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Research report

Chronic cerebral hypoperfusion induces long-lasting cognitive deficits accompanied by long-term hippocampal silent synapses increase in rats

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HIGHLIGHTS

- Chronic cerebral hypoperfusion causes long-lasting cognitive deficit.
- Silent synapses exist in the adult brain.
- Chronic brain hypoperfusion induces long-term silent synapses increase in CA1 of rat.

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ABSTRACT

Synaptic dysfunction underlies cognitive deficits induced by chronic cerebral hypoperfusion (CCH). There are silent synapses in neural circuits, but the effect of CCH on silent synapses is unknown. The present study was designed to explore learning and memory deficits and dynamic changes in silent synapses by direct visualization in a rat model of CCH. Adult male Sprague-Dawley rats were subjected to permanent bilateral common carotid artery occlusion (BCCAO) to reproduce CCH. Learning and memory effects were examined at 1, 4, 12, and 24 weeks after BCCAO. In addition, immunofluorescent confocal microscopy was used to detect AMPA and *N*-methyl-D-aspartate receptors colocalized with synaptophysin, and Golgi-Cox staining was used to observe dendritic spine density. We found that BCCAO rats exhibited recognition memory deficits from 4 weeks; spatial learning and memory, as well as working memory impairment began at 1 week and persistent to 24 weeks after surgery. Following BCCAO, the percentage of silent synapses increased by 29.81–55.08% compared with the controls at different time points ($P < 0.001$). Compared with control groups, dendritic spine density in the CA1 region of BCCAO groups significantly decreased ($P < 0.001$). Thus, the present study suggests that CCH can induce long-lasting cognitive deficits and long-term increase in the number of silent synapses. Furthermore, the decrease in dendritic spine density was correlated with the decrease in the number of functional synapses. The results suggest a potential mechanism by which CCH can induce learning and memory deficits.

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1. Introduction

Among older people, dementia is the leading chronic disease contributing to disability and need for care. Thus, dementia significantly affects every health system in the world, and large amounts of resources and money are spent in caring for people with dementia [1]. Vascular cognitive impairment (VCI) encompasses vascular dementia and is the second most common cause of dementing illness after Alzheimer's disease (AD) [2]. Chronic cerebral hypoperfusion (CCH), which is a common event in elderly people, has been identified as a notable risk factor of dementia in patients with cerebrovascular disease [3,4]. However, its underlying mechanism is poorly understood and no effective treatment is available. An understanding of injury mechanisms is essential for the development of therapeutic strategies. Permanent bilateral common carotid artery occlusion (BCCAO) has been widely used

Abbreviations: CCH, chronic cerebral hypoperfusion; BCCAO, bilateral common carotid artery occlusion; MWM, Morris water maze; ORT, object recognition task; DI, discrimination index; GluR2, AMPA receptor 2; NMDAR, *N*-methyl-D-aspartate receptor; NR1, NMDAR1; VCI, vascular cognitive impairment; AD, Alzheimer's disease; CBF, cerebral blood flow; PICK1, protein interacting with C kinase 1.

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as a model of VCI due to CCH [5–8], which provide a moderate but persistent reduction in regional cerebral blood flow (CBF) that compromises memory processes and contributes to the development and progression of dementia [9]. The advantages of this model have been discussed previously [2,10].

The changes in CBF after BCCAO can be divided into 3 phases with a gradual transition with regard to the metabolic and homeostatic state of the tissue [9]. In the acute phase after the start of BCCAO in rats, the CBF drops sharply and reduces to 35–45% of the control level in the cerebral cortex and to 60% of the control level in the hippocampus. From 1 week to 3 months after BCCAO onset, the CBF values start to gradually increase in the cerebral cortex and hippocampus, but remain at significantly lower levels than the control. This is a phase of chronic hypoperfusion, which most closely resembles the condition of reduced CBF in human aging and dementia [9]. Afterward, the CBF values reach baseline at 6 months [11–13]. Previous studies have identified several neuropathological changes induced by CCH in the brain (especially in the hippocampus) such as neuronal damage [10,14], neuroglia cell dysfunction (astrocyte/microglial activation) [15], white matter injury [16,17], oxygen stress [18], cholinergic dysfunction [19], vascular plasticity damage [5,20], and decrease of synaptic plasticity [21,22]. Therefore, the rat model of BCCAO is a widely accepted experimental model to study neuropathological characteristics and potential treatments for CCH-induced cognitive dysfunction.

Increasing evidence demonstrates that alterations in function of glutamatergic synapses are the cellular basis for learning and memory [23]. Since the early 1970s, neuroscientist proposed the concept of silent synapses, which emerged from the recognition that there can be a mismatch between the number of morphologically identified synapses and the number of functional synapses [24,25]. Evidence for silent synapses, particularly in the mammalian central nervous system, has been reviewed in several articles [24–30]. What we often perceive as silent synapses are actually AMPA-silent synapses (postsynaptic silent synapses). Different electrophysiological and morphological methods are used to detect and identify AMPA-silent synapses [31–36].

Silent synapses have mostly been found and studied in the developing brain [31,34,37,38], as they are substantially downregulated in adult (>1 month old) rats [36,39,40]. However, recent studies indicated (first described) that postsynaptically silent synapses were present in hippocampal CA1 pyramidal neurons of adult and aged rats [41]. Thus, the reservoir of postsynaptically silent synapses does not disappear with brain maturation, but rather is maintained as an exclusive subtype of silent synaptic connection. The similarity of silent and functional synapses in the CA1 region of the hippocampus at resting membrane potentials throughout adulthood in rats may indicate that impairments in the mechanisms of synaptic plasticity and its subsequent stabilization, rather than deficient synaptic transmission, underlie age-related cognitive decline [41]. Similar findings have been presented from studies of layer 2/3 pyramidal neurons of the visual cortex in dark-reared adult mice [42] and interneurons in the rat CA1 stratum radiatum after the first postnatal month using electrophysiological methods [43].

Neurodegenerative disorders, including VCI, are characterized by synapse loss [44]. Early signs of synaptic dysfunction in models of VCI include inhibition of LTP [45], facilitation of LTD, and loss of synaptic AMPA receptors (AMPAR) [46]. As LTP is compromised under these pathological conditions, the likelihood that silenced synapses will become functional again is reduced [47]. However, the mechanisms underlying pathological synapse elimination in VCI are still poorly understood. The prototypical glutamatergic synapse contains 2 types of ionotropic glutamate receptors, AMPAR and *N*-methyl-D-aspartate receptors (NMDAR). In contrast to the ligand-gated AMPAR, NMDAR are also voltage-gated, so that they are largely non-conducting at the resting membrane potential but conduct at depolarized membrane potentials. AMPA-silent synapses, which express only NMDAR, are thus silent at the resting membrane potential but are revealed by the NMDAR-mediated current at depolarized membrane potentials [24,31]. Thus, these synapses are also called "deaf" synapses [28].

Based on the aforementioned evidence, a reasonable hypothesis is that silent synapses exist in the adult brain, and changes in silent synapses may underlie the cellular basis of cognitive impairment induced by CCH. Therefore, the purpose of the present study was to confirm the existence of silent synapses in the hippocampus of adult rat and to investigate dynamic changes in silent synapses using a triple immunofluorescent method in a rat model of CCH. Different post-ischemic survival intervals were used in our experimental protocol to increase the predictive validity of the model.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (weighing 280 ± 20 g) used in this study were obtained from Chengdu Dossy Biological Technology Co., Ltd. [Certificate Number: SCXK (Chuan) 2014-028, Chengdu, China]. The rats were housed in groups of 5 per cage and maintained in a temperature- and humidity-controlled room with 12-h light/dark cycle (lights on at 07:00 AM). Food and water were freely available throughout the experimental period. All procedures were in accordance with the Hospital Policies on the Use and Care of Animals and were approved by the Institutional Animal Experiment Committee of Chengdu Military General Hospital, Chengdu, China. Every effort was made to minimize the number of animals used and their suffering.

2.2. Surgery

The surgical procedures were performed as described previously [15,48,49], with a slight modification. Briefly, rats were anesthetized with 0.15% pentobarbital sodium (40 mg/kg). After a midline incision in the neck, common carotid arteries were exposed and carefully separated from the carotid sheath as well as the cervical sympathetic and vagal nerves. Bilateral common carotid arteries were double ligated with surgical silk; sham-operated controls were not subjected to artery ligature. After the surgical procedure, rats were placed on a heating pad to maintain body temperature at 37.5 ± 0.5 °C and were kept on it until recovery from anesthesia. At the beginning, there were 15 rats per subgroup in the BCCAO group and 10 rats per subgroup in the sham-operated group. Surgery survival rate was 78.33% (47/60) in the BCCAO group, and no rats died in the sham-operated group.

Experimental groups were consistent with previous studies [14,20,50] and focused on the phase of chronic hypoperfusion after BCCAO [9]. Furthermore, we wanted to investigate whether the changes in silent synapse could return to the normal level upon cessation of cerebral hypoperfusion. Therefore, the groups were as follow: (1) BCCAO + 1 week of survival (n = 11); (2) BCCAO + 4 weeks of survival (n = 13); (3) BCCAO + 12 weeks of survival (n = 12); (4) BCCAO + 24 weeks of survival (n = 11); (5) sham-operated + 1 week after procedure (n = 10); (6) sham-operated + 4 weeks after procedure (n = 10); (7) sham-operated + 12 weeks after procedure (n = 10); (8) sham-operated + 24 weeks after procedure (n = 10).

2.3. Behavioral analysis

Rats were submitted to behavioral testing using an object recognition task (ORT) and a Morris water maze (MWM) 1, 4, 12,

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