Neuroscience 233 (2013) 139–145

EXERCISE-INDUCED CHANGES OF THE CAPILLARIES IN THE CORTEX OF MIDDLE-AGED RATS

C.-X. HUANG,[a†] X. QIU,[b†] S. WANG,[a] H. WU,[c,d] L. XIA,[c,d] C. LI,[c,d] Y. GAO,[e] L. ZHANG,[b,d] Y. XIU,[f] F. CHAO[c,d] AND Y. TANG[c,d*]

[a] *Department of Physiology, Chongqing Medical University, Chongqing 400016, PR China*

[b] *Department of Laboratory Medicine, Key Laboratory of Diagnostic Medicine (Ministry of Education), Chongqing Medical University, Chongqing 400016, PR China*

[c] *Department of Histology and Embryology, Chongqing Medical University, Chongqing 400016, PR China*

[d] *Laboratory of Stem Cell and Tissue Engineering, Chongqing Medical University, Chongqing 400016, PR China*

[e] *Department of Geriatrics, The First Affiliated Hospital, Chongqing Medical University, Chongqing 400016, PR China*

[f] *Institute of Life Science, Chongqing Medical University, Chongqing 400016, PR China*

Abstract—Previous studies have shown that running exercise could increase regional cerebral blood flow. There have been previous studies investigating the effects of running exercise on capillary density in the brain and showing that running exercise could induce brain angiogenesis. However, there have been no studies investigating the effects of running exercise on the total volume, total length and total surface area of the capillaries in the cortex. Moreover, sex differences in the effects of running exercise on the capillaries of the cortex have not previously been investigated. The current study was designed to investigate the effects of running exercise on the capillaries in the cortex of middle-aged rats using the new unbiased stereological methods. The present study found that the total length and total surface area of the capillaries in the cortex of running middle-aged female rats were significantly increased, compared to control rats. Our results also reveal that there are sex differences in the effects of running exercise on the capillaries in the cortex of middle-aged rats. These results demonstrate that exercise-induced increases of the capillaries in the female rat cortex might be one of the structural bases for the exercise-induced improvement in the spatial learning capacity of middle-aged female rats. These results provide a baseline for further studies that search for strategies to delay the deleterious effects of brain aging.

Key words: capillary, cortex, rat, running exercise, immunohistochemistry, stereology.

INTRODUCTION

Insufficient cerebral perfusion and regional decrease of cerebral blood flow (CBF) have been reported in the elderly (Martin et al., 1991; Kawamura et al., 1993). Cerebrovascular insufficiency, such as reduced blood supply to the brain or disrupted microvasular integrity in cortical regions, might induce cognitive decline during aging (Goldman et al., 1987; Farkas and Luiten, 2001; Boles Ponto et al., 2006). The declining CBF of the aging brain appears to have well-described morphological correlates (Farkas and Luiten, 2001). Previous studies reported that there were age-related abnormalities in the basement membrane such as perivascular collagen deposits and basement membrane thickening (De Jong et al., 1990; Farkas and Luiten, 2001). Other age-related structural changes reported in previous studies included abnormalities such as the loss of elongation of capillary endothelial cells, a decrease in the number of endothelial mitochondria, pericytic degeneration and an increase in the size of pericytic mitochondria (De Jong et al., 1990; Farkas and Luiten, 2001). In addition, previous researchers have found a decline of the capillary density in the hippocampus and cortex of the aged brain. These researchers thought the ultrastructure changes might contribute to a slow and lowered CBF (Amenta et al., 1995a,b; Sonntag et al., 1997). The age-related changes in the brain microvascular structures might also be detected at the behavioral level as cognitive failure and memory dysfunction (Farkas and Luiten, 2001; Riddle et al., 2003).

Exercise has commonly been considered as a convenient intervention that has positive effects on the vascular system of the aged brain. Imaging studies have shown that physical exercise can increase cerebral blood volume (Swain et al., 2003; Pereira et al., 2007; Rhyu et al., 2010) and CBF (Yancey and Overton, 1993; Colcombe et al., 2004). There have been some studies investigating the effects of running exercise on cerebral capillaries. These previous studies showed that exercise could induce an increase in cortical capillary density and lead to the growth of new capillaries in the brain, especially in the motor cortex (Black et al., 1990; Kleim et al., 2002; Swain et al., 2003; Ding et al., 2006; Rhyu et al., 2010). Swain et al. (2003) found that the volume fraction of the cortex capillaries was increased in the II/III layer of the motor cortex of 30 days of running exercise in

*Correspondence to: Y. Tang, Department of Histology and Embryology, Chongqing Medical University, Chongqing, PR China. Tel: +86-13883933846; fax: +86-23-68485868.
E-mail address: ytang062@yahoo.com.cn (Y. Tang).
† These authors contributed equally.

Abbreviations: CBF, cerebral blood flow; IUR, isotropic, uniform random; PBS, phosphate-buffered saline; VEGF, vascular endothelial growth factor.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.12.046

6–12-month-old rats (Swain et al., 2003). Rhyu et al. (2010) found that the vascular volume fraction of the motor cortex was increased significantly in adult monkeys exercised by running for 5 months (Rhyu et al., 2010). However, due to age-related tissue atrophy and the tissue processing-induced shrinkage, these density measurements may not accurately reflect the changes in total structural quantities (Iwadare et al., 1984). Until now, there have been no studies investigating the effects of running exercise on the total volume, total length and total surface area of the capillaries in the cortex. Moreover, the sex differences in the effects of running exercise on the capillaries of the cortex have not been investigated. Therefore, in the current study, we used stereological methods to investigate the effects of running exercise on the cortex and on the capillaries in the cortex of middle-aged female and male rats.

EXPERIMENTAL PROCEDURES

Animals

Forty 14-month-old Sprague–Dawley female and male rats from the Third Military Medical University, PR China were randomly divided into a running female group, a non-running control female group, a running male group and a non-running control male group, with 10 rats in each group. Animals were group-housed (2–3 rats per cage) under controlled temperature (22 ± 2 °C) with a 12-h light/dark cycle and free access to food and water. All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering.

Physical exercise

In the running group, the animals run on a six-lane motorized treadmill for 20 min each day for five consecutive days each week for four consecutive months (Lu et al., 2009). The rats ran at a speed of 10 m/min during the first week, 15 m/min during the second week, and 20 m/min during the duration of the study. The control rats were housed under identical conditions without running.

Tissue processing and estimation of cortex volume

The rats were anesthetized with 4% chloral hydrate intraperitoneally (10 ml/kg) and perfused intracardially with 0.9% heparinized saline followed by 4% paraformaldehyde in phosphate-buffered saline (PBS, 0.6 M, and pH 7.4). The brains were removed and one hemisphere was randomly sampled from each brain and coronally cut into 2-mm consecutive slabs. A transparent counting grid with an area of 0.4 mm^2 associated with each point was placed randomly on the caudal surface of each slab. The points hitting the cortex were counted using a dissecting microscope with a magnification of 40×. The total volume of the cortex [V_c] was calculated using Cavalieri's principle (Tang et al., 1997; Tang and Nyengaard, 2004; Shao et al., 2010). Slabs were post-fixed for at least 2 h in 4% paraformaldehyde, and embedded in paraffin wax with the caudal surface faced down. The orientator technique for generating isotropic, uniform random (IUR) sections was used (Shao et al., 2010) (Fig. 1). Finally, the tissue blocks were sectioned at 4 μm parallel to the IUR surface.

Immunohistochemical procedures

The tissue sections were deparaffinized in xylol and rehydrated through a graded alcohol series. Antigen retrieval was accomplished by first immersing sections in citrate buffer (0.01 M, pH 6.0) and then microwaved for 15 min. After cooling, sections were washed twice in PBS (0.01 M, pH 7.4) and then soaked in 3% hydrogen peroxide for 10 min at room temperature to inactivate endogenous peroxides. Sections were then washed in PBS before being incubated with normal goat serum for 20 min at room temperature to exclude nonspecific staining. Next, sections were incubated in rabbit polyclonal anti-collagen IV (ab6586; Abcam, Cambridge, UK) as the primary antibody at a dilution of 1:200 in PBS at 4 °C overnight and then 37 °C for 1 h. The sections were next washed in PBS and transferred to a secondary antibody solution (Biotinylated goat-anti-rabbit immunoglobulin G), and incubated for 20 min at 37 °C. The specimens were incubated with S-A/HRP for 20 min at 37 °C followed by three 5-min washes in PBS. Sections were then transferred to a diaminobenzidine (DAB) solution (DAB, ZLL-9032, ZSGB; Beijing, China) for approximately 10 min. Finally, sections were dehydrated by sequential immersion in gradient ethanols and xylene and coverslipped. The sections were observed under light microscopy. From each section, 4–6 cortical fields of view were randomly photographed under a

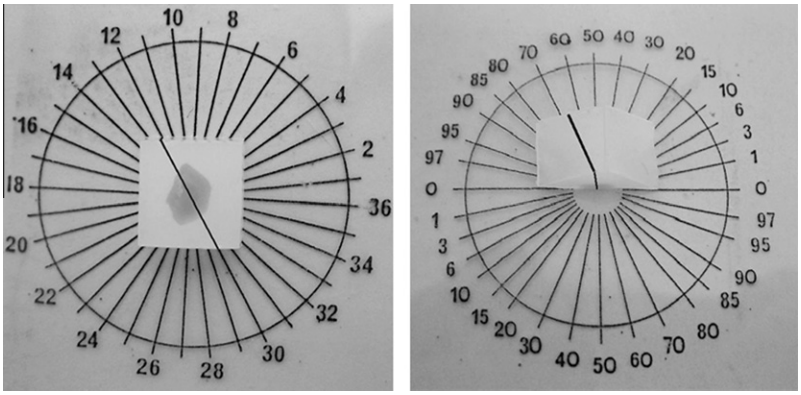

Fig. 1. The embedded tissue block with the orientator technique. *Left:* The tissue block is randomly put horizontally on the first circle. A random number is picked from 1 to 36. In this figure, 12 is the random number. The tissue block is vertically cut along the 12–30 direction in order to obtain the first vertical surface. *Right:* One of the two blocks cut in the first step is randomly sampled and put on the second circle. The intersecting line between the vertical surface and the caudal surface of the tissue block is put along the 0–0 direction. A random number is sampled from 0 to 97. In this example, 70 is the random number. The sampled tissue block is vertically cut again along the 70–70 direction to obtain the second vertical surface, which is an isotropic, uniform and random (IUR) surface.