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Research report

Impairment of intradimensional shift in an attentional set-shifting task in rats with chronic bilateral common carotid artery occlusion

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HIGHLIGHTS

- Rat received bilateral common carotid artery occlusion (BCCAo).
- Testing attentional function of BCCAo rats using an attentional set-shifting task.
- Impairment of intradimensional shift in the attentional task in BCCAo rats.
- Alterations of GABAergic neurons in the anterior cingulate cortex in BCCAo rats.
- Attentional impairment and dysfunction of ACC in the animal model for VaD.

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ABSTRACT

Studies of rats with chronic bilateral common carotid artery occlusion (BCCAo), an animal model for vascular dementia (VaD), have reported hippocampus-dependent memory impairment and associated neuropathologies. Patients with VaD also experience attentional shifting dysfunction. However, animal models of VaD have not been used to study attentional function. Therefore, the present study examined attentional function in rats with BCCAo, using attentional set-shifting task (ASST) that required rats to choose a food-baited pot from 2 possible pots. ASST included 6 consecutive sessions including simple discrimination, compound discrimination, intradimensional shifting, extradimensional shifting, and reversals. The BCCAo rats were significantly slower at learning the intradimensional set-shifting task compared to control rats. Previous studies have demonstrated that the cingulate cortex and medial prefrontal cortex are critical to intradimensional and extradimensional set-shifting, respectively. Additionally, inflammatory responses and neuronal dysfunction were observed in rats with chronic BCCAo. In addition, OX-6 positive microglia significantly increased in the forceps minor white matter of BCCAo rats, and glutamate decarboxylase signals co-localized with NeuN were reduced in the anterior cingulate cortex of BCCAo rats, compared to control rats. Impaired neuronal and GABAergic neuronal integrity in the anterior cingulate cortex, damage to white matter, and attentional impairments observed in BCCAo rats suggest dysfunction of brain structures that are associated with attentional impairments observed in patients with VaD.

1. Introduction

Persistent insufficient cerebral blood flow leads to vascular dementia (VaD), the second most common type of dementia following Alzheimer's disease (AD) [1]. Risk factors associated with VaD include transient ischemic attacks, silent and clinically evident strokes, and ischemic changes [1]. These vascular impairment reduce blood flow to the brain and thereby contribute to cognitive dysfunction [2]. Previous studies have reported episodic memory impairment and brain pathology in structures including the hippocampus, which is associated with memory deficits in patients with VaD and AD [3,4]. Studies using animal models of these diseases have also shown impairment of hippocampus-dependent memory and evidence for pathophysiological mechanisms underlying the memory impairments [5–9]. In addition, other cognitive dysfunctions such as attention deficits are observed in patients with VaD and AD [3]. Specifically, on the Wisconsin card sorting test

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(WCST), which assesses attentional function, patients with VaD or AD performed worse than non-patients, with those with VaD experiencing the most pronounced deficits [3]. However, there is no prior research using animal models of VaD to examine attention deficits or attention related neurobiological changes in the prefrontal cortex.

A rat model of chronic cerebral hypoperfusion via bilateral common carotid artery occlusion (BCCAo) is commonly used to study neuropathological characteristics and potential treatments for VaD [8,10]. In the BCCAo rat model, cerebral blood flow is immediately reduced to approximately 32% of the basal level, and is then gradually recovered via collateral blood supplies [11]. Neuropathological features in human patients with VaD, such as white matter damage, neuro-inflammatory responses, and memory impairments are reported in BCCAo rats [8,11–13]. In particular, memory impairment has been reported in BCCAo rats, as indicated by use of a Morris water maze test, novel object recognition/location test, and an 8-arm maze task [8,14,15].

Attention is an important component of cognitive function, as it underlies the ability to focus on relevant stimuli from the many stimuli presented simultaneously in everyday life [16]. In addition, attentional shifting is an important component of executive function in order to enable efficient responses to continuously changing stimuli in the environment [16]. Many studies have demonstrated that several cortical areas are involved in attentional shifting, using the WCST in humans [17–20] and non-human primates [16,21]. In rodents, attentional set shifting tasks (ASST) are adapted and modified from the WCST, using stimuli that are easily perceptible to rats, such as textures and odorants, instead of the visual, color, or shape stimuli used in the WCST [22]. Lesion and electrophysiological studies have revealed individual contributions of each cortical area to attentional processing. For example, the medial prefrontal cortex (mPFC) in rats, or the dorsolateral prefrontal cortex in primates, mediates attentional shifting for different perceptual features of the stimulus (i.e., extradimensional shift) [16,21–24]. The cingulate cortex (CgC) is involved in intradimensional shift within a learned perceptual dimension [23,25], whereas the orbitofrontal cortex (OFC) mediates reversal discrimination [24,26–29]. In particular, GABAergic neurons in these regions are involved in the performance of the attentional task [30]. Therefore, neuronal integrity and GABAergic neuron was examined in the subregions of frontal cortex of the BCCAo rats by neuronal nuclei (NeuN) and glutamate decarboxylase 67 (GAD67) immunoreactivity.

NeuN is a neuronal-specific nuclear protein expressing in mature neuron, which is mostly used as excellent neuronal marker [31]. Expression levels of NeuN immunoreactivity are decreased in the neurons with damage before degeneration occurs [32]. Decreased NeuN immunoreactivity was observed in the hippocampus of BCCA rats [33], but neuronal degeneration was also reported in the hippocampus [9]. GAD67, a GABAergic neuronal marker, is a key enzyme that produces a gamma-amino butyric acid (GABA) inhibitory neurotransmitter from glutamate. Decreased GAD67 immunoreactivity in prefrontal cortex was reported in the patient with attentional impairments [34].

Patients with VaD have attentional deficits, but previous studies have not examined attention in an animal model of VaD. Therefore, the present study evaluated attention in BCCAo rats, using the modified and adapted WCST, which is an ASST for rodents [22], and examined associated damage in brain structures involved in attention. BCCAo rats had impaired performance on the intradimensional task compared to control rats. Microglial activation was significantly increased in white matter of the forceps minor of BCCAo rats and immunoreactivity of glutamate decarboxylase-positive signals that co-localized with NeuN-positive signals were decreased in the anterior cingulate cortex (ACC) of BCCAo rats, compared to sham-operated controls.

2. Material and methods

2.1. Subjects

Twenty male Wistar rats (10-weeks-old, Charles River Co., Gapeung, South Korea) were used in this study. The rats were housed in a vivarium at Konkuk University for 2 weeks prior to the start of the experiment, under controlled temperature ($22 \pm 1\,^{\circ}C$) and humidity ($50 \pm 10\%$) on a 12 h light/dark cycle (lights on at 0700 h). Rats were handled by the experimenter for 2 weeks prior to surgery. The Institutional Animal Care and Use Committee of Konkuk University approved all protocols described in the report. All surgical procedures and behavioral testing took place during the light phase.

2.2. BCCAo surgery

We conducted the BCCAo surgery when rats were at twelve weeks old on the ground of the reports followed. First, it is reported that brain development in rat is almost finished at twelve weeks old [35–37]. Second, we usually used 12 week old rats for BCCAo and reported hippocampal dependent memory impairments and neuropathological features [8,50,51], which might provide a reference to understand behavioral impairment in the ASST. Rats were anesthetized using a 5% isoflurane and oxygen mixtures, and anesthesia was maintained with 3% isoflurane during the surgical procedure. A midline incision was made to expose bilateral common carotid arteries (CCA). Bilateral CCAs were then tightly double-ligated with silk sutures. In addition, control animals experienced a sham-operation consisting of the same procedure without CCA ligation. Experimental rats were segregated into 2 groups: a sham-operated control group (Sham, $n = 9$) and a BCCAo group (BCCAo, $n = 11$).

2.3. Attentional set-shifting task (ASST)

For 1 week after surgery, rats recovered with food and water available ad libitum. Rats were then food restricted 1 week prior to the behavior task, in order to reduce their body weight to 85%. But water was available ad libitum. Food restriction was continuing until end of behavior task. Body weights were maintained at 85% during the ASST.

2.3.1. Apparatus

All behavior tests took place in an opaque acrylic box ($50 \times 37.5 \times 25$ cm) with a transparent front side. The box was equally divided into 2 compartments by a black sliding door, which created a starting area and a test area. Two ceramic pots (7 cm diameter, 3.5 cm height) were located in the test area in order to create the odorant and texture medium. Two pots were filled with texture medium, and 50 μl of odorant was placed on top of the medium. The reward was placed at the bottom of the ceramic pot and concealed by medium. The food reward was 1/2 piece of Kellogg's® Froot Loops cereal.

2.3.2. Shaping and habituation

Habituation and testing procedures were adapted and modified from the protocol of Birrell and Brown [22]. One day prior to testing, rats were placed in the testing apparatus and allowed to explore the apparatus, including the 2 digging pots. Food rewards were buried in the 2 pots and rats were permitted to dig freely in the pots. The reward was put on the medium of 2 pots at the start of shaping, and then the consecutive baited reward was placed increasingly deeper in the bowl. In the shaping session, home cage bedding was used for medium. If rats found the reward in both pots, the trial was