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Cognitive type susceptibility in ICR mice model of chronic cerebral hypoperfusion.

Sirilak Somredngan[1] and Wachiryah Thong-asa[1]*

[1]*Physiology Division, Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand.*

ABSTRACT

Animal model of chronic cerebral hypoperfusion (CCH) was found to be beneficial as pathophysiological provided relevance to vascular dementia (VD) and subcortical ischemic vascular dementia (SIVD). There were many evidence kinds of CCH animal model and all were useful. The present study investigated the pathophysiology of mild CCH in ICR mice during 2 to 8 weeks of permanent right common carotid artery occlusion Sensorimotor, cognitive abilities and anxiety-like behavior were assessed in Morris water maze (MWM) and elevated plus maze (EPM), respectively. Frontal cortex, striatum and hippocampus infarctions were evaluated using 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 2, 4 and 8 weeks of permanent right common carotid arteryocclusion. Sensorimotor, spatial learning and memory in the acquisition paradigm of the MWM and anxiety-like behavior assessed in the EPM were not affected during the experimental period of 2, 4 and 8 weeks of arterial occlusion.Significant deficit of learning flexibility and memory of reverse platform location in the reversal paradigm were found, though reversible of learning flexibility deficit presented at 8 weeks. Significant infarction early appeared in striatum since 4 weeks while the frontal cortex and hippocampus stated at 8 weeks. The present study suggested that mild CCH induced by permanent right common carotid artery occlusion in ICR mice induced reversible learning flexibility deficit but not memory of the reverse platform location in the MWM.

KEY WORDS: CHRONIC CEREBRAL HYPOPERFUSION, ELEVATED PLUS MAZE,LEARNING FLEXIBILITY, SPATIAL LEARNING AND MEMORY.

INTRODUCTION

Animal model of chronic cerebral hypoperfusion (CCH) can be induced by permanent ligation of major cerebral arterial supplies. Severity of pathological appearances depended on the number of vessel occlusion and persistent of cerebral reduction. CCH study in animal model is associated with cerebral blood flow (CBF) reduction, metabolic insufficient, oxidative stress, neuroinflammation, neurotransmitter system dysfunction, mental confusion, cognitive decline, white matter and neuronal degeneration. These pathological relevancies of

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CCH model were variable due to the difference of vessel occlusion type and animal strain. Among rodent strain, the difference in susceptibility has been reported, (Reid et al. 2010). The rapid onset and significant pathophysiological appearance clearly revealed in permanent bilateral common carotid artery occlusion (BCCAO) model. BCCAO model has provided beneficial data of causative role played by cerebral hypoperfusion in neurodegenerative diseases and can be further useful in neuroprotective and therapeutic researches, (Farkas et al. 2007 and Du et al. 2016).

Knowing of pathomechanism is relevant to vascular dementia (VD) and subcortical ischemic vascular dementia (SIVD) using a milder model of CCH such as unilateral carotid artery occlusion (UCO) model. There was frequency used of right common carotid artery occlusion (rCCAO), and it revealed useful data of CCH outcome both in rats and mice. Pathological relevance for instance significant reduction of ipsilateral CBF, activation of pro-inflammatory cytokine (IL-1β, TNF-α), inhibition of anti-inflammatory cytokine (IL-4, 10), downregulation of A1 adenosine receptors, white matter damage, hippocampal neuronal degeneration,and correlation with cognitive impairments such as spatial learning and memory (Yoshizaki et al. 2008, Thong-asa et al. 2013, Thong-asa and Tilokskulchai 2014, Cheng et al. 2015, Thong-Asa 2015).

Reversible of cognitive deficit was reported in short-term but not long-term study of rCCAO without neuronal degeneration correlation (Thong-asa et al. 2013, Thong-asa and Tilokskulchai 2014, Thong-Asa 2015). Cognitive ability outcomes from rodent assessed in cognitive tasks might improve by repetitive test, previous experience and compensatory mechanisms of cerebral ischemia in CCH model were also suggested. Data comparing from rats and mice rCCAO model, focus only on cognitive which varied due to the difference of maze paradigm, repetitive test or previous experience and effect of global ischemia on behavioral measures of emotion, locomotion as well as habituation(Choy et al. 2006, Coyle and Panzenbeck 1990, Dellu et al. 1997, Kim et al. 2008). Rats exhibited more susceptibility than mice as more spatial ability deficits were found since 6 days of rCCAO(Thong-asa et al. 2013) but it was only 4 weeks in mice, (Cheng et al. 2015).

There are reports about global cerebral ischemia induced hyperactivity, anxiety and locomotion which might help reversible of cognitive ability deficit as well(Milot and Plamondon 2009, Plamondon and Khan 2005). It is interesting that strain of rat and mouse used as CCH model provide differences of pathophysiological outcome. It is important to clarify the pathomechanism, susceptibility and neuropathology correlated with behavioral deficit in each CCH rodent model. Regarding the importance of basic knowledge, the present study aimed to investigate the pathophysiologyof CCH induced by permanent right common carotid artery occlusion focus on ICR mice strain.

MATERIAL AND METHODS

Animals: Thirty-six male ICR mice, 40 – 50 grams, were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom.Mice were housed under 12h/12h light-dark cycle with well-controlled temperature (23 ± 2 °C), humidity (55 ± 5%) with appropriated ventilation. Mice were allowed free access to standard food pellets and RO water. The present study was conducted in accordance with internationally accepted principles for laboratory animal use and care of the European Community (EEC directive of 1986; 86/609/EEC) and the experimental protocol was approved by theAnimal Ethics Committee, Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand (ID#OACKU 04559).

Experimental protocol: In brief, mice were randomly assigned to two main groups of Sham and unilateral (right) common carotid artery occlusion (UCO). After fasting, mice were anesthetized by sodium pentobarbital (45 mg/kg) intraperitoneal injection. After checking their reflexes, a skin incision was made on the midline ventral neck, right common carotid artery was exposed, and it was cleared from nerves and surrounding connective tissues then permanently occluded with silk suture. After wound sutured, antibiotic was given intramuscular injection and mice were placed under heat lamps and blankets in a recovery chamber. Sham and UCO main groups were further randomly divided into 3 experimental groups based on the period of arterial occlusion at 2, 4 and 8weeks.Evaluation of sensorimotor, cognitive abilities usingthe Morris water maze (MWM), anxiety-like behavior in the elevated plus maze(EPM), and infarction volume of brain tissues were conducted at these period as well.

Sensorimotor and cognitive abilities evaluation in the Morris water maze: The Morris water maze was a 150 cm diameter plastic pool and 50 cm tall. It was filled with 30 cm depth of water (25 °C). Prior to the cognitive tests, the sensorimotor evaluation was done in order to assess visual and motor abilities. Sensorimotor test was conducted using the visible platform paradigm (cue test). Briefly, a visible platform was placed and clearly seenabove the water surface about 2 cm. Mice were given four trials to swim, search, climb and sit on the visible platform. The maximum time for each trail was 120 sec-

onds. The swimming speed of each group was compared for sensorimotor evaluation. On the following day, spatial learning was tested and continued for five consecutive days as the acquisition trial. Briefly, the pool was divided into four quadrants: northeast (NE), northwest (NW), southeast (SE), and southwest (SW) on the computer monitorthat was connected to a ceiling camera. The hidden platform was placed under the water surface about 2 cm in the center of the NW quadrant (the target quadrant of acquisition trial).

A variety of visual cues were placed outside and around the pool. Mice were continuously given four trials a day with 120 minutes maximum timeof each trial. For ran out time case, mice were guided to the hidden platform by the experimenter. When the acquisition trial was completed, the probe trial began in order to determine spatial memory capacity. The hidden platform was removed from the target quadrant, and mice were allowed to swim for 60 seconds, then the time spent in each quadrant was recorded. The time spent in the target quadrant was then further converted to percentage of time spent in the target quadrant and represented as spatial memory capacity. After finish acquisition trial and probe, learning flexibility was continuously assessed in the reversal trial for three consecutive days. The only difference from the acquisition trial was the switching of hidden platform to the opposite quadrant (SE). The probe trial was delivered on the last test day as well in order to assess memory capacity of reverse platform location. All data were record by using Smart©3.0.04 (Planlab/Harvard Apparatus).

Anxiety-like behavior assessment in the elevated plus maze: After finishing the cognitive tests in the MWM, the anxiety-like behavior was further evaluated. The EPM was a cross shape maze comprised of two open (25x5x0.5cm) and two closed (25x5x16cm) arms with a central platform (5x5x0.5cm). EPM was placed in dry circular tank (normally used as the MWM) and about 40 cm above the floor. The EPM was started at 6.00 pm. by transferring all mice into the experimental room 30 min prior to the test. Illumination was maintained at 100 lux in the experimental room. The testing of anxiety-like behavior was started when mice were placed on the central platform facing to the closed arm. Mice were allowed to move freely in the EPM for 5 min with continuously video recording. Arm entries defined as the center of mass of the mouse enters the arm.The number of open arm entries and duration were analyzed and served as an index of anxiolytic behavior, (Komada et al. 2008).

Infarction area analysis: After finishing all behavioral tests in each experimental period, all mice were sacrificed by lethal dose of intraperitoneal injection of sodium pentobarbital (>60 mg/kg). Brains were quickly removed after decapitation, and briefly washed in cold 0.9 % normal saline solution (NSS) and cut with surgical blade to yield 2 mm of thickness. Brain pieces were stained with 2 % 2, 3, 5-triphenyltetrazolium chloride (TTC) at 37 °C for 10 min. After staining with TTC, brain pieces were kept in 10 % neutral buffer formalin (NBF) for 24 h, then images were captured and analyzed. Infarction area was calculated by using UTHSCSA Image Tool 3.0by differentiated pale tissue area among reddish areas. About TTC technique, colorless TTC was reduced to a deep-red precipitate by dehydrogenases in the presence of NADH of viable tissue. The lethally damaged cells do not retain these reactants, nonviable areas were not stained and appear pale, while viable cells were stain red(Fishbein et al. 1981).

Statistical analysis: All data were interpreted as mean ± SEM., spatial learning ability and learning flexibility (represented by escape latencies) were analyzed by repeated-measure analysis of variance (ANOVA) followed by Fisher's PLSD post hoc test. Memory capacity (% time spent in the target quadrant), anxiety-like behavior index (represented by open arm entries and duration), infarction area (represented by % infarction) were analyzed by ANOVA followed by Fisher's PLSD post hoc test. Statistical significance was accepted at p-value < 0.05.

RESULTS

Sensorimotor evaluation at 2, 4 and 8 weeks: All mice exhibited normal ability of swimming, seeing and climbing on the platform during cue test in the MWM. Results indicated no significant difference of the swimming speed (cm/min) at 2 (Sham 2W = 17.18±1.97, UCO 2W = 17.81±1.48, $p > 0.05$), 4 (Sham 4W = 19.25±0.99, UCO 4W = 19.87±1.37, $p > 0.05$) and 8 (Sham 8W = 18.89±1.09, UCO 8W = 19.23±0.81, $p > 0.05$) weeks after permanent right common carotid artery occlusion.

Spatial cognitions and learning flexibility at 2, 4 and 8 weeks: Swimming pathof acquisition and reversal trials, the target selected quadrants, spatial learning ability, spatial memory capacity, learning flexibility and memory capacity of reverse platform location at 2, 4 and 8 weeks of right common carotid artery occlusion showed in Fig.1. Spatial learning ability indicated by the escape latency was not difference at all period of the experiment ($p > 0.05$).Similar to spatial memory capacity indicated by the percentage of time spent in the target quadrant of the acquisition probe ($p > 0.05$). These results imply that the permanent right common carotid artery occlusion for a period of 2, 4 and 8 weeks did not induce deficit on spatial learning ability and memory capacity of ICR mice CCH model.

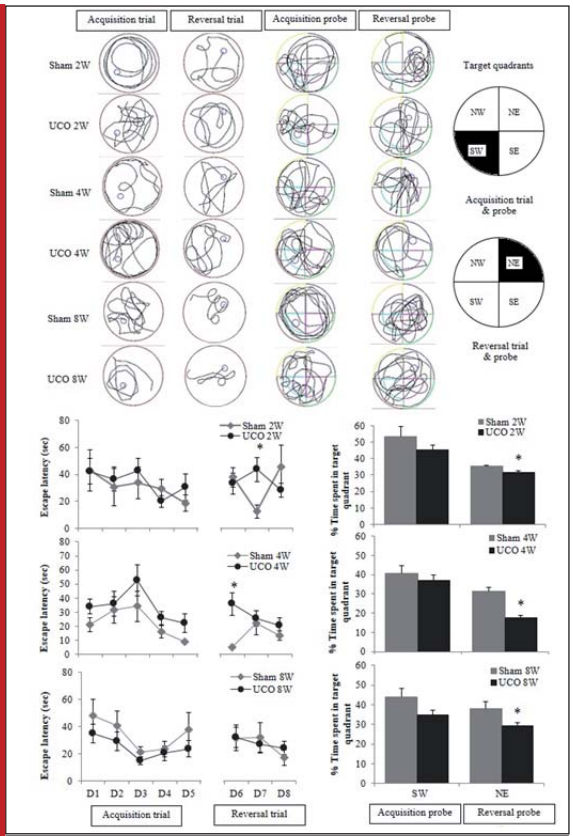

FIGURE 1. Representation of swimming paths, target quadrants, spatial learning and memory in the acquisition paradigm, learning flexibility and memory in the reversal paradigm assessed in Morris water maze (MWM); spatial learning ability and learning flexibility represented by escape latencies (sec); memory capacity represented by the percentage of time spent in the target quadrant (%); at 2, 4 and 8 weeks of permanent right common carotid artery occlusion, * $p < 0.05$.

The escape latency in the reversal trial(learning flexibility) significantly increased in UCO group at 2 ($p < 0.05$) and 4 ($p < 0.05$)but not 8 weeks ($p > 0.05$) of permanent right common carotid artery occlusion which indicated reversible of learning flexibility deficit at 8 weeks. Memory capacity of the reverse platform location in the reversal probe significantly decreased in UCO group at all periods ($p < 0.05$) with no such the reversible as found in learning flexibility. Our data revealed about cognitive type susceptible to CCH that was induced by permanent right common carotid artery occlusion and it was the cognitive flexibility.

Anxiety-like behavior at 2, 4 and 8 weeks: Anxiety-like behavior indicated by the number of open arm entries and duration at 2, 4 and 8 weeks showed in Fig.2. The result indicated that CCH induced by permanent right common carotid artery occlusion at 2, 4 and 8 weeks did not induce difference in anxiety-like behavior ($p > 0.05$).

Infarction area at 2, 4 and 8 weeks: Percentage of infarction area in Fig. 3 indicated the significant difference in the frontal cortex and hippocampal areas found at 8 weeks ($p < 0.05$). Striatum infarction found significantly at 4 and 8 weeks of arterial occlusion ($p < 0.05$). Infarction areas from TTC staining in our present study suggested about early appearance of infarction on striatum in ICR mice CCH model.

DISCUSSION

The present study used ICR mice as CCH model induced by permanent right common carotid artery occlusion-and persistent from 2 to 8 weeks. This CCH model did not induce spatial learning and memory deficits, but revealed about cognitive type susceptible to CCH that-was the learning flexibility. The present study also found reversible of flexibility deficit but not the memory capacity of new platform location. Mice strain that used as CCH model induced by permanent right common carotid artery occlusion such as ICR and B57BL/6 gave such differences of outcome. Using of ICR mice as the present study revealed about CCH did not induce spatial learning and memory deficits during 2 to 8 weeks, but B57BL/6 mice the deficit of spatial memory capacity appeared since 2 weeks and spatial learning deficit found at 4 weeks, (Cheng et al. 2015).

It was not surprise because of B57BL/6 was the most susceptible to global cerebral ischemia rather than ICR or other mouse strains, based on neurological signs, histological findings, cortical microcirculatory and perfusion patterns(Yang et al. 1997).There were reports about the differences of pathophysiology and behavioral outcome among mouse strains(Adams et al. 2002, Brosnan-Watters et al. 2000). Not only strain difference, sex also concern especially in the cognitive behavioral tests(Ge et al. 2013). There was evidence indicated that locomotor activities such as the basal open-field activity of the ICR was greater than that of the C57BL/6, the hippocampal-dependent learning and memory such as novel object was lower in the ICR and the strength of memory retention in the ICR mice was relatively weak (Kim et al. 2008). This evidence correlated with our present study that the flexibility of learning and memory retention especially for the new platform location were impaired. As the differences of brain circuit and mechanism of cognitions, affected of cognitive functions depended on location of circuit area andneuronal cell damage.

We suggest that cognitive type specific factor that early affected in ICR mice CCH model was the flexibility.Adaptive behavior associated with prefrontal cortex-

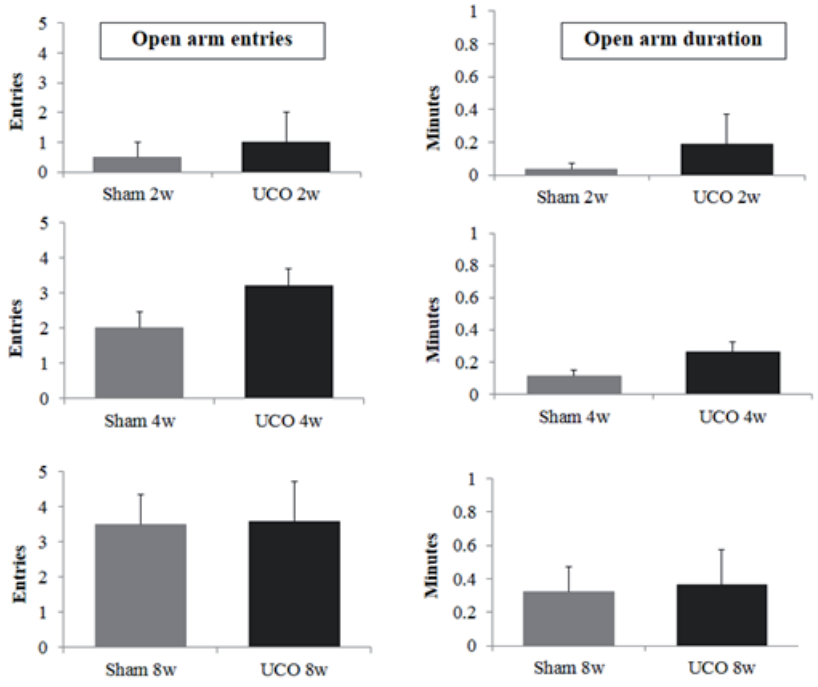

FIGURE 2. Representation of anxiety-like behavior assessed in the elevated plus maze (EPM). Data interpreted as open arm entries and duration at 2, 4 and 8 weeks of right common carotid artery occlusion.

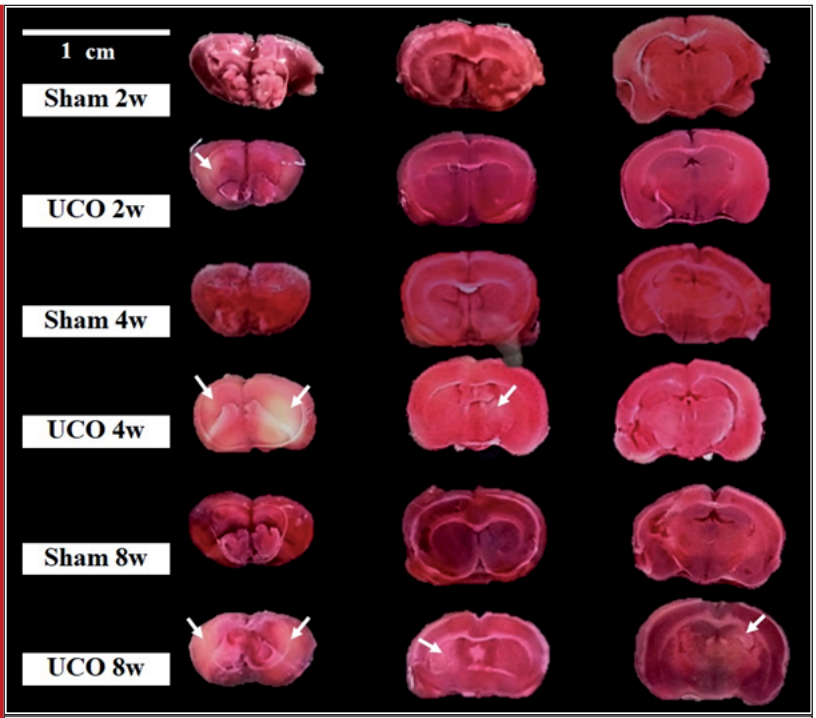

FIGURE 3. Representation of brain tissue stained with 2 % 2, 3, 5-triphenyltetrazolium chloride (TTC) of frontal cortex, striatum and hippocampus. White arrow indicated example of pale TTC area as identification of tissue infarction. Scale bar = 1 cm.

basal ganglia circuitry, facilitating a shift in strategies and response pattern(Ragozzino et al. 2009).The present study found significant infarction of striatum since 4 weeks that might correlated with this flexibility deficit. However, reversible of learning flexibility deficit did not correlate with significant frontal cortex and striatum infarction at 8 weeks. TTCtechnique use the reaction of dehydrogenase to reduce TTC and turn to red color in healthy tissue (Fishbein et al. 1981). Tissue lack of blood perfusion was found to lead to lack of $O_2$and further NADH in cellular respiration. TTC technique had high degree identification of infarct area and volume and suitable for producing accurate measurements of cerebral experimental infarcts as the use of cresyl violet staining (Tureyen et al. 2004).

The present study identified pale area of TTC as not a pure white, but pink-white, so we got a trend. It might describe as penumbra area of ischemic tissue, perfusion still present and cells are viable with hypofunction because of metabolic insufficient. This area was destined as delayed cell dead or survive (Heiss and Graf 1994). As we had found in the frontal cortex and striatum, the reversible of learning flexibility deficit at 8 weeks that not correlated with infarction volume might involve compensatory mechanisms such as vascular remodeling and improvement of collateral blood sup-

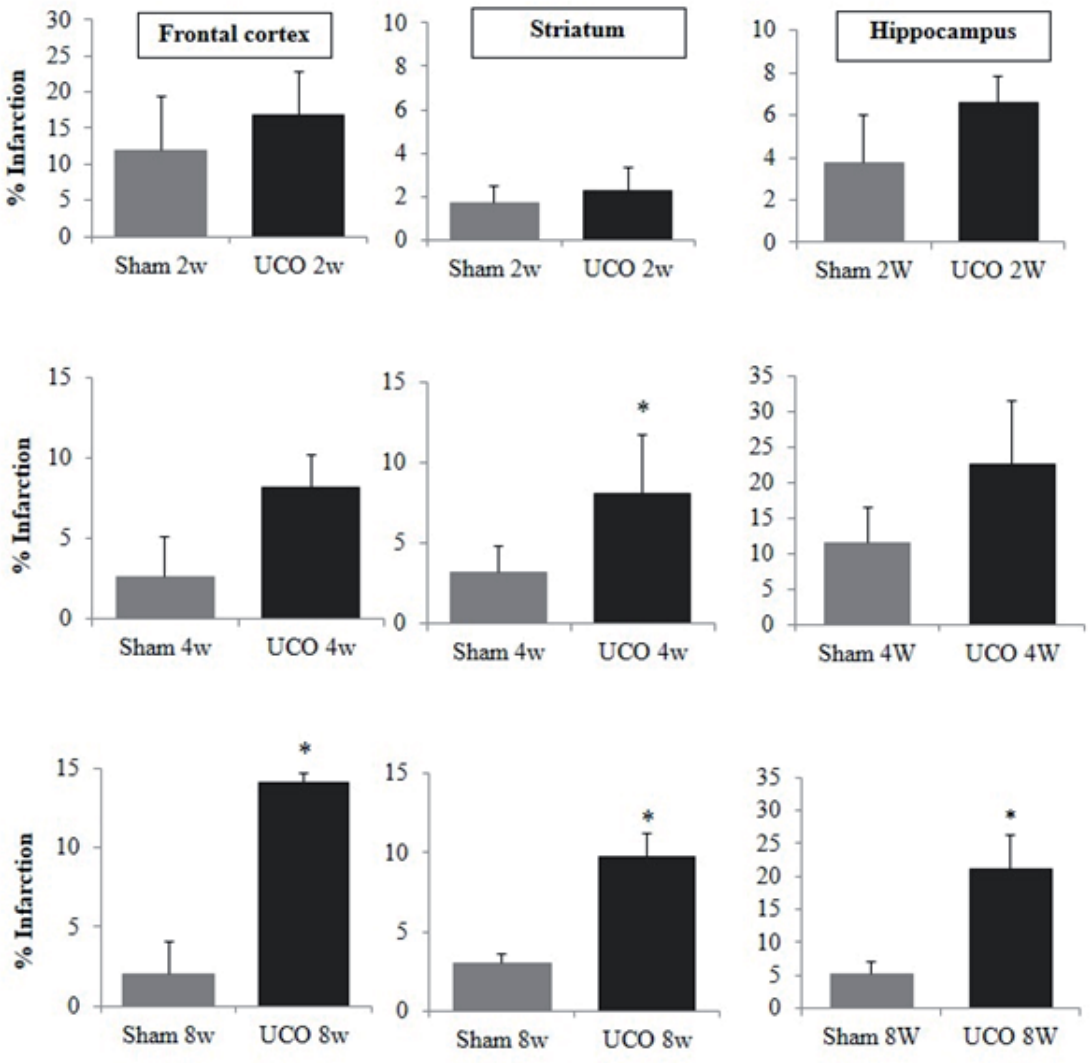

FIGURE 4. Representation of infarction area of frontal cortex, striatum and hippocampus using TTC staining technique. The infarction area indicated by the percentage of infarction at 2, 4 and 8 weeks of permanent right common carotid artery occlusion (UCO), * $p < 0.05$.

ply that help survive of cells in this penumbra-like area in CCH model(Choy et al. 2006, Coyle and Panzenbeck 1990).The ipsilateral cerebral blood reduction in rCCAO mice returned to normal level with 4 weeks was reported (Yoshizaki et al. 2008), however, hemispheric perfusion asymmetry was found in some challenge situation such as hypercapnia. It was report about progressively less pronounced with time but a slight asymmetry still persists one month after unilateral carotid occlusion(Ley et al. 1985). This hemodynamic insufficient might last long as the artery was occluded. Early affected of CCH was on cognitive behavior while pathological appearance of damaged neuron not significantly presented as has been reported by Thong-asa et al. (2013), Thong-asa and Tilokskulchai (2014) and Thong-Asa (2015).

Unlike present study, spatial learning and memory in the acquisition trial and probe did not affected but learning flexibility. The difference between acquisition and reversal paradigm in the MWM produced a difference outcome of stress and lead to difference effect on synaptic density of the hippocampus. The divergent effects of experiences on CA3 hippocampal synaptic activity, i.e. stress as a suppressor and learning as a promoter of synaptic plasticity (Sandi et al. 2003). Susceptibility of cognition type to CCH found in the present study might involve stress inducing during reversal paradigm as well. Learning flexibility associated with prefrontal cortex-basal ganglia circuitry while spatial acquisition was hippocampal-dependent cognitive type. It was not surprising that hippocampus-dependent learning ability in acquisition trial did not correlate with hippocampus infarction found in the present study.

The percentage of infarction area in the hippocampus higher than the frontal cortex and striatum but it was not significantly different until 8 weeks. There was a report suggested that hippocampal-dependent spatial learning only requires a minislab (down to 26% of total) of dorsal hippocampal tissue (Moser et al. 1995).

The present study found infarction area not more than 25% and not all of this area appear dead. It is interesting that how long after appearance of significant infarction in the hippocampus that the spatial learning and memory in the acquisition paradigm will appear? There are reports suggesting about increase of locomotor activity and mental confusion such as anxiety-like behavior in the ischemic experimental animals(Milot and Plamondon 2009, Plamondon and Khan 2005) and these factors might involve facilitation of cognitive ability in the cognitive tasks. Early after ischemic onset as 24 hours and 1 week, it appeared significantly anxiolytic promoting of ischemia.This anxiolytic promoting disappeared in long term as time-dependent effects of global cerebral ischemia. These reports provided data only ischemic-reperfusion model unlike in the present study. We assessed anxiety-like behavior at 2, 4 and 8 weeks of CCH model and we found only trend that UCO mice had higher open arm entries and duration than Sham with no significant difference. We first report about anxiety-like behavior in CCH mice model, and it did not involve or facilitate the cognitive abilities in this study.

In conclusion, the present study suggested that mild CCH induced by permanent right common carotid artery occlusion in ICR mice induce reversible learning flexibility deficit but not memory of the reverse platform location paradigm of the MWM. Our study imply about cognitive type susceptible to mild CCH in ICR mice and it was the learning flexibility.

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Conflict of interest: None.

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