

Upregulation of aryl hydrocarbon receptor nuclear translocator 2 in the hippocampi of post-stroke depression rats

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Abstract. Aryl hydrocarbon receptor nuclear translocator protein 2 (ARNT2), a member of the basic helix-loop-helix superfamily of transcription factors, may serve a vital role in neuronal survival and cell proliferation via formation of heterodimers with hypoxia-inducible factor-1 α . Previous studies indicated that ARNT2 levels were elevated in the brains of ischemic rats; however, the involvement of ARNT2 in post-stroke depression (PSD) rats is not well understood. Therefore, the present study aimed to investigate the levels of ARNT2 in the hippocampi of PSD rats, and to clarify the potential association between ARNT2 and behavioral performance. A PSD rat model was established by middle cerebral artery occlusion (MCAO) followed by a 4-week chronic unpredictable mild stress (CUMS) regimen. A sucrose preference test and open field test (OFT) were conducted, and body weight was measured. In addition, reverse transcription-polymerase chain reaction and immunohistochemistry were performed

to measure ARNT expression. Results indicated that MCAO+CUMS rats had lower weight gain, consumed less sucrose and moved less compared with controls. Furthermore, the mRNA and protein levels of ARNT in MCAO+CUMS rats were increased compared with in controls. The sucrose preference index and horizontal movement distance in the OFT were positively correlated with ARNT mRNA level. Thus, from these findings it was suggested that ARNT2 may be positively associated with improvement of cognitive impairment, and therefore may be a potential target in PSD treatment.

Introduction

Stroke is among the most common causes of mortality and disability worldwide (1), and is defined as loss of blood supply to the brain by thrombotic, embolic or hemorrhagic events (2). The main consequences include local hypoxia, mitochondria dysfunction and increases in intracellular calcium, all of which lead to neuronal death and subsequent tissue damage due to oxygen and glucose deprivation (3). According to a report by the American Heart Association in 2015, up to 85% of strokes are ischemic, and 12% are hemorrhagic (4).

Recently, neuropsychiatric disorders resulting from cerebrovascular diseases have gained increasing attention. An epidemiological study across a diverse population suggested that post-stroke depression (PSD) was among the common complications of stroke, with an incidence rate of more than 30% within 5 years of stroke occurrence (5). In addition, PSD has been reported to be accompanied by sleep disorders, cognitive impairments, and may also be a predictor of poor recovery, high mortality and morbidity, and a cause of considerable health care costs (6-8). The underlying mechanism of PSD is not clearly understood, although several studies have suggested that it is associated with altered neurotrophic signaling, dysregulation of the hypothalamus-pituitary-adrenal axis, neuroinflammation and biological amine neurotransmitter disorders (1,8,9).

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Drutel *et al.* (10) reported that aryl hydrocarbon receptor (AHR) nuclear translocator protein 2 (ARNT2) may serve a vital role in neuron survival and cell proliferation by forming heterodimers with hypoxia-inducible factor (HIF)-1 α , a factor involved in adaptation to hypoxia.

ARNT2 is established as a member of the basic helix-loop-helix (bHLH) superfamily of transcription factors, and shares an amino acid sequence of almost 90% similarity with ARNT (11). It is primarily expressed in the central nervous system and developing kidney (12), forming a dimer with other basic helix-loop-helix Per-Arnt-Sim homology (bHLH-PAS) transcription factors including AHR and HIF-1 α when responding to xenobiotics and hypoxia, respectively (13). It has been suggested that under hypoxic conditions, HIF-1 α alternatively binds with ARNT or ARNT2, also designated as HIF-1 β and HIF-2 β , respectively, and subsequently activates the hypoxic response element, leading to the expression of target genes involved in glycolysis, erythropoiesis and angiogenesis (14,15). Interestingly, a study involving a population from a high altitude area of Ethiopia demonstrated an association between ARNT2 and hemoglobin levels (16), which aided to further clarify the key role of this protein in the hypoxia response in humans.

Magnetic resonance images have shown that hippocampal volume is reduced in patients with depression (17,18). Depression following stroke events is associated with cognitive dysfunction, which may be indirectly associated with the damage to the hippocampus (19-21). In hypoxic environments, neurons, particularly CA1 pyramidal neurons, are susceptible and vulnerable to post-ischemia cell death (22). It has also been reported that the hippocampal CA3 region regulates learning and memory abilities, particularly the normal operations of learning and memory processes (23). Although Valen *et al.* (24) reported that ARNT2 was highly and preferentially expressed in the hippocampus, and previous research indicated that ARNT2 was also potently expressed in ischemic brain tissue in rats (16), less is known about its expression levels in PSD rats. Therefore, the present study aimed to investigate the levels of ARNT2 in the hippocampus of PSD rats, as well as the potential association between ARNT2 levels and cognitive behavioral function.

Materials and methods

Animals and groups. Male Sprague-Dawley rats (n=32) aged 8 weeks, weighing 160 \pm 20 g, were purchased from the Experimental Animal Center of Zhengzhou University, Zhengzhou, China. Rats were housed at 22 \pm 2°C with 57 \pm 2% relative humidity under a 12-h light/dark cycle, and were given food and water *ad libitum* except during specific times of the experiment. All experiments were conducted in accordance with the National Research Council: Guide for the Care and Use of Laboratory Animals (8th edition), and the rat experimental procedures were approved by the Laboratory Animal Management Commission of the Henan Key Laboratory of Biological Psychiatry, Xinxiang, China.

Rats were allowed to adapt to the environment for 1 week before baseline body weight (BW) and sucrose consumption were measured, and open field tests (OFTs) were performed to ensure the homogeneity of rats. Rats

were then randomly divided into four groups (n=8/group): A control group; a chronic unpredictable mild stress (CUMS) group; a middle cerebral artery occlusion (MCAO) group; and an MCAO+CUMS group. All rats in the MCAO and MCAO+CUMS groups underwent MCAO surgery, while the rats in the CUMS group were treated with a 4-week CUMS regiment. Finally, rats in the MCAO+CUMS group were exposed to the same 4-week CUMS regiment following surgery.

MCAO. Rats were fasted overnight prior to the day of the experiment but were allowed free access to tap water. MCAO was conducted as previously described (25). Briefly, anesthesia was induced with 10% chloral hydrate [350 mg/kg BW, intraperitoneally (i.p.); Dalian Meilun Biological Technology Co., Ltd., Dalian, China] and a ventral side incision was made to expose the left carotid bifurcation, the left common carotid artery (CCA), the left internal carotid artery (ICA) and the left external carotid artery (ECA). The left ECA and proximal CCA were ligated with a 4-0 silk suture, while the left ICA was occluded by a microvascular clip; a small incision was made in the CCA proximal from the left carotid bifurcation. The microvascular clip was then removed and a nylon thread was inserted through the left CCA and left ECA to occlude the middle cerebral artery. Rats in the control and CUMS groups underwent the same surgery as above excluding threading of the nylon through the left CCA and left ECA. Following surgery, rats were selected according to Longa score, as previously described (25).

CUMS procedure. Following recovery for 1 week, rats in the CUMS groups that underwent surgery were subjected to a CUMS procedure adapted from Willner (26) with minor modifications. A total of seven different stressors were used: Water/food deprivation for 24 h; behavioral restriction for 2 h; wet cage for 24 h; electric foot shocks (average 60 V, 10 sec duration, average 1 shock/5 sec); forced ice water swimming (4°C, 5-15 min); and tail pinch for 1-2 min (27,28). Rats in the CUMS and MCAO+CUMS groups were housed in separate cages. Stressors were individually administered and separated from one another by a 1-2 day period over a total of 28 days to account for adaptation of rats to the CUMS regimen.

Behavioral tests BW. The BW of rats was measured prior to surgery and on days 1, 7 and 28 thereafter, and was obtained only during normal eating regimens (29).

Sucrose preference test. The animals were allowed to consume water and 1% sucrose solution for 12 h following 24 h food and water deprivation on the day prior to surgery and on days 7 and 28 thereafter. All other components of the test were performed as previously described (21,25).

OFT. The device for this test consisted of an open-field response box (100x100x50 cm with opaque black metal walls; RWD Life Science, Shenzhen, China) and an automatic data acquisition and processing system (Panlab Smart version 2.5.16; Panlab, Barcelona, Spain). Behavior, in terms

of horizontal and vertical movements, was determined in the open-field response box over 5 min to measure spontaneous activity, independent ability to explore and cognitive function (30).

Immunohistochemistry. Rats were anesthetized with 10% chloral hydrate (350 mg/kg, i.p.) and perfused with 0.9% saline followed by 4% paraformaldehyde. The samples of 4 rats per group were used for immunohistochemistry. Brains were removed and dehydrated, after which brain tissue was embedded in Tissue-Tek® O.C.T. Compound (Sakura Finetek USA, Inc., Torrance, CA, USA). Frozen tissue was then sectioned at 15- μ m thickness on a microtome (Leica CM1850; Leica Microsystems GmbH, Wetzlar, Germany), and immunohistochemistry was performed using a rabbit SP-9001 detection kit (OriGene Technologies, Inc., Beijing, China) according to the manufacturer's instructions. Briefly, sections were blocked with 3% H₂O₂ in deionized water at room temperature for endogenous peroxidase ablation, rinsed in phosphate buffered saline (PBS) three times and blocked in blocking buffer (normal goat serum for 10-15 min at room temperature). Following removal of blocking buffer, sections were incubated with primary antibody in PBS for 1 h at 37°C, washed three times with PBS, and incubated with biotinylated secondary antibody (immunoglobulin G, anti-rabbit) for 10-15 min at room temperature. Sections were then incubated with horseradish enzyme-labeled streptavidin for a further 10-15 min at 37°C. ARNT-positive staining in the CA1 and CA2 hippocampal fields was observed under an optical microscope (Leica DM2000; Leica Microsystems GmbH). All data were analyzed with Image-Pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Reverse transcription-polymerase chain reaction (RT-PCR). All tissue preparation steps for the isolation of mRNA was performed as previously described (25). The samples not subject to immunohistochemistry were used (n=4 rats per group). Primer pairs for *GAPDH* and *ARNT2* were designed based on sequences in the PubMed database (<https://www.ncbi.nlm.nih.gov/pubmed/>). The primers for *ARNT2* were forward, 5'-ACC AGCGAGACGGGCTGTCA-3' and reverse, 5'-GTGCCCGGC AGGGAATGGAC-3', and for *GAPDH* were forward, 5'-GGG CTCTCTGCTCCTCCCTCT-3' and reverse, 5'-CCGTTGAAC TTGCCGTGGGT-3'. The cycling parameters for PCR were as follows: An initial cycle (pre-denaturation step) at 94°C for 3 min, followed by 35 cycles at 94°C for 15 sec, 68°C for 30 sec and 72°C for 30 sec and 72°C for 10 min. Band intensities were measured and analyzed using an GAS7001B gel image analysis software (UVIband 10.02; UVItect, Cambridge, UK). The expression of target mRNA was measured based on its quantity relative to that of *GADPH* (relative quality=target gene optical density/*GADPH* optical density).

Statistical analysis. Data were presented as the mean \pm standard error of the mean and were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). All data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference post hoc tests. Pearson's correlation analysis was performed to analyse the relationship between target gene expression and behavioral

performance. A two-tailed P-value of <0.05 was considered to indicate statistical significance.

Results

Behavioral assessment BW. Rats in the MCAO and MCAO+CUMS groups gained less weight than controls at 7 and 28 days after surgery (P<0.01). After 28 days of the CUMS regimen, rats in the CUMS group exhibited significantly lower BW compared with the controls (P<0.01), while rats in the MCAO+CUMS group gained significantly less weight compared with those in the MCAO group (P<0.01; Table I).

Sucrose preference. On day 7 after surgery, sucrose preference in the MCAO and MCAO+CUMS groups was decreased compared with in the control group, albeit non-significantly (P>0.05); likewise the sucrose preference index of the CUMS group did not differ significantly compared with the control group (P>0.05). After 28 days of the CUMS regimen, the CUMS and MCAO+CUMS groups exhibited significantly decreased sucrose preference indexes compared with the control group (P<0.01), although no significant difference was determined between the MCAO and control groups (P>0.05). Meanwhile, the CUMS and MCAO+CUMS groups displayed significantly decreased sucrose preference indexes compared with the MCAO group (P<0.01; Table II).

OFT. The OFT assessed horizontal motion distance traveled and number of rearings of each rat. On day 7, rats in the MCAO and MCAO+CUMS groups showed significantly less horizontal movement compared with controls (P<0.01), although rats in the CUMS group did not (P>0.05; Table III). Similarly, rats in the MCAO and MCAO+CUMS groups performed a significantly lower number of rearings compared with the control group (P<0.01), while rats in the CUMS group exhibited no significant difference compared with the controls (P>0.05; Table IV). After 4 weeks of the CUMS regiment, rats in the CUMS and MCAO+CUMS groups exhibited significantly less horizontal movement (P<0.05) and a lower number of rearings (P<0.01) compared with controls (Tables III and IV).

ARNT2-positive staining. ANOVA demonstrated that the mean optical density of ARNT2-positive neurons in the CA1 and CA3 hippocampal areas of MCAO and MCAO+CUMS rats was significantly higher than in the control group (P<0.05; Fig. 1A,C and D). Although staining in the CUMS group revealed more ARNT2-positive neurons in CA1/3 than controls, the difference was not significant (P>0.05; Fig. 1A,C and D). Additionally, rats in the CUMS group exhibited increased ARNT2 staining in the CA2 area compared with controls (P<0.05; Fig. 1B and D); and decreased ARNT2 staining in CA2 compared with MCAO+CUMS rats (P<0.01; Fig. 1B and D).

PCR of ARNT2 mRNA. ARNT2 mRNA levels in the hippocampi of MCAO and MCAO+CUMS rats were significantly increased compared with in controls (P<0.05 and <0.01, respectively); while there was no significant difference between the MCAO and MCAO+CUMS groups (P>0.05). Additionally, ARNT2 mRNA expression was higher

Table I. Results of weight changes in rats.

Group	BW, g		
	Baseline	Postoperative day	
		7	28
Control	278.38±9.89	299.19±9.15	360.69±16.39
MCAO	277.50±21.93	194.31±27.13 ^a	295.44±41.45 ^a
CUMS	273.00±21.66	279.75±16.66	273.25±20.98 ^a
MCAO+CUMS	279.75±31.57	208.31±21.50 ^a	249.56±38.44 ^{a,b}
F-value	0.134	55.126	18.718
P-value (ANOVA)	0.939	<0.001	<0.001

BW prior to enrolment, and 7 and 28 days after surgery in the control, MCAO, CUMS and MCAO+CUMS groups (n=8/group). Data are presented as means ± standard error of the mean. ^aP<0.01 vs. the control group; ^bP<0.01 vs. the MCAO group. BW, body weight; MCAO, middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress; ANOVA, analysis of variance.

Table II. Sucrose preference index.

Group	Sucrose preference index		
	Baseline	Postoperative day	
		7	28
Control	0.854±0.023	0.735±0.010	0.668±0.014
MCAO	0.890±0.014	0.708±0.017	0.633±0.007
CUMS	0.873±0.011	0.710±0.020	0.457±0.015 ^{a,b}
MCAO+CUMS	0.874±0.020	0.698±0.025	0.406±0.025 ^{a,b}
F-value	0.660	0.683	60.314
P-value (ANOVA)	0.584	0.570	<0.001

Sucrose preference index prior to enrolment and 7 and 28 days after surgery in the MCAO, CUMS and MCAO+CUMS groups (n=8/group). Data are presented as means ± standard error of the mean. ^aP<0.01 vs. the control group; ^bP<0.01 vs. the MCAO group. MCAO, middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress; ANOVA, analysis of variance.

Table III. Horizontal movement in the OFT.

Groups	Movement, cm/5 min		
	Baseline	Postoperative day	
		7	28
Control	10,526.62±746.72	10,798.12±1,155.86	10,674.85±1,914.86
MCAO	9,855.28±2,037.12	2,578.43±718.61 ^a	10,980.34±939.91
CUMS	10,621.12±2,139.89	10,543.91±1,975.83	6,630.28±4,449.65 ^b
MCAO+CUMS	10,432.58±1,332.78	2,504.37±1,220.60 ^a	6,385.03±3,738.11 ^b
F-value	0.343	97.340	5.216
P-value (ANOVA)	0.794	<0.001	0.005

Horizontal movement in the OFT prior to enrolment and 7 and 28 days after surgery in the control, MCAO, CUMS and MCAO+CUMS groups (n=8/group). Data are presented as means ± standard error of the mean. ^aP<0.01, ^bP<0.05 vs. the control group. OFT, open field test; MCAO, middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress; ANOVA, analysis of variance.

Table IV. Number of rearings in the OFT.

Groups	No. of rearings/5 min		
	Baseline	Postoperative day	
		7	28
Control	20.00±4.57	20.50±2.33	20.38±8.03
MCAO	20.75±5.04	7.50±4.21 ^a	20.50±3.85
CUMS	20.25±6.67	21.00±3.70	10.13±10.23 ^a
MCAO+CUMS	19.63±2.39	7.13±5.77 ^a	8.00±4.87 ^a
F-value	0.074	27.496	6.757
P-value (ANOVA)	0.974	<0.001	0.001

Number of rearings in the OFT prior to enrollment and 7 and 28 days after surgery in the control, MCAO, CUMS and MCAO+CUMS groups (n=8/group). Data are presented as means ± standard error of the mean. ^aP<0.01 vs. the control group. OFT, open field test; MCAO, middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress; ANOVA, analysis of variance.

Table V. Correlation (r-values) between rat behavioral traits and ARNT mRNA expression.

	Body weight	Sucrose preference index	Horizontal movement	Number of rearings
ARNT mRNA expression	0.067	0.547 ^a	0.485 ^a	0.014

^aP<0.01.

in the hippocampi of rats in the MCAO+CUMS group compared with in CUMS rats, albeit non-significantly (P>0.05; Fig. 2).

Partial correlation analysis results. The sucrose preference index and horizontal movement distance in the OFT were positively correlated with ARNT mRNA level (r=0.547 and 0.485; P<0.01). ARNT mRNA expression was also positively correlated with body weight (r=0.067) and number of rearings (r=0.014), though this was not significant (P>0.05; Table V).

Discussion

The results of the current study illustrated that ARNT2 expression in the hippocampus differed among rats following control, CUMS, MCAO and MCAO+CUMS treatments, which is consistent with a previous study describing the effect of transcription factors from the bHLH super-family on post-stroke depression (25). The increased expression of ARNT2 detected in the hippocampi of rats has previously been confirmed to be associated with the vulnerability of distinct dopaminergic projections to hypoxia (16,31), but to our knowledge, the increased expression in the MCAO+CUMS group, representing a model of PSD, is a novel finding. Additionally, the observation that there was a trend toward increased ARNT2 levels in the MCAO+CUMS group compared with in the MCAO group is also novel. These alterations in ARNT2 expression were also confirmed at the protein level, which is consistent with a previous study that found HIF-1 α -mediated increases

in ARNT2 protein expression in neuronal PC12 cells (32). By contrast, another study detected decreased ARNT2 expression in response to HIF-1 α signaling in oral squamous cell carcinoma-derived cell lines (33), which may be attributed to variable cell types and altered cell cycle progression. Although these conflicting findings warrant further research, the focus of the current study was primarily on the change of ARNT2 in a PSD model, and its specific mechanism is still unclear, worthy for further study.

A notable finding was that the hippocampi isolated from the MCAO and MCAO+CUMS groups exhibited increased levels of ARNT2 protein expression as well as mRNA expression, reflecting its potential protective role in resisting stress and promoting neuron survival. Ischemic brain damage leads to secondary ischemic brain tissue hypoxia, brain edema, degeneration, tissue softening, necrosis and inflammation, which in turn induce apoptosis and cell death (34).

Behavioral tests were also utilized to investigate the association between the expression of ARNT2 and behavioral performance. On day 7 after surgery, rats that underwent MCAO gained less weight, consumed slightly less sucrose solution and showed reduced activity, albeit temporarily, as a result of the procedure. After 28 days, rats with MCAO appeared to somewhat recover, although the BW of MCAO rats remained significantly lower compared with that of control rats, potentially as a result of a systemic response to the surgical injury. Moreover, the BW and sucrose consumption of rats subjected to the CUMS regimen were significantly decreased compared

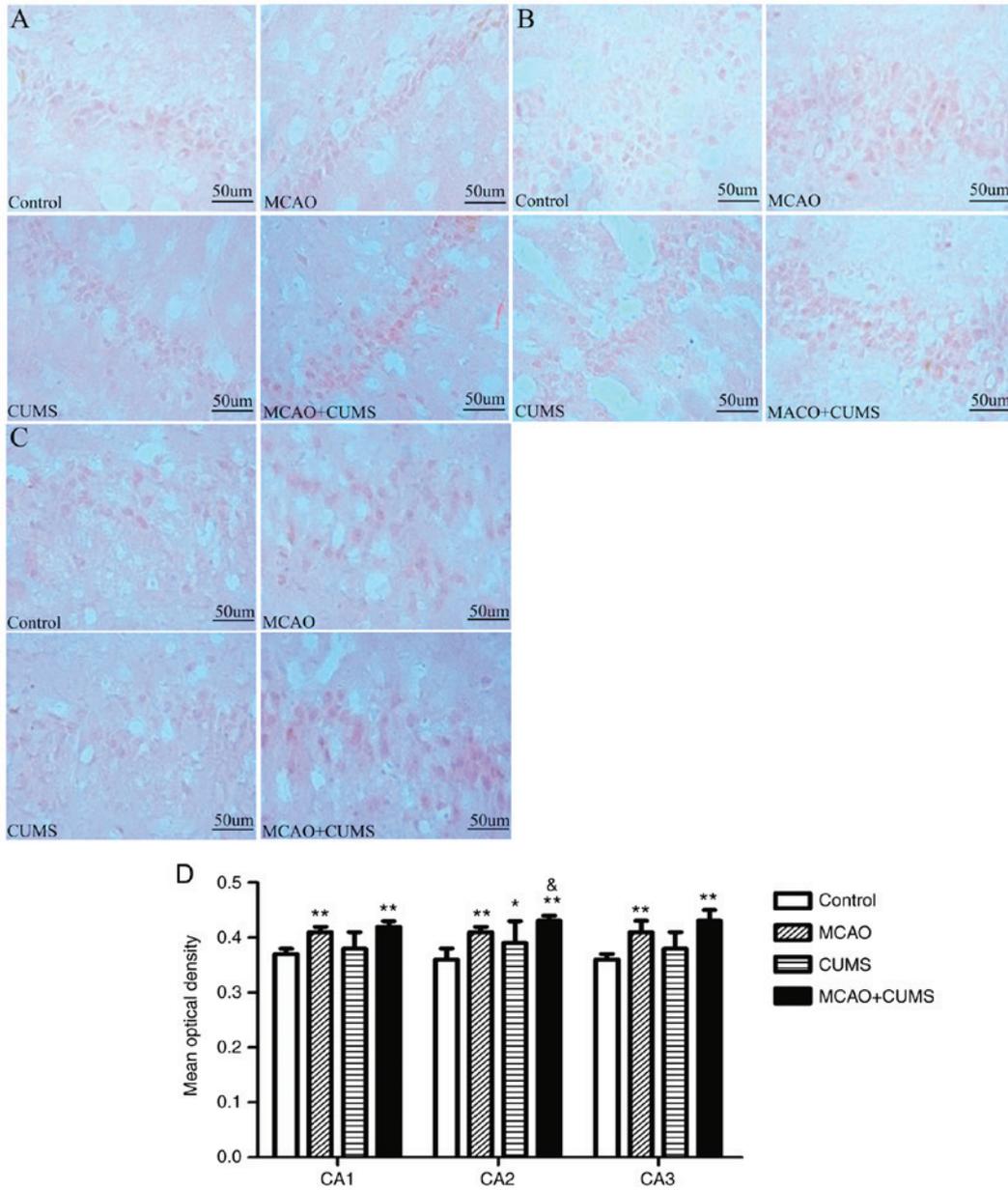


Figure 1. (A-C) Immunohistochemical results of ARNT2-positive staining and (D) mean optical density of staining after 4 weeks in the control, MCAO, CUMS and MCAO+CUMS groups (n=4/group). The hippocampal subfields CA1, CA2 and CA3 were observed at x200 magnification. (A-C) show CA1, CA2 and CA3, respectively. *P<0.05, **P<0.01 vs. the control group; &P<0.01 vs. the CUMS group. ARNT2, aryl hydrocarbon receptor nuclear translocator protein 2; MCAO, middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress.

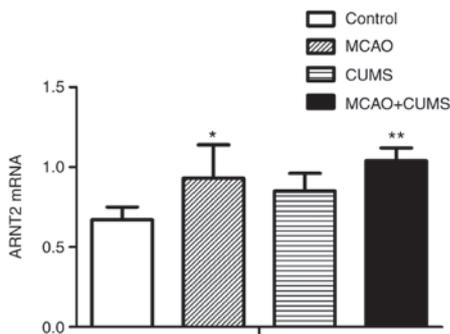


Figure 2. Expression of ARNT2 mRNA relative to GAPDH in the hippocampi of rats after 4 weeks in the control, MCAO, CUMS and MCAO+CUMS groups (n=4/group). *P<0.05, **P<0.01 vs. the control group. ARNT2, aryl hydrocarbon receptor nuclear translocator protein 2; MCAO, middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress.

with in controls; further, MCAO+CUMS rats gained less weight and consumed less sucrose solution compared with MCAO rats. Collectively, these results indicate successful establishment of the depression and PSD models.

OFT was performed to measure spontaneous activity and independent exploratory behavior, which has previously been used to evaluate depressive behavior (35). In the current study, CUMS and MCAO+CUMS rats subjected to a 4 week-CUMS regimen moved shorter distances and performed a lower number of rearings compared with controls. Furthermore, MCAO+CUMS rats moved shorter distances and performed less rearings than those of the CUMS group, although these differences were not significant. Overall these results indicate that cerebral ischemia together with stress may serve a role in behavioral performance.

Interestingly, ARNT2 was significantly elevated in the MCAO and MCAO+CUMS groups within penumbra regions of the hippocampus, and therefore may be part of a protective response to this type of injury. Moreover, levels of ARNT2 were increased, which was previously observed in white blood cells of patients with depression (36). Expectedly, MCAO+CUMS rats also exhibited higher levels of ARNT2 compared with MCAO and CUMS rats. However, the behavioral performance of MCAO+CUMS rats was significantly worse compared with the other groups. Several factors may account for this. First, it may be that the protective role of ARNT2 is limited to compensation for damaged tissue, via upregulation in the hippocampi; however, the regulation underlying this ARNT2 expression has yet to be fully elucidated. Second, the HIF-1 α -dependent feed-forward loop that promotes the upregulation of ARNT2, thus inhibiting apoptosis and necrosis of neurons (31), may be prolonged in disease states in order to ensure the survival of neurons in the brain. Maltepe *et al* (15) demonstrated that ARNT2/HIF-1 α complexes associated with p53 and B-cell lymphoma 2 family members to modulate apoptosis in response to hypoxia/ischemia in neurons and other tissues. It has also been indicated that chronic stress has a negative impact on neurogenesis, with a previous study showing that cell proliferation and survival, as well as neuronal differentiation, declined in stressed rodents (25). Therefore, it may be worthwhile investigating whether and how the survival and apoptosis of neurons in the prefrontal cortex and hippocampus are affected in PSD.

Previously, our group demonstrated that the bHLH-PAS factor, NPAS4, which dimerizes with ARNT2 and subsequently binds to brain-derived neurotrophic factor promoter-I to modulate nerve-excitability-dependent transcription, was significantly decreased in CUMS and MCAO+CUMS rats (25). Therefore, it is possible that ARNT2 is involved in mechanisms underlying gene-environment interactions in psychiatric models, but the specific biological signaling pathways require further investigation. Additionally, further research is warranted to clarify HIF-1 activity in neurons, using ARNT2-deficient rats or ARNT2 antagonists/agonists. Nevertheless, the results of the present study regarding the expression of ARNT2 in the hippocampus may serve as a basis for further study into the etiology of psychiatric disorders following cerebral ischemia.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

WW made substantial contributions to conception and design of the study. LZ performed the experiments on animal behavior. SG was responsible for the polymerase chain reaction and immunohistochemistry experiments. XW collected the data. PF analyzed and interpreted data. CP drafted the manuscript. XZ revised the manuscript critically for important intellectual content. WL and JM ensured that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. ZZ and JS gave final approval of the version to be published.

Ethics approval and consent to participate

All experiments involving animals were approved by the Laboratory Animals Management Commission of the Henan Key Laboratory of Biological Psychiatry, Xinxiang, China.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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