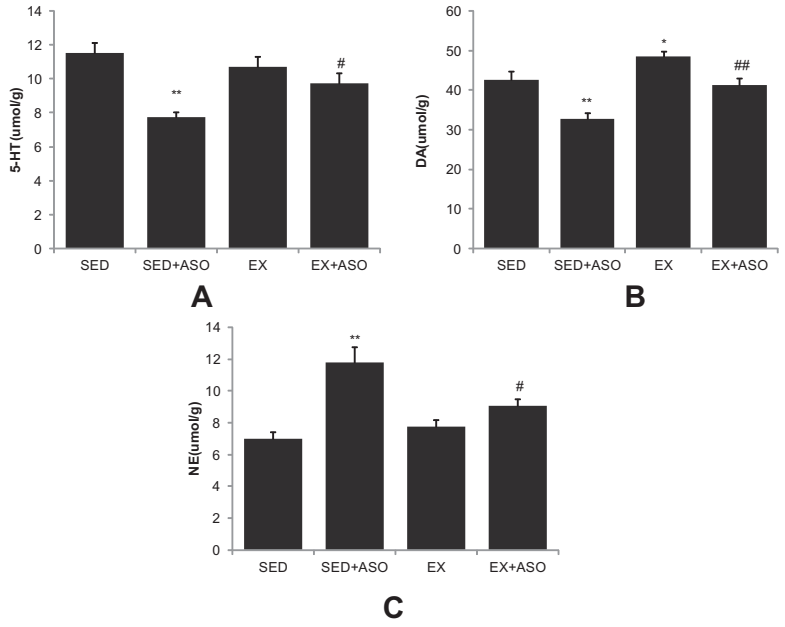

Fig. 6. Effect of ASO administration and exercise on hippocampal monoamines. Data are expressed as mean ± SEM (n = 6–8). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. SED; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. SED + ASO.

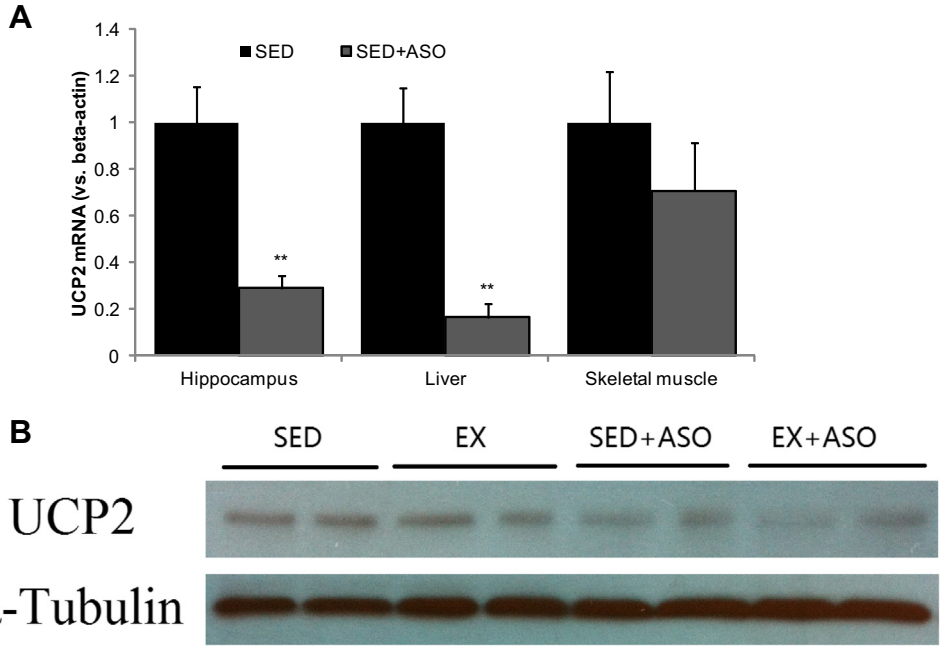

Fig. 7. UCP2 expression in the hippocampus. (A) Real-time PCR analyses demonstrate lower *UCP2* mRNA levels in the hippocampus and liver of mice treated with UCP2 ASO (n = 8, two-tailed unpaired t test). Data are expressed as mean ± SEM. $^{**}p < 0.01$ vs. SED. (B) Representative examples of Western immunoblots in hippocampal samples assessed for UCP2 protein levels.

ORT tests. Also, ASO treatment produced anxiety-like behaviors in mice as shown in OPT, light/dark box test, and EPM. Obviously, long-term exposure to wheel running ameliorated cognitive dysfunction and anxiety in mice as shown in the behavioral tests. However, UCP2 protein expression in the hippocampus was not associated with the benefits of exercise.

The hippocampus plays a central role in both cognition and emotion, such as spatial cognition and anxiety. However, cognition and anxiety are mediated respectively by separate neural mechanisms. The dorsal hippocampal lesions impaired spatial learning in rodents rather than anxiety, whereas the ventral hippocampal lesions relieved anxiety but had no effect on spatial

memory (Barkus et al., 2010). UCP2 was involved in hippocampal neuroprotection in ischemic brain injury, where UCP2 as a mitochondrial antioxidant reduced the content of oxidized proteins in hippocampal CA1 neuron (Chen et al., 2010a). UCP2 expression was increased in the mitochondria of hippocampal CA1 after ischemia/reperfusion. Rosiglitazone administration to hippocampus further enhanced mitochondrial UCP2 expression following ischemia/reperfusion (Chen et al., 2006). Upregulation of UCP2 by cerebral ischemia was mediated by PGC-1alpha, because knockdown of PGC-1alpha reduced UCP2 expression with exacerbated oxidative stress and increased neuronal cell death in the hippocampus after transient global ischemia (Chen et al., 2010b; Chuang et al., 2012). In addition, UCP2 expression was increased 7 days after kainic acid-induced hippocampal injury (Lebedev and Arkhipov, 2010). These findings strongly indicate that UCP2 plays an important neuroprotective role in the hippocampus by decreasing oxidative stress. Assuming that ASO treatment impairs UCP2 expression in the hippocampus, the hippocampus-related cognition ability and anxiety homeostasis may be impaired as a result. Our results firstly demonstrated that UCP2 ASO successfully produced knockdown of UCP2 in the hippocampus. The findings in the behavioral tests also provided a causal and significant evidence for an essential role of UCP2 in learning, memory and anxiety disorders. Functional loss of UCP2 is induced by ASO treatment leading to cognitive impairment and anxiety, thereby indicating a resultant hippocampal dysfunction.

Accumulating data indicated that the beneficial effects of exercise on cognitive ability and anxiety depend on a variety of neural biochemical mechanisms. An exciting study revealed that UCP2 is essential for exercise-induced synaptogenesis in the hippocampus. Voluntary exercise increased UCP2 expression and mitochondrial oxygen consumption in coupled as well as uncoupled respiratory states in the hippocampus. In UCP2 knockout mice, however, there were no increase in mitochondrial number and dendritic spine synapses in hippocampus after exercise (Dietrich et al., 2008). The findings suggest that UCP2 is essential for neuronal bioenergetic adaptation and synaptic plasticity in response to exercise. However, this study did not involve rodents' cognition and emotion. According to the data retrieval, we found no other findings involving the role of UCP2 in the behavioral and neural benefits of exercise. Our findings provided a contrary and interesting evidence for a role of UCP2 in hippocampal adaptations to exercise. Our results demonstrated that UCP2 knockdown produced cognitive decline and anxiety-like behaviors in mice, whereas voluntary exercise was able to prevent cognition deficits and anxiety without UCP2 expression in the hippocampus. These findings suggest that the benefits of exercise for cognition and anxiety are UCP2 independent.

In addition, our results showed that ASO treatment decreased the levels of 5-HT and DA in the hippocampus. A large number of studies have pointed out that monoamine transmission is closely associated with memory and emotion processing. For instance, 5-hydroxytryptamine (5-HT) depletion can lower memory performance, while depression is also linked to memory deficits (Meeter et al., 2006). 5-HT transporter is regarded as a neural marker for memory mechanisms and cognitive function (Meneses, 2013). The anxious rats showed significantly lower levels of 5-HT release from the hippocampus (File et al., 1987). 5-HT(1A) receptor agonists attenuated anxiety by reducing serotonergic transmission in brain subareas (Cervo et al., 2000). Further, UCP2-dependent mitochondrial mechanisms were involved in the maintenance and protection of normal nigrostriatal DA function (Andrews et al., 2009). Our result was consistent with the previous studies. As shown in our findings, UCP2 knockdown significantly regulated monoamine release from the hippocampus (5-HT, DA and NE), thereby producing cognitive impairment and anxiety-like behaviors in mice. In UCP2- deficient mice, abnormal monoamine release from the hippocampus may mediate cognitive impairment and anxiety-like behaviors. However, we also observed that voluntary exercise significantly reversed ASO-induced abnormal monoamine release from the hippocampus. Therefore, UCP2 is dispensable for the effects of exercise on monoamine production in the hippocampus.

CONCLUSIONS

Our results suggest that UCP2 knockdown in the hippocampus produced by ASO treatment impairs learning and memory and produces anxiety disorder in young mice. UCP2 plays an essential role in the development of cognitive ability and the resistance to anxiety, as well as hippocampal monoamine transmission. Nonetheless, our results also suggest that voluntary exercise has a reversal effect on ASO-induced cognitive impairment and anxiety, whereas exercise cannot rescue UCP2 deficiency in the hippocampus. Therefore, hippocampal UCP2 is not required for the benefits of exercise.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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