ABSTRACT

Purpose. In this study, we explore the effects of aging on pericytes and capillaries using mice. Pericytes are important components of the neurovascular unit and function as contractile cells around the walls of capillaries. They play many important roles in the brain, such as blood vessel formation, cerebral brain blood flow, maintenance of the blood-brain barrier, and regulation of immune cell entry into the CNS. Dysfunction of pericytes contribute to a wide range of illnesses that result in cognitive impairments such as cerebrovascular disease, stroke, Alzheimer's disease (AD), and other neurological disorders. Aging has been studied and shown to be an established risk for vascular dysfunction that affects the integrity of the neurovascular unit. Furthermore, studies have shown significant reductions in pericyte density during age-related disorders, but these studies are few. Most nutrients in the brain are supplied by capillaries, and because pericytes are embedded on capillaries, studying their patterns and effects may lead to a better understanding of the pathophysiology and preliminary triggers of age-related disorders. In this study, we explore whether both pericyte and capillary numbers are affected in the adult brain of mice as they age.

Methods. All experiments were performed on young (3 month old; n=3) and old (20-23 month old; n=3) C57BL/6 male mice. To identify pericytes and capillaries for quantification, immunohistochemistry and immunofluorescence were used. Pericytes were stained using the biomarker PDGFrβ and capillaries were stained using Lectin. CA1, CA2, CA3, and DG sites were chosen for quantification in the hippocampus, and layers I-VI in the somatosensory cortex of each mouse. Confocal imaging was used to study and quantify the population of PDGFrβ and lectin-positive cells. T-tests were performed to compare the number of pericytes in the hippocampus and somatosensory cortex of the two groups of mice (young and old).

Results. Old mice exhibited significantly lower capillary (via lectin) and pericyte (via PDGFrβ) numbers than young mice ($p < 0.0001$) in the hippocampus. There was no significant reduction in the number of pericyte ($p = 0.1448$) and capillary ($p = 0.0967$) in the somatosensory cortex. Pericytes that expressed PDGFrβ were only classified as such when colocalized to capillaries. To record the number of pericytes embedded on capillaries, the number of PDGFrβ + Lectin that expressed a "bump-on-a-log" morphology was also quantified and showed a significant reduction in the hippocampus ($p < 0.0001$) and somatosensory cortex ($p = 0.0110$) with age.

Conclusion. Since cerebrovascular dysfunction plays a vital role in the development of cognitive impairment disorders, understanding the aging patterns of neurovasculature cells such as pericytes may aid in the early prevention of age-related illnesses.

PERICYTE AND CAPILLARY MAY DECLINE DEPENDING ON THE AGING PROCESS IN MICE

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INTERNSHIP PRACTICUM REPORT

Presented to the Education Council of

the School of Biomedical Sciences at

University of North Texas Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Oluwaseun Omoba, B.S.

Fort Worth, Texas

April 2022

ACKNOWLEDGEMENTS

I want to thank Dr. Kunlin Jin for extending his lab to my education. I respect Dr. Jin's intellect and I am grateful for his guidance and mentorship. One of the many things I learnt from him this year is to embrace the discomfort of not knowing, because you can always find the answer.

Thank you also to my other committee advisors, Dr. Caroline Rickards and Dr. Keisa Mathis, for their support as well. I hope to be as self-assured as they are in my own career.

I also want to thank my fellow lab colleagues, Siyang Lin and Benjamin Landon. I will miss Siyang and Ben dearly. I came to lab with no prior experience, and they guided me to the best of their abilities. They truly are amazing kind-hearted people, and I wish them the best in everything.

Lastly, I want to thank my family and friends for their continuous support.

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CHAPTER I - BACKGROUND AND LITERATURE

Aging

The effects of aging on the brain are extensive and may be attributed to a variety of causes. Aging has an impact on molecules, cells, vasculature, physical morphology, and cognition[26]. During aging, the brain undergoes specific pathophysiological changes, such as shrinkage in volume,[31], loss of neuronal circuits[6] and brain plasticity, decrease in blood flow, and increased susceptibility to inflammation.[20,21].

While cognitive loss is an inevitable outcome of aging, cognitive decline does not necessarily result in the development of aberrant cognitive diseases. Stress, hereditary factors, and lifestyle choices, among other variables, may result in the manifestation of abnormal aging. With aberrant aging comes a more rapid and severe deterioration in cognition, which may emerge as dementias such as Alzheimer's disease (AD), cerebrovascular diseases, motor diseases such as Parkinson's disease, and Lou Gehrig's disease, as examples[1].

Memory loss is the most widely seen cognitive change related to aging, and brain changes during aging do not occur to the same extent in all brain regions. Studies have shown the prefrontal cortex and hippocampus are the most impacted during aging, whereas the occipital cortex is the least affected[31,7]. Sex may also play a role in the brain regions most impacted by aging. Age-related brain tissue loss was significantly greater in males than females in the entire brain, but also in the frontal and temporal lobes, and loss was greater in females than males in the hippocampus and parietal lobes[14,27]. However, when examining the individual, both sexes exhibit considerable declines in these regions. Females are also more likely to develop AD than men, which cannot solely be explained by their longer life expectancy, given that women also have greater disease severity and a higher age-adjusted prevalence of AD than men[14]. In rodents, age-

related declines also vary between sexes but tend to be greater in males[34]. Further investigation is required into the subject of sex and age-related disorders.

Capillaries and their role in age-related cognitive disorders.

Capillaries are the smallest blood vessels in the vascular system. They transport blood and nutrients (including oxygen) to the end-organs. There are two types of capillaries in the body; continuous fenestrated capillaries located in the kidneys, small intestine, and endocrine glands, and continuous nonfenestrated capillaries located in the nervous system, skin, and lungs. Continuous capillaries are composed of endothelial cells that line the capillary walls, basement membrane that support endothelia cells, and pericytes that can contract to reduce capillary diameter[30,40]. In the brain, continuous nonfenestrated capillaries make up the blood-brain barrier (BBB). They regulate the movement of water, oxygen, and other essential substances between the blood and the brain, and prevent the passage of toxins, thus protecting the brain from injury and diseases[16].

The brain is densely packed with capillaries that can span 400 miles, indicating that they are responsible for the majority of the effort involved in nourishing this vital organ[13]. As we age, a number of factors, including vascular stiffness, autonomic disruption, neurovascular uncoupling, and BBB degradation, can affect the dynamics of brain blood flow and perfusion[23]. Vascular contributions to cognitive impairments such as dementia and Alzheimer's disease (AD) are increasingly recognized, particularly dysfunctions affecting the BBB and capillary blood flow, which may deprive the brain of its necessary nutrients [17,18,20]. Recent studies suggest that the breakdown of the BBB is an early biomarker of human cognitive dysfunction[8], and others indicate that AD is associated with a 20-30% decrease in cerebral blood flow (CBF) [29,35].

Quantitative analysis of capillary density during aging has shown severe reduction in the cerebral cortex[38] as well as morphological changes and declines in the hippocampus[37]. These significant changes and declines in capillary function as we age provide evidence that brain microvascular dysfunction is a critical factor in the development of age-related illnesses.

Impact of aging on neurovasculature

The neurovascular unit (NVU) in the central nervous system (CNS) is composed of endothelial cells, pericytes, basal lamina, astrocytes, capillaries, peri-capillary microglia, and neurons[12]. Several studies have demonstrated that during aging, the integrity of the NVU is compromised, notably at the capillary component of the NVU [38,37]. These studies also suggest a decrease in blood flow and capillary density during aging[2].

Although it is well established that capillary function declines with age, little is known about the interactions between other components of the NVU and their own individual responses to aging. Pericytes are one of those components. Due to the scarcity of studies on pericytes throughout the aging process, they remain a relatively unexplored area of investigation. One study assessed whether pericyte and vascular smooth muscle cell (VSMCs) decline in the adult brain of transgenic mice genetically predisposed to disrupt platelet-derived growth factor receptor β (PDGFrβ) signaling. They found early and progressive reductions in pericytes which were more pronounced in the cortex, hippocampus, and striatum[28]. Similar to this study, our project quantifies the progression of pericytes during aging, though we do not use transgenic mice.

Pericytes and cerebral blood flow.

Pericytes are contractile cells that surround the walls of capillaries and post-capillary venules[5]. They are embedded in the basement membrane of CNS blood vessels and lie adjacent to endothelial cells (Figure 1)[25]. Pericytes perform critical functions in the CNS, namely blood vessel formation, regulation of CBF, BBB maintenance, and regulation of immune cell entry into the CNS[4,5,30]. Studies have demonstrated that direct activation by neurotransmitters and ischemia can modulate vascular diameter at pericyte sites, causing the vessels in which they are embedded on to constrict or dilate [15,19,30,]. The consequent effect can either decrease or increase red blood cell movement through the vessels, thus affecting the effectiveness of oxygenation extraction from the blood to the surrounding areas. In the event of ischemia, for instance, pericytes contract and tighten around capillaries, further restricting the movement of blood cells[30].

Pericytes morphology.

Pericyte morphology can be difficult to classify, and studies have been conducted to accurately categorize the various types of pericytes in human and rodent brains[10,33,18]. Pericytes are a subset of mural cells that are classified into four distinct types: smooth muscle cells (SMCs) on pial and penetrating arterioles, ensheathing pericytes on the arteriole-capillary transition, capillary pericytes on capillaries, and venular SMCs on venules. Within these four types of mural cells are two subclasses of pericytes: the ensheathing pericytes and capillary pericytes[19]. Identification of these subclasses is critical because variations in pericyte categorization suggest diversity in pericyte function. Subsequent studies have demonstrated that certain markers are more specific for the different subclasses of pericytes, further demonstrating the diverse functions pericytes play in the brain[19]. Given the dense capillary network stationed in the brain,

it is critical to analyze and investigate the subclass of pericytes embedded on capillaries. This is because studies credit insufficient blood flow as a contributing factor in numerous neurologic conditions[5]. The precise role pericytes directly play on cerebral blood flow is still uncertain.

Ex vivo investigations employing histology, electron microscopy, or slice physiology have provided most of our understanding of pericytes, and pericytes can be recognized by their morphology when stained with protein markers. While protein markers such as neural/glial antigen 2 (NG2) and platelet-derived growth factor receptor (PDGFrβ) are extensively used to identify pericytes embedded on capillaries, these markers are also produced by other brain cells, thus limiting their specificity for pericytes[12].

Pericytes and Aging

Aging is an important factor that influences the integrity of the NVU. Considering that pericytes are a component of the NVU, age-related deterioration of this unit can have an aberrant effect on the critical functions pericytes perform in our brain. Stroke, neural dysfunction, cognitive decline, and neurodegenerative disorders can all be associated with defects in pericyte function[8]. Additionally, previous research has demonstrated that individuals with age-related diseases, such as AD, have a 25–50% reduction in pericytes compared to age-matched controls[17]. The precise mechanism by which aging impacts pericytes is a subject in need of further investigation.

SPECIFIC AIMS

The purpose of this study is to examine the influence of aging on the number of pericytes and capillaries in the hippocampus and somatosensory cortical regions of mice. We hypothesize that pericytes and capillaries decline significantly in the hippocampus and somatosensory cortical regions of old mice.

Specific Aim 1: **Determine the morphology of pericytes embedded on capillaries.** When examining pericytes, one of the challenges that exist is the wide range of morphology that they exhibit. They are frequently described as a "bump on a log," although their appearance can vary depending on their location. For instance, some studies have revealed that they resemble thin-strands, while others characterize them as mesh-like[9]. According to current research, there are two types of pericytes currently recognized: ensheathing pericytes and capillary pericytes. Due to the abundance of capillaries in the brain, we will focus on capillary pericytes that express PDGFrβ in this study[39]. The sole purpose of this aim is to describe how these cells appear in order to maintain a consistent categorization throughout this project. This study utilized C57BL/6 male mice aged 3 months ("young"), 20 and 23 months ("old"). To visualize pericytes embedded on capillaries, we co-stained pericytes labeled with PDGFrβ with Lycopersicon esculentum (Lectin), a well-known capillary marker, and 4′,6-diamidino-2-phenylindole (DAPi) was used to stain the nuclei of the cells. **Hypothesis:** Pericytes colocalized on capillaries will express a "bump on a log" morphology when identified with PDGFrβ, lectin, and DAPi.

Specific Aim 2: Determine the number of pericytes and capillaries in old and young mice at the hippocampal and somatosensory cortex. The purpose of this aim is to quantify and

compare the number of pericytes and capillaries found in old and young mice in the hippocampus and somatosensory cortex. To accomplish this goal, pericytes and capillaries will be stained with PDGFrβ and lectin, respectively, and then quantified in young and old mice. DAPi will be used to stain nuclei. **Hypothesis:** Pericytes and capillaries in the hippocampal and somatosensory cortex of old mice will be significantly reduced compared to young mice.

SIGNIFICANCE

Significance and Novelty

Notable studies have suggested a link between vascular function deficiency and age-related neurodegenerative diseases[8,17,18,20, 29,35] . Since the brain is densely packed with capillaries and their colocalized pericytes, knowing the impact of failure of this unit can aid in understanding the pathological disorders associated. Numerous neurodegenerative diseases are age-related; therefore, understanding what affects pericytes and capillaries throughout aging might offer insight on the health of the brain before or during the onset of these diseases. For instance, AD in particular results from a variety of vascular abnormalities such as BBB breakdown and microvasculature irregularities. Given that current research is focused on determining the underlying preliminary causes of AD, a vascular perspective focusing on cells such as pericytes and capillaries can aid in this discovery.

The literature is still scarce when examining pericyte population in aging mice. Typically, these studies focus on transgenic mice that have been purposefully altered to investigate pericytes, rather than on wildtype mice without genetic modification. In this study, we add to the discussion of whether pericytes increase or decrease in the brain during aging in the hippocampus and somatosensory cerebral cortex of mice, regions that prior studies have shown to be affected by aging[14,27].

CHAPTER II - MATERIALS AND METHODS

Animal and fixation of brain tissue

The UNTHSC Institutional Animal Care and Use Committee (IACUC) approved the procedures used in this study and all experiments were performed within the IACUC guidelines.

C57BL/6 male mice purchased from Charles River Laboratories were used for this project. Mice were housed in plastic cages on a 12 h light cycle with access to water and a standard laboratory diet. Three young mice aged 3 months, and three old mice aged 20-23 months were either sacrificed (N=2; young, ~26 g) or already sacrificed (N=4) by previous lab members for this project. Mice were placed in a chamber under 4% isoflurane anesthesia. Once the animals were under anesthesia, their heart and lungs were surgically exposed for perfusion. A needle was inserted into the left ventricle until it entered the ascending aorta. A small incision was made in the right atrium to release venous blood. 20 ml of PBS was perfused through the needle to flush out the blood, followed by 40 ml of 4% paraformaldehyde (PFA) to fix the tissues and cells. The animals were then decapitated, and their brain extracted and soaked in 4% PFA overnight at 4 degrees Celsius. Mice brains were then sliced in a mice brain slicer and embedded in paraffin before sectioning of 7 μm onto each slide for immunofluorescent staining.

Research design

For each mouse, we focused on the hippocampus and the somatosensory cortex based on studies that have shown these regions to be affected in volume during aging[27]. The hippocampus region was chosen because it is one of the first brain regions to be damaged in a variety of age-related cognitive impairments, and while the somatosensory cortex is not as severely affected as the hippocampus during aging, it is affected, nonetheless. To detect pericytes embedded on

capillaries (PDGFrβ+Lectin), we stained each section with PDGFrβ, a pericyte and endothelia cell identifier, lectin, a capillary label, and DAPi, a nuclei marker. Quantified hippocampus areas were (Box width: 516px, height: 347px): CA1a and CA1c, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3 and DG, dentate gyrus. These locations represent the main anatomical makeup of the hippocampus and were thus the locations chosen. Layers I-VI were quantified for the somatosensory cortex (Box width: 717px, height: 527px) (Figure 2). For easier measurement, the somatosensory cortex was separated into four sections. (Figure 2).
The workflow is summarized in Figure 3.

Immunofluorescence staining.

Immunofluorescence was performed using the markers PDGFrβ, Lectin, and DAPi in order to determine and classify the morphology and number of pericytes, capillaries and their nuclei respectively.

Brain sections were perfused with ice-cold PBS followed with cold 4% paraformaldehyde. The brain was then fixed in 4% paraformaldehyde, dried, paraffin-embedded, and sectioned at 7μm-thickness. The sections of the hippocampus and somatosensory cortex regions of the brain were rehydrated and treated with citrate buffer (pH 6.0) and microwaved for 10 minutes at 100°C. We then used 5% horse serum at room temperature for 1 h to block non-specific anti-bodies that created excessive background in our staining. After that, the sections were treated overnight at 4°C with the primary antibodies PDGFrβ (1:200, abcam; ab32570, UK) and lectin (1:200, vector labs; Burlingame, CA). Following a PBS wash, Alexa Fluor 594 donkey anti-rabbit IgG (a21207) fluorescent secondary antibodies obtained from ThermoFisher were applied for 1 h at room temperature in the dark. Images showing the morphology of pericytes,

capillaries and their corresponding nuclei were taken using an Olympus Fluoview FV1200 confocal microscope.

Categorizing the morphology of pericytes.

In this report, immunofluorescent staining on 3 month old and 20-23 month old mice revealed distinct capillary-pericyte morphology that was used to define pericytes. Pericyte-positive cells (Figure 4B) were stained alongside lectin-capillary positive cells (Figure 4C), and DAPi was used as a nuclear counterstain (Figure 4D). Overlaying DAPi, PDGFrβ, and lectin, revealed pericytes colocalized on capillary cells (Figure 4A & 5). Cells expressing PDGFrβ are not automatically considered pericytes unless they 1) expressed PDGFrβ 2) possessed a nucleus stained by DAPi, and 3) colocalized with Lectin (Figure 4 & 5).

Statistical analysis.

Graph Pad Prism 8 was used for the statistical analysis. An unpaired t-test was performed to determine any significant difference between old and young mice. $P < 0.05$ was deemed statistically significant.

CHAPTER III – RESULTS

PDGFrβ expression declines in old mice at the Hippocampus.

Using confocal imaging analysis for PDGFrβ, an established pericyte marker, along with lectin fluorescent to visualize capillaries, we found a significantly loss of pericyte coverage in old mice, as illustrated in figure 6A at the hippocampus ($p<0.0001$*) (Figure 6A).

Lectin expression declines in old mice at the Hippocampus.

The number of Lectin cells in the CA1a, CA1c, CA2, CA3, and Dentate Gyrus of the Hippocampus significantly decreased in old mice compared to young mice, thus allowing us to infer a decrease in capillaries during aging ($p< 0.0001$*) (Figure 6B).

PDGFrβ + Lectin expression declines in old mice at the Hippocampus.

The number of PDGFrβ cells embedded on lectin capillary (PDGFrβ + Lectin) was significantly lower in old mice compared to young adult mice ($p< 0.0001$*) (Figure 6C). Pericyte coverage was quantified as the number of PDGFrβ-positive pericytes co-localized on lectin-positive capillary surface area in old mice, confirming a reduction of pericyte coverage.

Somatosensory cortex

When young mice were compared to old mice, the number of PDGFrβ ($p = 0.1448$) and Lectin cells ($p = 0.0967$) showed no significant differences in the somatosensory cortex (Figure 7A&B). When PDGFrβ + Lectin was counted, there was a significant reduction in old mice ($p = 0.0110$*) (Figure 7C).

CHAPTER IV – DISCUSSION

In this study, we investigated whether aging decreases the number of pericytes and capillaries in our mice model. We discovered that 1) pericytes colocalized with capillaries do indeed exhibit a bump-on-a-log shape. It was important that we classified this since pericyte morphology can vary; 2) pericytes and endothelia cells marked by PDGFrβ and capillaries marked by lectin were significantly reduced in the hippocampus of old mice, and; 3) There was no significant difference in PDGFrβ and lectin cells individually at the somatosensory cortex level, but there was a significant difference in pericytes embedded on capillaries, as indicated by PDGFrβ + Lectin colocalization.

Pericytes have been labeled using a number of markers, including PDGFrβ, neural glial antigen 2 (NG2), desmin, and aminopeptidase N. (CD13)[12]. The dilemma is that these markers are also expressed on other cells, making it difficult to properly classify pericytes[5, 12]. PDGFrβ was used in this project because, while it also stains endothelia cells, it stains pericytes much more intensely. We co-stained with DAPi and Lectin to ensure that only pericytes embedded on capillaries were identified as capillary pericytes. Because PDGFrβ also stains other endothelial cells, cells expressing this marker were not automatically classified as pericytes unless they 1) expressed PDGFrβ; 2) had a nucleus stained by DAPi, and 3) colocalized with Lectin generating a bump (Figure 4A & 5). Once these three conditioned were met, we counted them as capillary pericytes. To eliminate bias, we double-checked that pericytes had the "bump-on-log" as shown in Figure 4A & 5 and did not quantify any cells that didn't clearly look like a bump. If there was an issue categorizing a particular cell, lab members not involved in this study weighed in on its morphology.

Pericytes

Our findings indicate that old mice have a considerable reduction of pericytes and capillaries in the hippocampus. This decrease in capillaries observed with aging is consistent with what previous research predicted, and was expected. On the other hand, the decrease in pericytes seen in our study might be interpreted in a variety of ways. When considering the physiological role of pericytes on capillaries, we know that they constrict the capillaries, but this does not always indicate that they alter blood flow. Our findings of a significantly reduced number of pericytes are consistent with those of other researchers, such as Nikolakopoulou et al., 2017. They also observed a similar drop in pericyte numbers with age in the hippocampus and somatosensory cortex of transgenic mice, using the CD13 pericyte marker. The significance of this decline remains uncertain. Numerous studies have been conducted to assess if pericytes do indeed impact blood flow. They demonstrated that ischemia induces pericyte-mediated capillary constriction followed by pericyte death, which may irreversibly constrict capillaries and disrupt the blood–brain barrier[35,41]. These few studies thus concluded that pericytes do influence blood flow. However, others have contested those claims. Some studies dispute the proposed physiological role of pericytes on brain CBF. One study demonstrated that although pericytes do indeed exhibit contractility to capillaries in vivo, they do not control CBF in the brain. They suggested that other cells such as precapillary and penetrating arterioles play that role instead[15]. Other research discovered that smooth muscle cells, not pericytes, constrict and control CBF responses in the brain[22]. These inconsistencies demonstrate that the involvement of pericytes in neurovascular coupling remains disputed and should be investigated further.

How do these interpretations relate to a deficiency of pericytes? And what happens to blood flow in the absence of pericytes? Our research concluded with a considerable reduction in

pericytes, but what this indicates is uncertain. According to one study, the loss of pericytes inhibits capillary CBF responses to neuronal inputs, resulting in neurovascular uncoupling, reduced oxygen delivery to the brain, and metabolic stress[24], but others readily dispute this claim.

Additionally, AD is linked to a 20-30% decrease in CBF[29,35] and a 25-50% decrease in pericytes[17], raising the question of whether these declines are contingent on each other. This demonstrates even more why it is vital to understand the interplay between pericytes and cerebral blood flow in order to have a better understanding of these age-related diseases.

Somatosensory cortex

Interestingly, we detected no significant changes in the individual levels of PDGFrβ and lectin in the somatosensory cortex of old mice, but we did observe a significant decrease when they were co-localized (PDGFrβ + Lectin). A potential explanation for this lack of decline in the somatosensory cortex is that, since our models were wild-type mice with no known defect, their somatosensory cortex did not undergo aberrant aging.

The significant decrease in the number of pericytes seen when PDGFrβ + Lectin was counted was particularly striking, given that these cells did not demonstrate a significant decrease when evaluated individually. This could be due to the ability of pericytes to detach from capillary walls and contribute to fibrosis in a variety of organs by transforming into scar-forming myofibroblasts[32]. Since we exclusively counted pericytes attached to capillaries as PDGFrβ + Lectin, this might explain why PDGFrβ + Lectin levels decreased but the number of individual PDGFrβ-expressing pericytes (both attached and detached) remained constant.

Limitations

This exploratory study harbors certain limitations: 1) I exclusively used male specimens to control for confounding variables that may exist with female specimens. The findings in this paper may not hold true for female models; 2) the sample size of mice used in this investigation was small due to restricted laboratory resources and time. Six mice were used in total. A greater sample size will lend credibility to the study's conclusions; 3) Because the cells were manually counted by a single individual, selection bias may exist. Although precautions were taken by consulting with peers regarding the morphology of pericytes, bias may have remained, and; 4) because pericytes lack a specific marker, PDGFrβ was used to identify them, despite the fact this marker also stains other endothelial cells that can occasionally resemble pericytes.

Future studies

The purpose of studies like this one is to gain a better understanding of the degenerative changes that occur in the brain just before it reaches the aging extremes associated with cognitive impairment diseases.

In this study, we employed wild-type normal mice. It is more effective to study these cells in mice and human models with Alzheimer's disease, moderate cognitive impairment, and other age-related diseases. Future research should include male and female subjects as well, particularly because females are more prone to developing AD. Further research must be done because the literature on why females are more vulnerable to this age-related illness is scarce. Some researchers have related these sex variations to estrogenic substances that may protect females against amyloid-mitochondrial beta toxicity when they are young, but expose them to the disease more severely than males after menopause[36].

Also, little is known whether these mice have a stable loss of pericytes in the CNS following birth or whether brain pericyte population can regenerate and/or compensate for a loss of pericyte coverage. Additional studies demonstrating a direct relationship between pericytes, capillaries, and cognitive impairment disorders should be conducted.

SUMMARY AND CONCLUSIONS

Aging is a significant risk factor for developing neurodegenerative illnesses. The purpose of this study was to examine the effect of aging on the number of pericytes and the capillaries in which they are embedded. We discovered that old male mice exhibited fewer pericytes and capillaries in the hippocampus and somatosensory cortex than young mice.

Age-related vascular cognitive impairment/dementia and AD account for more than three-quarters of all dementias. Vascular pathology is frequently observed in a host of neurodegenerative disorders, particularly in AD, where pericytes are considered to play an important role. Targeting pericytes as a therapeutic technique has become increasingly important due to their molecular mechanisms and functions with nearby cells. Research in this area is anticipated to increase in the future.

FIGURES & TABLES

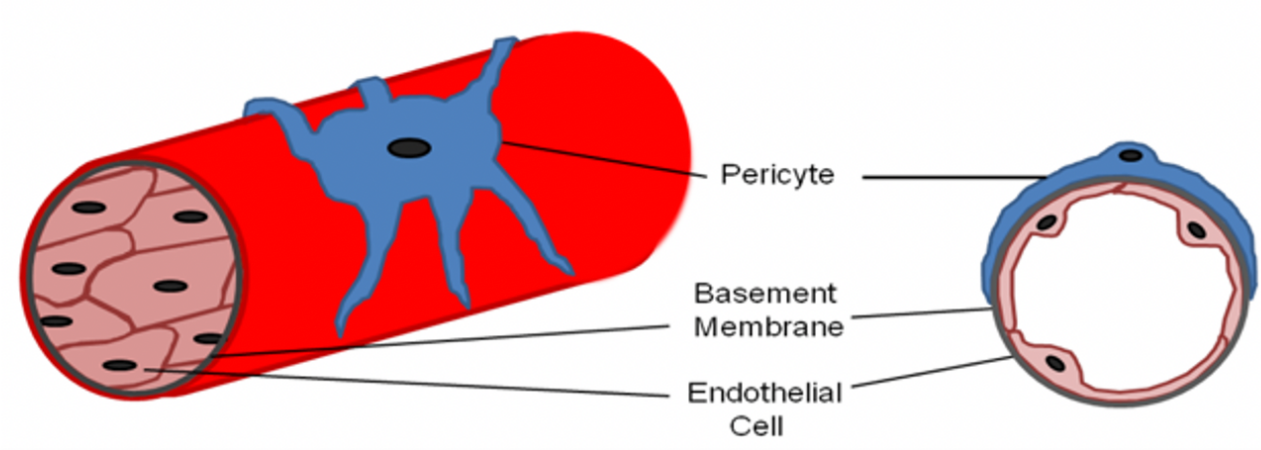

Figure 1. Pericytes, also known as mural cells, are present in many forms throughout the body. They can arise directly from endothelial cells, and they are usually found on the outside of blood capillaries, where they interact with the underlying endothelial cells[25].

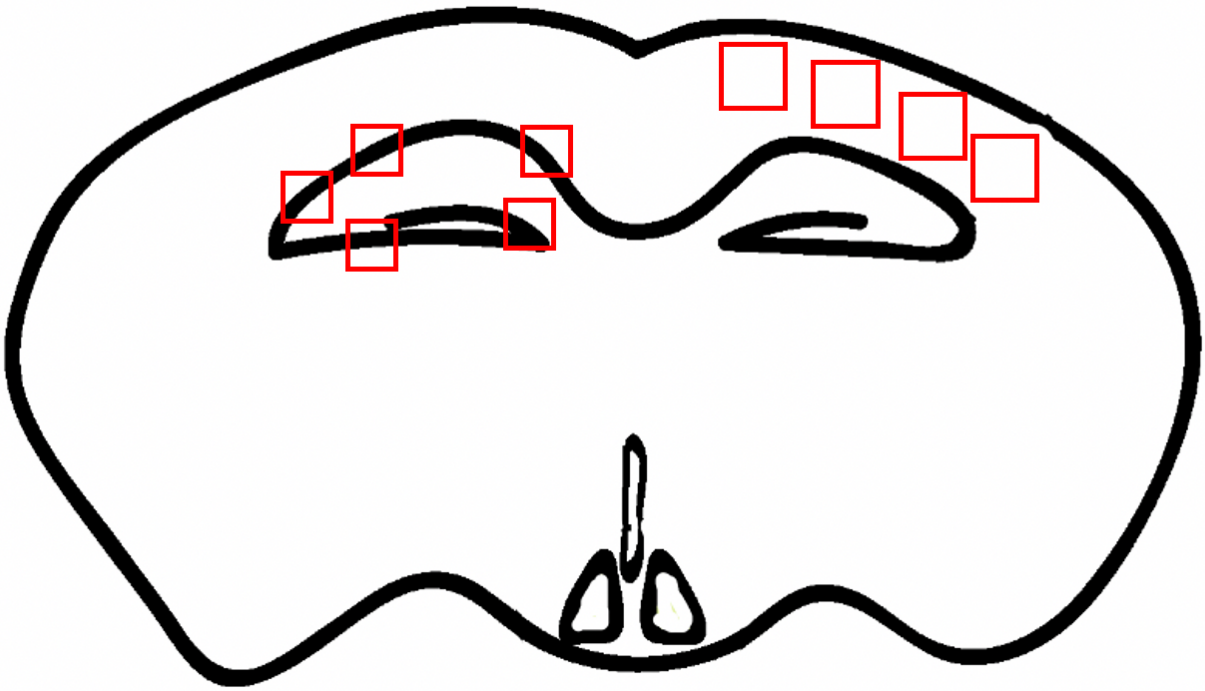

Figure 2. Overview of hippocampal and somatosensory cortex areas used for quantitative measurements from the mice. Cresyl violet coronal sections stained. Quantification Hippocampus areas are (Red Box width: 516px, height 347px): CA1a and CA1c, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3 and DG, dentate gyrus. 4 sections quantified for the Somatosensory cortex (Red Box width: 717px, height 527px).

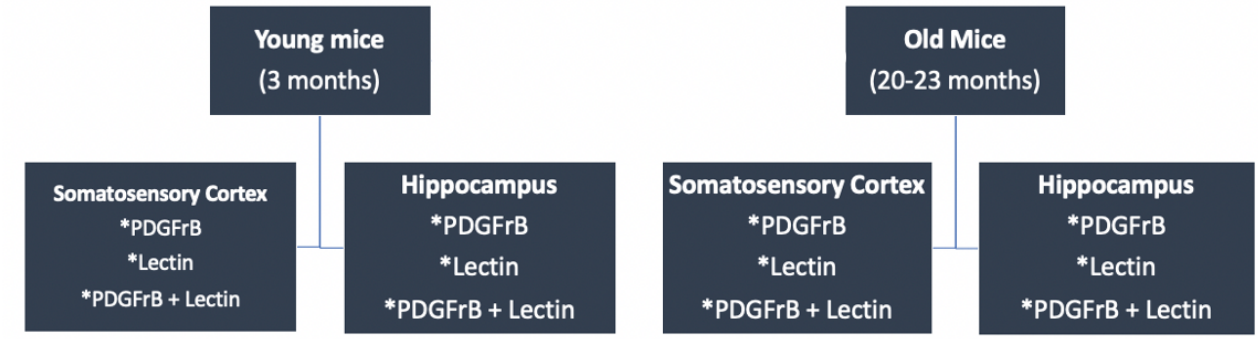

Figure 3. Research design workflow.

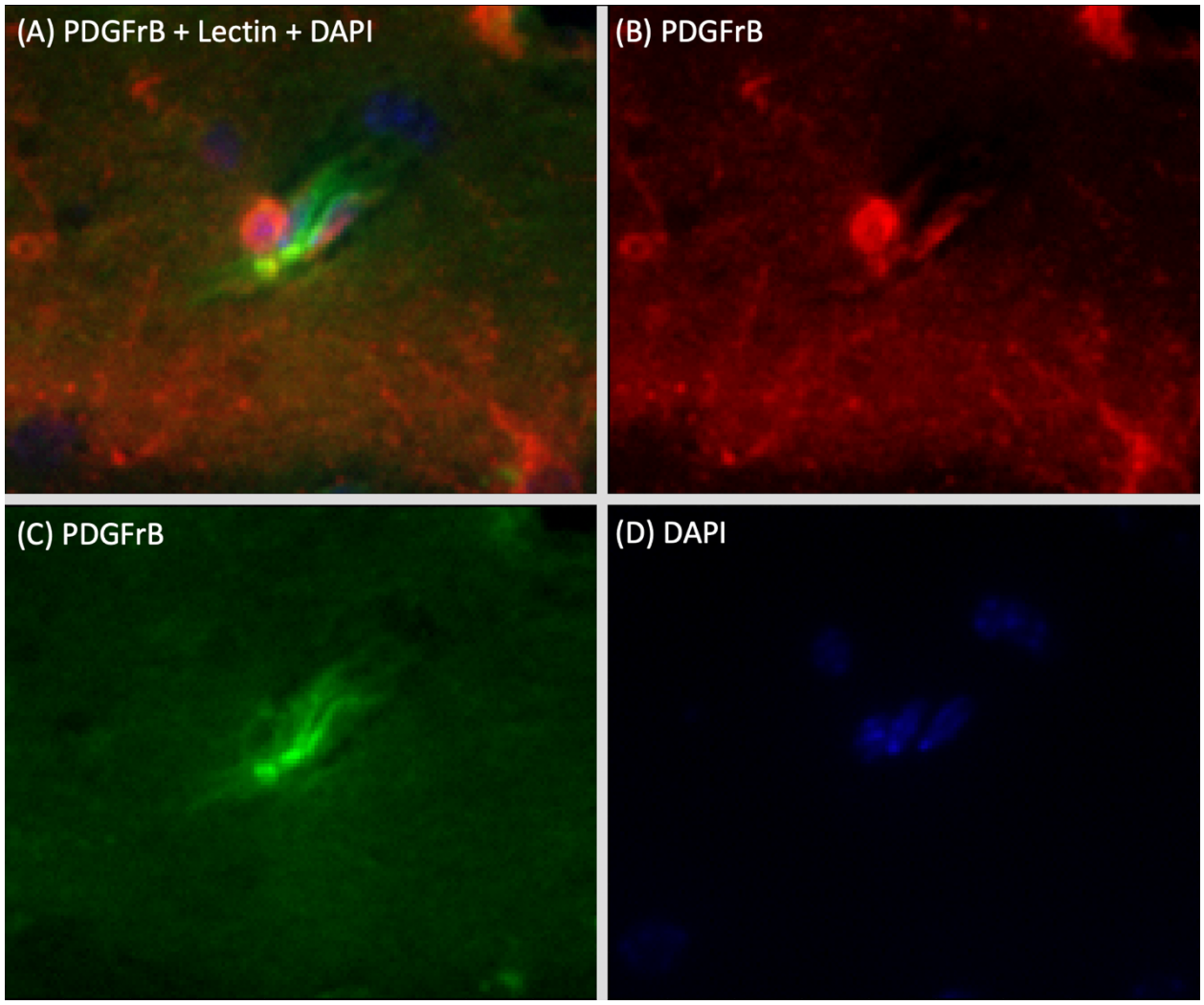

Figure 4. (A) Confocal imaging of PDGFrβ, Lectin, and DAPi staining together. (B) Simultaneously captured Texas Red fluorescence showed PDGFrβ expressing pericytes at each stratification (C) Lectin-stained Capillaries are also visible under green fluorescence with (D) blue florescent DAPi staining of the nucleus.

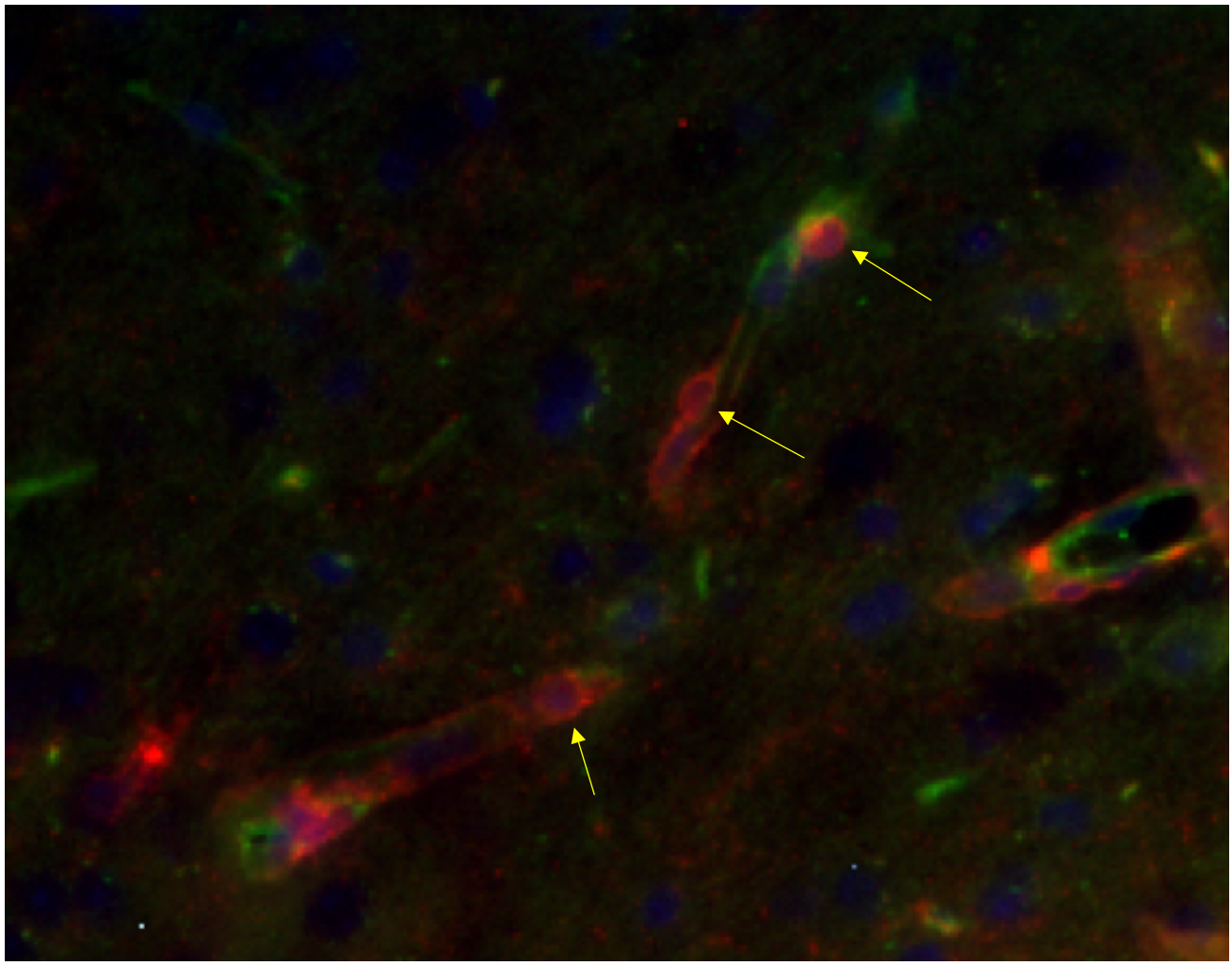

Figure 5. PDGFrβ, DAPi, and Lectin combined. Yellow arrows point to a bump on a log morphology indicative of pericytes.

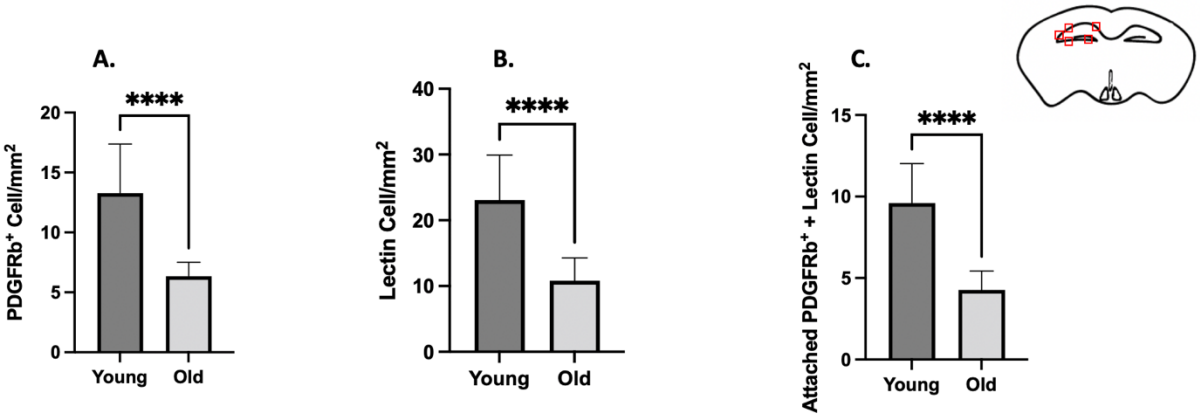

Figure 6. Immunofluorescence performed at CA1, CA1c, CA2, CA3, and DG hippocampal region. Unpaired t-test was used to compare the number of pericytes, capillaries, and capillary-pericytes between old and young mice. [A] Number of PDGFrβ^{+} (pericytes and endothelia cells) in old and young mice. P value < 0.0001*** [B] Number of Lectin (capillaries) in old and young P value < 0.0001*** [C] Number of attached PDGFrβ + Lectin representing pericytes embedded on capillaries in old and young P value < 0.0001***.

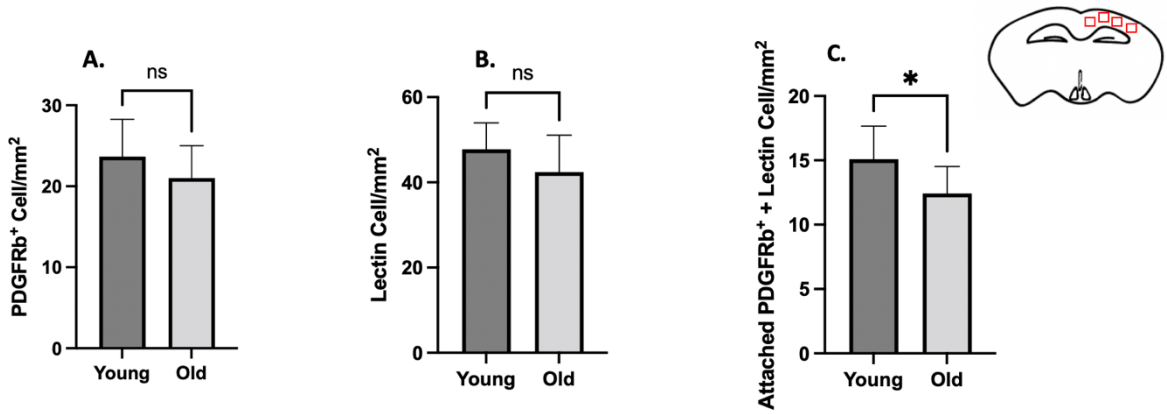

Figure 7. Immunofluorescence performed at somatosensory cortex. Unpaired t-test was used to compare the number of pericytes, capillaries, and capillary-pericytes between old and young mice [A] Number of PDGFrβ^{+} (pericytes and endothelia cells) in old and young mice. P value = 0.1448 [B] Number of Lectin-capillaries in old and young P value = 0.0967 [C] Number of PDGFrβ + Lectin (attached pericytes on capillaries) in old and young P value = 0.0110*

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