UNIVERSIDAD AUTÓNOMA METROPOLITANA
Unidad Iztapalapa

División de Ciencias Biológicas y de la Salud
Posgrado en Biología Experimental

Characterization of cellular and molecular changes in brain pericytes during sleep restriction

T E S I S

Que para tener el grado de
Doctora en Biología Experimental

P R E S E N T A

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DEDICATORIAS

Este proyecto de investigación lo dedico a Dios, a mis padres y amigos por su apoyo incondicional durante este viaje académico.

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Resumen

Los pericitos cerebrales estabilizan los vasos sanguíneos mediante el contacto directo con las células endoteliales. En ratas, diez días de restricción del sueño promueven el desprendimiento de pericitos de la pared capilar al disminuir la expresión de las proteínas PDGFR-β y conexina-43. El desprendimiento de pericitos se acompaña de una disminución en la expresión de proteínas de uniones ocluyentes y de hiperpermeabilidad de la barrera hematoencefálica a moléculas de bajo y alto peso molecular. Estos cambios están relacionados a altas concentraciones de MMP-9 y del receptor de adenosina A_{2A}. Para estudiar la disfunción temprana de la barrera hematoencefálica, las ratas fueron sometidas a cinco días de restricción del sueño. Un periodo corto de restricción del sueño también aumentó la permeabilidad de la barrera hematoencefálica a fluoresceína sódica en el hipocampo y la corteza cerebral. El desprendimiento de pericitos del endotelio se observó a los 5 días de la pérdida del sueño. El evento inicial fue la disminución de conexina-43 en ambas regiones del cerebro, mientras que PDGFR-β sólo disminuyó en la corteza cerebral. La pérdida del sueño durante 5 días aumentó la expresión de MMP-9 y TNF-α en microvasos aislados de ambas regiones del cerebro y aumentó el receptor de adenosina A_{2A} en microvasos aislados del hipocampo. Una dosis única del antagonista del receptor de TNF-α, R-7050 en el quinto día de pérdida del sueño restableció la expresión de conexina-43 y PDGFR-β y restableció la permeabilidad de la barrera hematoencefálica a fluoresceína sódica en ambas regiones del cerebro; pero no modificó la expresión de MMP-9, A_{2A} y NFκB. Nuestros resultados sugieren que la pérdida de uniones entre pericitos y células endoteliales, seguida de la pérdida de la vía de señalización

regulada por PDGFR-β, son los eventos clave que regulan la alteración de la barrera hematoencefálica durante la pérdida del sueño, donde TNF-α juega un papel central en la modulación de las interacciones pericito y célula endotelial.

Abstract

Brain pericytes stabilize blood vessels through direct contact with endothelial cells. Ten days of sleep restriction promotes pericyte detachment from the capillary wall by decreasing PDGFR-β and connexin-43 protein expression. Pericyte detachment is accompanied by a reduced expression of tight junction proteins and blood-brain barrier hyperpermeability to low- and high-molecular-weight molecules. These changes are related to high concentrations of MMP-9 and A_{2A} adenosine receptor. Five days of sleep restriction were induced to study the earlier state of the blood brain barrier dysfunction. The short-sleep restriction also increased blood-brain barrier permeability to sodium-fluorescein in the hippocampus and cerebral cortex. Pericyte detachment from the capillary wall began already at 5 days of sleep loss; the initial event was the downregulation of connexin-43 in both brain regions, with PDGFR-β lost only in the cerebral cortex. Sleep loss for 5 days increased the expression of MMP-9 and TNF-α in isolated microvessels of both brain regions and elevated the A_{2A} adenosine receptor in isolated microvessels from the hippocampus. A single dose of the TNF-α receptor antagonist R-7050 on the 5^{th} day of sleep loss restored connexin-43 and PDGFR-β expression and normalized blood-brain barrier permeability to sodium-fluorescein in both brain regions; but did not modify MMP-9, A_{2A}, and NFκB expression. Our results suggest that the loss of gap junctions between pericytes and endothelial cells, followed by the loss of the signaling pathway regulated by PDGFR-β are the key events regulating blood-brain barrier disruption during sleep loss, where TNF-α plays a central role in modulating pericyte-endothelial cell interactions.

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1. INTRODUCTION

The brain is the organ with the highest rate of energy consumption. It requires approximately 20% of the total oxygen metabolism, being the neurons the cells that consume between 70-80% of the energy produced in the brain (Hyder et al., 2013). Therefore, an extensive small blood vessel network that facilitates oxygen and glucose delivery, solutes exchange, the efficient transport of nutrients and hormones between the blood and the brain tissue (Roy and Sherrington, 1890). Microvascular network architecture is organized mainly by vessels with variable sizes: arterioles, capillaries and venules. In the most superficial part of the brain, at the level of the pia-arachnoid layer, there are arterioles with a diameter that varies from 10 to 100µm. They regulate cerebrovascular tone and cerebral perfusion through vasodilation and vasoconstriction (Hamel, 2006). Arteries branch into penetrating arterioles, with a diameter ranging from 10 to 30µm. Penetrating arterioles lie within the Virchow-Robin space and enter into the cerebral cortex (Jones, 1970; Nishimura et al., 2007); they are characterized by responding to neurotransmitters released during neuronal activity, such as serotonin and norepinephrine (ladecola, 2017). Penetrating arterioles branch into capillaries that have a diameter ranging from 5 to 8µm (Grant et al., 2017). Capillaries are the most abundant blood vessels in the brain, with approximately a total length of 400 miles (Begley and Brightman, 2003). Particularly, the microvascular endothelial cells lining the cerebral capillaries form the blood-brain barrier.

The German scientist Paul Ehrlich (1854-1915) performed pioneering experiments that described the presence of a physical compartment that separates the central nervous system (CNS) from the rest of the body (Ehrlich, 1885). Ehrlich injected a

vital dye into the mice peritoneum, which stained all organs except the brain and the spinal cord. Later, Edwin Goldmann (1862-1913), a student of Ehrlich, injected the trypan blue dye (MW 960 Da) directly into the cerebrospinal fluid (CSF), finding that it stained the brain but not the rest of the mice's body. These results demonstrated the presence of a highly specialized structure called the blood-brain barrier (BBB). The BBB facilitates the exchange of oxygen and nutrients, debris clearance, and prevents the passage of potentially toxic molecules from the circulatory system into the brain parenchyma (Figure 1) (Hartmann et al., 2015).

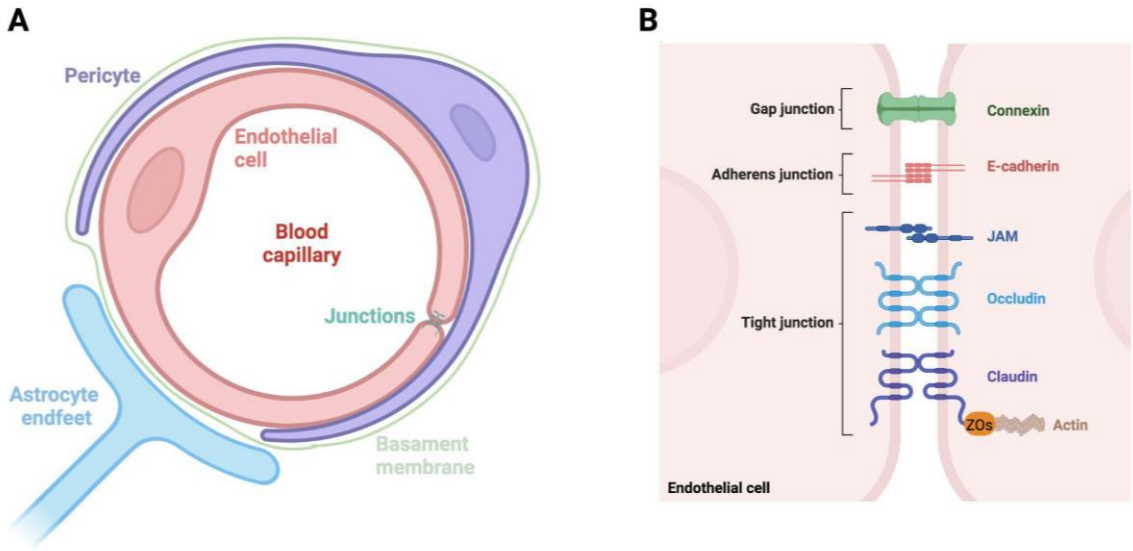

Figure 1. General structure of the blood-brain barrier. The integrity of the blood-brain barrier is formed by the interaction between endothelial cells, pericytes and astrocytes (A). Endothelial cells establish tight junctions to regulate paracellular transport (B).

1.1 Brain endothelial cells

The endothelial cells that constitute the brain microvasculature regulate the passage of molecules to and from the brain parenchyma (Alvarez et al., 2011). This specialized function of the brain endothelium is provided by the intercellular

expression of tight junction (TJ) and adherens junction proteins, the specific polarized expression of receptors and transporters, as well as the expression of drug export pumps that prevent a large number of harmful molecules reaching the central nervous system (CNS) (Sweeney et al., 2019). The tight junctions are formed of the integral transmembrane proteins claudins, occludin and junctional adhesion molecules (JAMs). In order to assemble, these proteins interact with the actin cytoskeleton through the scaffold proteins zonula occludens (ZO)-1, ZO-2, ZO-3 (Wolburg et al., 1994; Liebner et al., 2000; Urich et al., 2012; Roux and Couraud, 2005). The adherens junctions are involved in TJ assembly (Bazzoni and Dejana, 2004; Bazzoni, 2011) by initiating the cell-to-cell contacts and promoting their maturation, maintenance and plasticity through homophilic interactions between the transmembrane proteins: vascular endothelial cadherin (VE-cadherin) and epithelial cadherin (E-cadherin) (Vorbrodt and Dobrogowska, 2003).

Endothelial cells are interconnected via gap junctions (Hautefort et al., 2019). Gap junctions regulate endothelial intercellular communication and modulate solute transport between the cell and the extracellular space (Nagasawa et al., 2006; Meşe et al., 2007). Gap junctions are formed by two hemichannels or connexons located on opposite cell membranes; each one is composed of 6 transmembrane proteins called connexins (Cx). The connexin superfamily comprises 21 isoforms in humans and 20 in mice, which are named according to their molecular weight (Dobrowolski and Willecke, 2009). The variability of connexins is due to the C-terminal tail that might be different in length, aminoacidic sequences, tertiary structure, and sites for phosphorylation. Connexin channels mediate the cytoplasmic communication by the interchange of ions and small metabolites to maintain metabolic cooperation and

the propagation of electrical signals and calcium waves between adjacent cells (Bruzzone et al., 1996; Laird, 2006). In rodents, Cx-32 and Cx-43 are highly expressed in the brain; however, their expression differs during brain development (Kumar and Gilula et al., 1996; Nadarajah et al., 1997). Connexin-32 expression predominates at the late stage of cortical maturation, while connexin-43 expression persists during postnatal development and in the adult cortex (Nadarajah et al., 1997).

The transendothelial transport is highly limited by the expression of specific transporters and multiple efflux transporters of the ABC cassette superfamily (Pardridge, 1988). The main transport mechanisms present in the BBB are:

1.- Carrier-mediated transport: used by small hydrophilic molecules to enter the brain from the blood stream. The glucose transporter GLUT-1 is a protein that carries extracellular glucose into the cell (Dwyer et al., 2002). Other types of specific transporters include the protein LAT-1, that carries large neutral amino acids like tyrosine, tryptophan, and leucine; and the monocarboxylate transporter (MCT-1), which introduces pyruvate, lactate, and ketone bodies.

2.- Adsorptive endocytosis: the mechanism consists of the invagination of the plasma membrane to introduce solutes from the extracellular space. Vesicles are enriched by caveolin-1.

3.- Receptor-mediated transport: this occurs when clathrin-coated vesicles internalize the complex ligand-receptor (Smith and Gumbleton, 2006).

4.- ABC (ATP-binding cassette) transporters: a superfamily of energy-dependent active transporters formed by 48 members classified into 7 families (ABC-A, -B, -C, -D, -E. -F and -G). In particular, the P-glycoprotein (P-gp) has been widely studied.

P-gp is an ATP-dependent efflux bomb of 170 kDa located in the apical side of brain endothelial cells (Schinkel, 1999); it restricts the passage of H1 histamine receptors antagonists, verapamil and other hydrophobic molecules. Jodoin and colleagues (2003) reported that in the cerebral endothelium P-gp co-localizes with caveolin-1 and caveolin-2 to form a molecular complex of 669 KDa. This interaction improves the activity of P-gp by facilitating its oligomerization and accelerating drug metabolism (Demeule et al., 2000; Barakat et al., 2008; Guo et al., 2010).

1.2 Pericytes

Pericytes were first described more than 150 years ago by the French scientist Charles-Marie Benjamin Rouget; so they used to be called *Rouget cells* (Rouget, 1873). Rouget cells were renamed *pericytes* (surrounding -peri, cells -cytes) by the German anatomist and histologist Karl Wilhelm Zimmermann in 1923, and he defined them as a mural cell population surrounding the blood vessels (Zimmermann, 1923).

Pericytes are embedded in the same basal lamina than endothelial cells and are morphologically characterized by having a large nuclear region that gives them the appearance of an elongated cell body, with long, thin and circumferential cytoplasmic processes that surround and cover between 2 to 4 endothelial cells (Zimmermann, 1923); these processes can even overlap, forming one or two layers (Diaz-Flores et al., 2009). Pericytes can display different morphologies depending on the endothelial bed in which they are located (Attwel et al., 2006). The unsheathing pericytes or mural contractile cells show dense ramifications wrapping the precapillary vessels (Grant et al. 2019; Hariharan et al. 2020). At the first and

second branches of post-arteriole capillaries, pericytes are named mesh pericytes and present mesh-like ramifications surrounding the capillary wall. After the second post-arteriole branch, thin-strand pericytes extend fine processes along the capillary wall (Hartmann et al. 2015; Grant et al. 2019; Hartmann et al. 2021). Furthermore, pericytes present a variety of specific markers, depending on the vascular bed and their developmental state; including Platelet-derived growth factor receptor β (PDGFR-β), neural/glial antigen 2 (NG2), Alpha smooth muscle actin (α-SMA), desmin, vimentin, and aminopeptidase N (CD13) (Armulik et al., 2011). These markers are also expressed by other cell types such as oligodendrocyte precursor cells and vascular smooth muscle cells (VSMC). So, to identify a pericyte in *in vivo* or *in vitro* conditions, two or more pericyte-specific markers need to be used or, due to their proximity to endothelial cells, pericyte markers can be co-localized with vascular markers such as fluorescently conjugated lectins (e.g., FITC-tomato lectin) or endothelial cell markers (e.g., CD31) (Nehls and Drenckhahn, 1991; Trost et al., 2013; Hill et al., 2015; Robertson et al., 2015).

Pericytes are located very close to endothelial cells, so their intercellular communication is regulated by specialized surface junctions, such as peg-socket junctions, gap junctions and adhesion plaques (Díaz-Flores et al. 1991; Armulik et al, 2005; Sweeney et al. 2019). Peg-socket junctions are anchoring sites in areas without basement membranes and involve reciprocal and interdigitated evaginations of each cell (Díaz-Flores et al., 1991). These junctions can only be visualized with electron microscopy and are identified because the pericyte has a cytoplasmic projection (peg) oriented towards the endothelial cell where it is inserted (socket) or vice versa (Armulik et al. 2011; Ornelas et al. 2021). The pericyte pegs

are mainly located on the edge of the pericyte nucleus, and a proportion of around 30 pegs of pericytes per soma is estimated (Ornelas et al. 2021). Peg-socket junctions facilitate the adhesion and biochemical crosstalk between the pericytes and the endothelial cells (Vandenhaute et al. 2011; Kovacs-Oller et al. 2020; Ornelas et al. 2021).

Gap junctions are established between endothelial cells and pericytes, and also between pericytes. Gap junctions are mainly located between the cytoplasmic processes of pericyte and abluminal plasmalemma of endothelial cells (Fujimoto, 1995; Kovacs-Oller et al., 2020). Single-cell transcriptomic studies have shown that capillary pericytes express high mRNA for Cx37 and Cx45, and low mRNA expression for Cx26 and Cx43 (Aasen et al. 2018; Vanlandewijck et al. 2018). This type of cell-to-cell communication through gap junctions allows the passive diffusion of small molecular weight solutes ranging from 1.0 to 1.5 kDa, such as inorganic ions, ATP, cyclic nucleotides, siRNA, glucose, and polypeptides (Harris, 2007). However, the permeability of these solutes through connexins is selective according to ionic charge and molecular size; for example Cx43 channels are more permeable to metabolites like ADP and ATP than Cx32 channels, while Cx43 channels are less permeable to adenosine and calcein than Cx32 channels (Goldberg et al., 1999; Valiunas et al., 2005). In addition, the pericyte-endothelial cell relationship allows that gap junctions to maintain interactions with tight junction proteins. The interaction of ZO-1 and Cx43 is important for the stabilization of Cx43 in the plasma membrane. The association of ZO-1 and F-actin binding protein drebrin with Cx43 is necessary for anchoring gap junction plaques to the actin cytoskeleton (Olk et al., 2009). Conversly, a dysfunction of the BBB is accompanied by a redistribution of

gap junctions (Alonso et al., 2010; Medina Flores et al., 2020). Hence, that gap junctions not only function as a channel to transport molecules between cells, but are also essential for the stabilization and maintenance of the endothelial barrier.

The crosstalk between pericytes and endothelial cells is modulated by different signaling pathways, including transforming growth factor β (TGF-β), angiopoietin, sphingosine-1-phosphate, vasoactive molecules such as vasopressin and platelet-derived growth factor (PDGF), being this one the most widely studied (Figure 2) (Armulik et al., 2005; Armulik et al., 2011; Winkler et al., 2011). Activation of this signaling pathway begins when endothelial cells secrete PDGF-B, which binds to its platelet-derived growth factor receptor-β (PDGFR-β) expressed on pericyte's surface. The activation of PDGFR-β, a transmembrane receptor with tyrosine kinase activity, induces receptor autophosphorylation, ensuing the activation of the rat sarcoma virus protein (Ras)/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), phospholipase C (PLC)/protein kinase C (PKC), and the signal transducer and activator of transcription (STAT) family, resulting in the recruitment, migration and establishment of the pericyte into the endothelial cell (Heldin and Westermark, 1999).

Pericytes are heterogeneous, dynamic and complex cells that actively participate in the maintenance of the CNS homeostasis and, in particular, the BBB, so their absence, loss or detachment from the endothelium will contribute to its disruption (Sweeney et al., 2018).

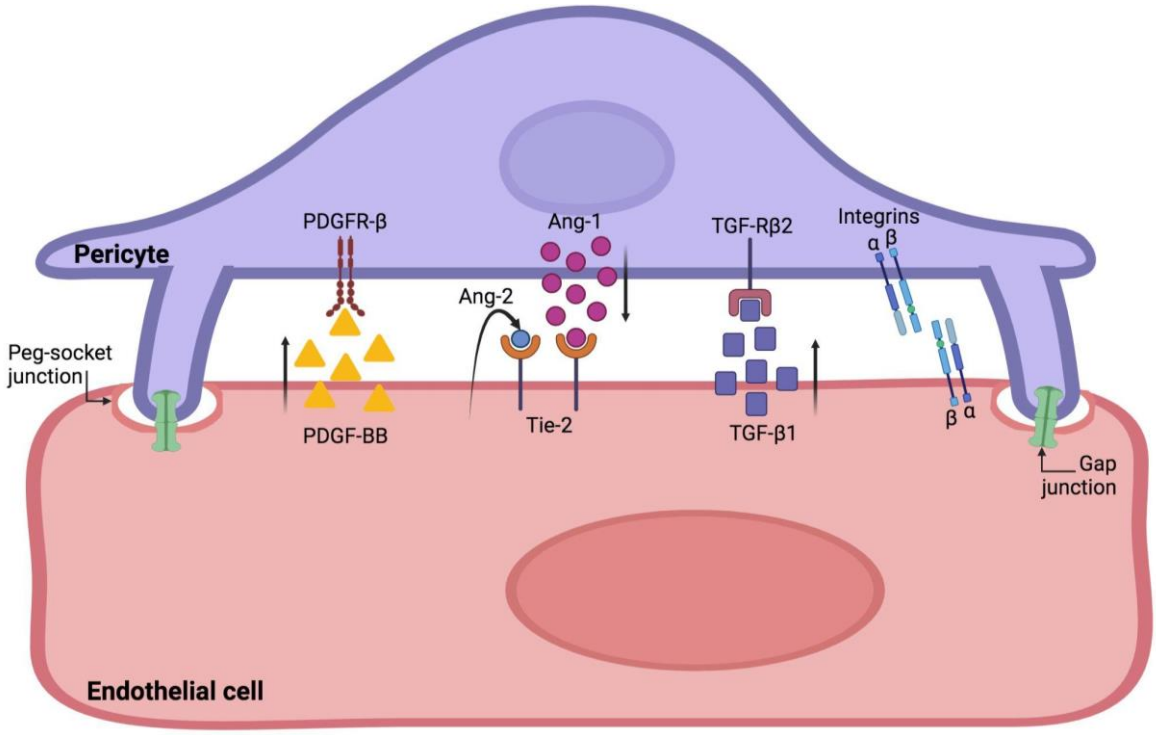

Figure 2. Pericyte and endothelial cell crosstalk. The physical interaction between the pericyte and the endothelial cell allows the activation of different signaling pathways that contribute to the maintenance of the integrity and functioning of the blood-brain barrier.

1.3 Sleep regulates blood-brain barrier function

Sleep is a physiological state characterized by a reduction of locomotor activity and alertness, the acquisition of a typical posture for sleeping and the presence of a characteristic pattern of brain electrical activity (Huang et al., 2011; Potter et al., 2016). The World Health Organization Technical Meeting on Sleep and Health noted that *"sleep is a basic human need and is essential for good health, good quality of life and performing well during the day"* (World Health Organization. Regional Office for Europe, 2004). This is associated with the fact that during sleep, various physiological phenomena that guarantee the optimal functioning of the CNS are carried out, including immunological, metabolic, and clearance (CNS) (Krueger et al., 2016).

Sleep is characterized by typical cerebral electrical activity with a characteristic pattern of ocular movements and muscular activity. In most of species, sleep can be classified into two states according to changes in the cerebral electrical activity: non-rapid eye movement (Non-REM) and rapid eye movement (REM), which follow a cyclic pattern (from 90 to 120 minutes in humans) during the night. In humans, Non-REM sleep is divided into 3 stages: N1 and N2 are considered light sleep, and N3 corresponds to the slow-wave sleep, also known as delta sleep (Saper et al., 2010). In the rest of the mammals, the non-REM sleep can be subdivided into slow-wave sleep stages 1 and 2. During non-REM sleep, the electroencephalographic activity is characterized by the presence of slow-waves of high amplitude; in addition, there is a decrease in muscle tone and slow eye movements. In REM sleep, the electroencephalographic activity is of high frequency and low amplitude, like the one observed during wakefulness, eye movements are rapid and muscular atony is present (Staunton, 2005). In addition to changes in brain electrical activity, each sleep phase is accompanied by variations in some physiological parameters. During non-REM sleep, there is a decrease in blood pressure, heart rate, respiratory rate and body temperature; whereas, in REM sleep there are stochastic variations in respiratory rate, blood pressure, and heart rate, also, peripheral thermoregulation is reduced (Wehr, 1992; Porkka-Heiskanen et al., 2013).

Experiments with animal models and clinical observations in humans have been used to study the sleep function. Experimental procedures generally involve continuous periods of sleep loss (a procedure called sleep deprivation); periods in which the number of sleep hours *per* night is reduced (called sleep restriction); and periods in which each night the subject is awakened on numerous occasions to

induce sleep fragmentation. After sleep loss, subjects can freely sleep during different periods (from hours to days) to study the restoration of the physiological functions with sleep recovery. Several studies have shown that sleep is necessary for body restoration, clearance of toxic brain metabolites, and memory consolidation (Xie et al., 2013; Rasch and Born, 2013). On the other hand, continuous sleep loss impairs health status and eventually leads to death in experimental animal models (Rechtschaffen et al. 1989; He et al., 2014). Multiple studies have shown central and peripheral effects of sleep loss ranging from alterations in hormones circulating levels to pathological changes in various organs and tissues (Gómez-González et al., 2012). In general, sleep loss induces a low-grade pro-inflammatory state at the systemic level, characterized by the increase in circulating levels of pro-inflammatory molecules and cytokines, such as C-reactive protein, pro-inflammatory cytokines IL-1α, IL-1β, TNF-a, IFN-γ along with alterations in circulating immune cells (Yehuda et al., 2009; He et al., 2014; Hurtado-Alvarado et al., 2018).

Sleep loss alters brain neurochemistry in the central nervous system, by increasing excitatory amino acids levels, such as glutamate and aspartate mainly in the cerebral cortex and hippocampus (Mohammed et al., 2011). Sleep loss also decreases the hippocampal volume (Novati et al., 2011) and neurogenesis (Guzman-Marin et al., 2008), which results in learning and memory deficits (Novati et al., 2011). Sleep is, therefore, necessary to maintain the homeostasis of the CNS.

2. BACKGROUND

Pericytes maintain the neurovascular stability and their absence compromises the BBB physiology (Bastide et al., 2007; Kovács et al., 2012). The total inactivation of

the signaling pathway PDGFR-β/PDGFBB leads to vascular aberrations as a result of pericyte deficit ensuing perinatal death (Leveen et al., 1994). Previous studies showed that mice with a mutation that produces the constitutive activation of PDGFR-β (PDGFR-βret/ret) are viable, but show microvascular dysfunction (Lindblom et al., 2013). In the PDGFR-βret/ret mouse model, decreased pericyte density is observed, accompanied by an increase in BBB permeability to Evans blue, immunoglobulin G and fibrinogen (Armulik et al., 2010; Villaseñor et al., 2017); as well as a reduction in the tight junction protein expression of claudin-5 and zonula occludens-1 in regions like the cerebral cortex, basal nuclei, and hippocampus (Armulik et al., 2010; Bell et al., 2010). Similar results have been reported by our laboratory during sleep restriction conditions, where a general BBB dysfunction was observed (Gómez-González et al., 2013; Hurtado-Alvarado et al., 2017; Medina-Flores et al., 2020). In a male young rodent model, ten days of chronic sleep restriction increased the BBB permeability to a variety of exogenous dyes, such as Evans blue (Gómez-González et al., 2013), 10 kDa- and 70 kDa-dextrans (Hurtado-Alvarado et al., 2016), sodium-fluorescein and rhodamine 123, a substrate for P-glycoprotein (Hurtado-Alvarado et al., 2017; Medina-Flores et al., 2020). The dye extravasation is associated with decreased tight junction proteins claudin-5 and occludin (Hurtado-Alvarado et al., 2018, Medina-Flores et al., 2020). Furthermore, ten days of sleep restriction modified the BBB ultrastructure by increasing endocytic vesicle density, promoting the formation of cytoplasmic projections oriented towards the lumen of the capillary, and increasing the basal lamina thickness (Gómez-González et al., 2013; Hurtado-Alvarado et al., 2017). He et al. (2014) described that six days of sleep deprivation decreased mRNA expression of claudin-1, claudin-

5, and ZO-2, leading to an increase in the paracellular permeability in the cerebral cortex, brainstem and cerebellum. However, these BBB alterations can be reversible during short periods of sleep recovery, ranging from 40 to 120 minutes; those periods are enough to restore the BBB basal permeability in almost all the studied brain regions, except for the hippocampus (Gómez-González et al., 2013). Yet, the mechanism by which sleep modulates the BBB integrity and function is not completely understood, some molecules that may participate have been proposed. Adenosine is a ubiquitous nucleoside that modulates cellular activity, and its concentration increases throughout the waking time and decreases during the sleep period (Basheer et al., 2004). Adenosine levels in the extracellular space could increase during sleep restriction and actively modulate BBB function; a previous study described that the administration of selective A_{2A} adenosine receptor antagonist immediately after completing the ten days of sleep restriction reestablished the BBB function by reducing its permeability to 10-KDa dextrans and restoring claudin-5 protein expression. Adenosine antagonism also decreased the expression of pro-inflammatory markers such as Iba-1 and glial fibrillary acidic protein (GFAP) (Hurtado-Alvarado et al., 2016). Our laboratory has proposed that sleep restriction may lead to pericyte detachment from the endothelium. In a rodent model subjected to ten consecutive days of sleep restriction, through electron microscopy, a slight separation between the membranes of the endothelial cell and the pericyte was observed, suggesting partial pericyte detachment (Hurtado-Alvarado et al. al., 2017). Subsequently, we reported that ten days of sleep restriction significantly decreased the expression of PDGFR-β and Cx43 in brain microvessels isolated from the cerebral cortex and hippocampus, which resulted in

the loss of pericyte-endothelial cell interactions and subsequent BBB dysfunction (Medina-Flores et al., 2020). These altered interactions between pericytes and endothelial cells may be a consequence of the onset of a proinflammatory event. During sleep loss, there is an upregulation in the central expression of pro-inflammatory cell markers, such as COX-2 levels (He et al., 2014), Iba1, and GFAP (Hurtado-Alvarado et al., 2016, 2018), and also increased serum levels of C-reactive protein, as well as the inflammatory cytokines interleukin (IL)-1α, IL-1β, IL-6, IL-17, Interferon (IFNɣ) and Tumor necrosis factor (TNF-α) (Yehuda et al., 2009; He et al., 2014; Hurtado-Alvarado et al., 2018).

Pericytes maintain the physiology and function of the BBB, and due to their capability to respond quickly to harmful stimuli, they should be considered modulators of the cellular and molecular mechanisms involved in sleep regulation.

3. JUSTIFICATION

The contemporary society is characterized by decreased sleep time due to environmental, behavioral factors, including travel long distances from home to work or school, spend more time at work and watching media entertainment. Approximately two-thirds of adults worldwide do not get the recommended 8 hours of sleep time each night (Walker, 2017). Poor sleep has become a major public health problem due to the increased risk for the development of long-term pathologies, such as obesity, diabetes, cardiovascular disease, depression, anxiety, cognitive deficit, and Alzheimer's disease (Medic et al., 2017; Tobaldini et al., 2017). Therefore, the impact of sleep duration and sleep quality on health has become highly relevant in current research. Sleep is a physiological process that preserves

the integrity of the neuro-immune-endocrine network through specialized structures like the BBB, which maintains the bidirectional communication between the periphery and the CNS. Previous studies have shown a direct relationship between sleep restriction and BBB dysfunction. However, there are no studies that describe how sleep restriction modifies the cellular and molecular mechanisms governing the interaction between endothelial cells and pericytes. Thus, the aim of the study was to elucidate the role of pericyte-endothelial cell interactions in mediating the changes in BBB function induced by sleep restriction.

4. HYPOTHESIS

The inflammation generated by sleep restriction will decrease the interactions between the pericytes and the endothelial cells, which will lead to BBB dysfunction.

5. MAIN OBJECTIVE

Evaluation of the pericytes-endothelial cells interactions after sleep restriction.

6. SPECIFIC OBJECTIVES

1.- Characterization of the proteins expression that regulate the interactions between pericytes and endothelial cells in isolated brain microvessels during sleep restriction.

2.- Evaluation of local and systemic pro-inflammatory mediators during sleep restriction.

3.- Characterization of the effect of pro-inflammatory cytokine pathway on pericytes-endothelial cells interactions during sleep restriction and their effect on blood-brain barrier function.

7. MATERIAL AND METHODS

7.1 Experimental subjects

Three-month-old male Wistar rats were used (n=95). Animals were housed in standard conditions in our laboratory vivarium under a 12 h light-dark cycle (lights on at 2:00 am) at room temperature of 20–25 °C. Commercial rat chow and tap water were provided *ad libitum*. All experimental animal procedures were performed following the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2010), the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines (www.nc3rs.org.uk/arrive-guidelines) and were approved by the Academic Ethic Committee of the Biological Sciences Division of the Universidad Autónoma Metropolitana, Unidad Iztapalapa.

7.2 Sleep restriction

Rats were subjected to sleep restriction using the multiple platform technique. Rats were placed in an acrylic chamber (82×59×48 cm) with 7 cm diameter platforms surrounded by water for 20 h daily, with an opportunity to sleep in their home cages for the last 4 h of the light phase during 5 consecutive days (Medina-Flores et al., 2020). To reduce social stress, groups of five animals remained in their same social group. An extra platform was added to the deprivation chamber to facilitate the animals ’movement and reduce restraint stress. In the first experiment, rats were randomly divided into the sleep restriction group (n=11) and the control group (n=11). Control rats slept *ad libitum* in their home cages during the 5 days of the experiment.

7.3 Effect of TNF-α on sleep regulation

To elucidate the role of TNF-α in regulating pericyte-endothelial cell interaction during sleep loss the TNFR1 selective antagonist R-7050 (8-chloro-4-(phenylthio)-1-(trifluoromethyl)-[1,2,4]triazolo[4,3a]quinoxaline; (Sigma-Aldrich, SML1377) was dissolved in 5% dimethyl sulfoxide (DMSO) (5 mg/mL) was used. A single injection of R-7050 (ip. 6 mg/kg of body weight; King et al., 2013) was administered at the end of the sleep restriction period on the fifth day of sleep restriction.

Rats were randomly divided into the following groups: controls sleeping *ad libitum* (n=10), control rats treated with vehicle (n=13), R-7050 rats sleeping *ad libitum* (n=13), 5-day sleep-restricted rats (n=10), 5-day sleep-restricted rats treated with DMSO (n=13), and 5-day sleep-restricted rats treated with R7050 (n=13).

Sleep-restricted rats remained in the acrylic chamber of sleep restriction after the antagonist administration to prevent sleep and were euthanized 30 min after the R-7050 or DMSO administration. Animals sleeping *ad libitum* received R-7050 at the same time as sleep-restricted rats.

7.4 *In vivo* blood-brain barrier permeability assays

In the sleep restriction experiment, immediately after the fifth day of sleep restriction, rats from the control (Con, n=4) and from the sleep restricted groups (SR, n=4) were anesthetized with sodium pentobarbital (0.063 g/kg body weight ip.). In the TNF-α receptor antagonist experiment, control vehicle group (n=3), R-7050 control (n=3), sleep restricted group + DMSO (n=3), and sleep restricted group + R-7050 (n=3) groups were anesthetized with sodium pentobarbital in the same way. In both experiments, a cocktail containing sodium fluorescein (Sigma-Aldrich, F6377, 10

mg/mL) and rhodamine 123 (Sigma-Aldrich, 83702, 1 mg/mL) diluted in phosphate buffer solution was administered intracardiacly (0.2 ml/100 g of body weight) and left to circulate for 5 min. Then, animals were transcardially perfused with 0.9% saline solution, and the cerebral cortex and hippocampus were dissected. The samples were weighed, homogenized with cold-PBS, and centrifuged at 17135 *x g* for 10 min at 4 °C. Supernatants were collected, and 99.8% methanol was added; supernatants were mixed and centrifuged at 17135 *x g* for 10 min at 4 °C. The supernatants were placed in a 96-well plate and absorbance values were measured in an ELISA plate reader HLAB (H Reader 1) for fluorescein (485 nm excitation/535 nm emission) and rhodamine 123 (535 nm excitation/ 595 nm emission). Each sample was quantified in duplicate. A standard curve of known sodium fluorescein and rhodamine 123 concentrations was prepared in duplicate, measured, and used for calculating dye concentrations.

7.5 Brain microvessel isolation

In sleep restriction experiment, brain microvessels were isolated from control (n=6) and sleep restricted (n=6) groups. In the TNF-α receptor antagonist experiment, brain microvessels were isolated from intact control (n=6), control plus DMSO (n=6), control plus R-7050 (n=6), 5 days of sleep restriction (SR, n=6), 5 days of sleep restriction plus DMSO (n=6), and 5 days of sleep restriction plus R-7050 (n=6) groups. Animals were euthanized, and their brains were removed and treated as previously reported (Medina-Flores et al., 2020a, 2020b). The cerebral cortex and hippocampal regions were homogenized with 1 ml cold microvessel isolation buffer (0.3 M sucrose, 3 mM HEPES, 1% BSA in PBS, pH: 7.4) and centrifuged at 1000 *x*

g for 10 min at 4°C. The supernatants were eliminated, and the pellets were resuspended in 1 ml microvessel isolation buffer, then centrifuged at 1000 *x g* for 10 min. The supernatants were obtained and centrifuged at 200 *x g* for 5 min, and the pellets were washed with 1 mL PBS and centrifuged at 100 *x g* for 2 min. The isolated brain microvessels were frozen at -20ºC until use (Medina-Flores et al., 2020a, 2020b).

7.6 Western blot

Protein expression from isolated brain microvessels was analyzed in all groups. The pellet containing isolated brain microvessels was homogenized with 200 µL RIPA buffer containing protease inhibitors and centrifuged at 17135 *x g* for 10 min at 4 °C. Protein concentration was determined with the Bradford assay (BioRad, 500-0006). The proteins (30 µg) were resolved on a 10% SDS-PAGE gel and transferred to PVDF membranes. Membranes were blocked with 5% non-fat milk in PBS containing 0.1% Triton X-100 for 30 min and incubated overnight at 4°C with antibodies against PDGFR-β (ThermoFisher, G.290.3, 1:1000), connexin-43 (Invitrogen, 71-0700, 1:1000), claudin-5 (Biorbyt, orb160461, 1:1000), MMP-9 (Abcam, ab38898, 1:1000), A_{2A} adenosine receptor (Abcam, ab3461, 1:1000), TNF-α (Abcam, ab1793, 1:1000), phospho-NFκB p65 (Cell Signaling, 3033S, 1:1000) and active caspase-3 (BioVision, 3015-100, 1:1000). Total brain tissue protein expression from the control + DMSO, control + R-7050, sleep-restricted + DMSO, and sleep-restricted treated with R-7050 groups were incubated with IBA-1 (Abcam, ab48004, 1:1000), GFAP (Abcam, ab4648, 1:1000) and NeuN (Abcam, ab177487, 1:1000). Blots were incubated with secondary antibodies conjugated with

horseradish peroxidase (1:2500 dilution) at room temperature and revealed with the chemiluminescence detection system (Amersham, RPN2232). Images were acquired using the C-DiGit image generation and analysis system (LI-COR iS image studio, version 3.1).

7.7 Enzyme-linked Immunosorbent assay

In the sleep restriction experiment, animals from the control (n=4) and 5-day sleep restriction (n=4) groups were euthanized, trunk blood was collected, and the cerebral cortex and hippocampus were dissected. In the TNF-α receptor antagonist experiment, animals from the control + DMSO (n=4), control plus R-7050 (n=4), 5 days of sleep restriction plus DMSO (n=4), and 5 days of sleep restriction plus R-7050 (n=4) groups were euthanized, trunk blood was collected, and the cerebral cortex and hippocampus were dissected. Trunk blood was collected in heparinized tubes and was centrifuged at 5700 *x g* for 10 min; thereafter, plasma was recovered. The cerebral cortex and hippocampi were homogenized in an ice-cold lysis buffer containing 20 mM Tris-HCl, 0.25 M sucrose, 2 mM EDTA, 10 mM EGTA, 1% Triton-100, and a protease inhibitor cocktail. The lysates were centrifuged at 20000 *x g* for 30 min to 4ºC, and the supernatants were collected. Protein concentrations were determined using the Bradford protein assay. ELISA assay was performed according to the manufacturer's instructions (DY510-05, R&D Systems). TNF-α concentrations were measured by DuoSet TNF-α Rat ELISA Kit, from the cerebral cortex, hippocampi, and serum, according to the manufacturer's instructions (R&D Systems). Absorbance was determined at 450 nm with a microplate reader.

7.8 Immunofluorescence confocal microscopy

Control (n=1) and 5 day-sleep restricted (n=1) rats were ip. anesthetized with sodium pentobarbital. Animals were transcardially perfused with 0.9% saline solution for 5 min, followed by 4% paraformaldehyde (PFA) solution in PBS for 5 min. Thereafter, brains were removed and placed in 4% PFA overnight. Brains were maintained in a 30% sucrose solution in PBS with 0.05% sodium azide at 4ºC for tissue cryoprotection until use. Sucrose cryoprotected brains were cut and frozen at −18 °C. Serial coronal sections (30 µm) were collected using a cryostat (LEICA, CM1850) and collected in PBS 0.1 M with 0.1% sodium azide. The tissue was incubated with the antibodies to NeuN (Abcam, EPR12763, 1:2000), IBA-1 (Abcam, ab5076, 1:1000) and GFAP (Invitrogen, 14989282, 1:2000), to visualize neurons, astrocytes and microglia respectively. Free floating slides were incubated with secondary antibodies Donkey anti-mouse (Jackson ImmunoResearch, Alexa-Fluor 488, 715-545-150, 1:500), Donkey anti-rabbit (Jackson ImmunoResearch, Alexa-Fluor 647, 711-605-152, 1:500) or Donkey anti-goat (Jackson ImmunoResearch, 705-165-147, CY3 1:500). Images were acquired using a ZEISS LSM 880 confocal microscope with a 20X objective lens.

7.9 Statistical analysis

Data from the sleep restriction experiment comparing control and sleep-restricted groups were analyzed with a one-tail t-student test. A two-way analysis of variance (ANOVA) followed by a *post hoc* Turkey's multiple comparison test was used to evaluate the differences between the experimental groups (CON and SR) and the treatments (intact, vehicle and R-7050). All results are presented as the mean ±

standard error of the mean (s.e.m). A statistically significant difference was considered at $p<0.05$. Statistical analyses were conducted using GraphPad Prism 8.0 software (GraphPad Software, CA, USA).

8. RESULTS

Sleep restriction for 5 days increased blood-brain barrier permeability to sodium fluorescein but not to rhodamine 123

An *in vivo* permeability assay with sodium fluorescein tracer was performed to evaluate the effect of 5 days of sleep restriction on the BBB transport of small molecules. Sleep restriction increased the extravasation of sodium fluorescein in the cerebral cortex ($t=9.266$, $p<0.0001$; Figure 3a) and hippocampus ($t=18.56$, $p<0.0001$; Figure 3b) compared to the control group. To determine if 5 days of sleep restriction modified the P-glycoprotein efflux pump function, we evaluated the accumulation of rhodamine 123 in the cerebral cortex and hippocampus. As shown in Figure 3c-d, the concentration of rhodamine 123 did not change in the brain regions of the sleep-restricted rats as compared to intact control rats (cerebral cortex, $t=0.2217$, $p=0.6739$; hippocampus $t=1.894$, $p=0.5766$).

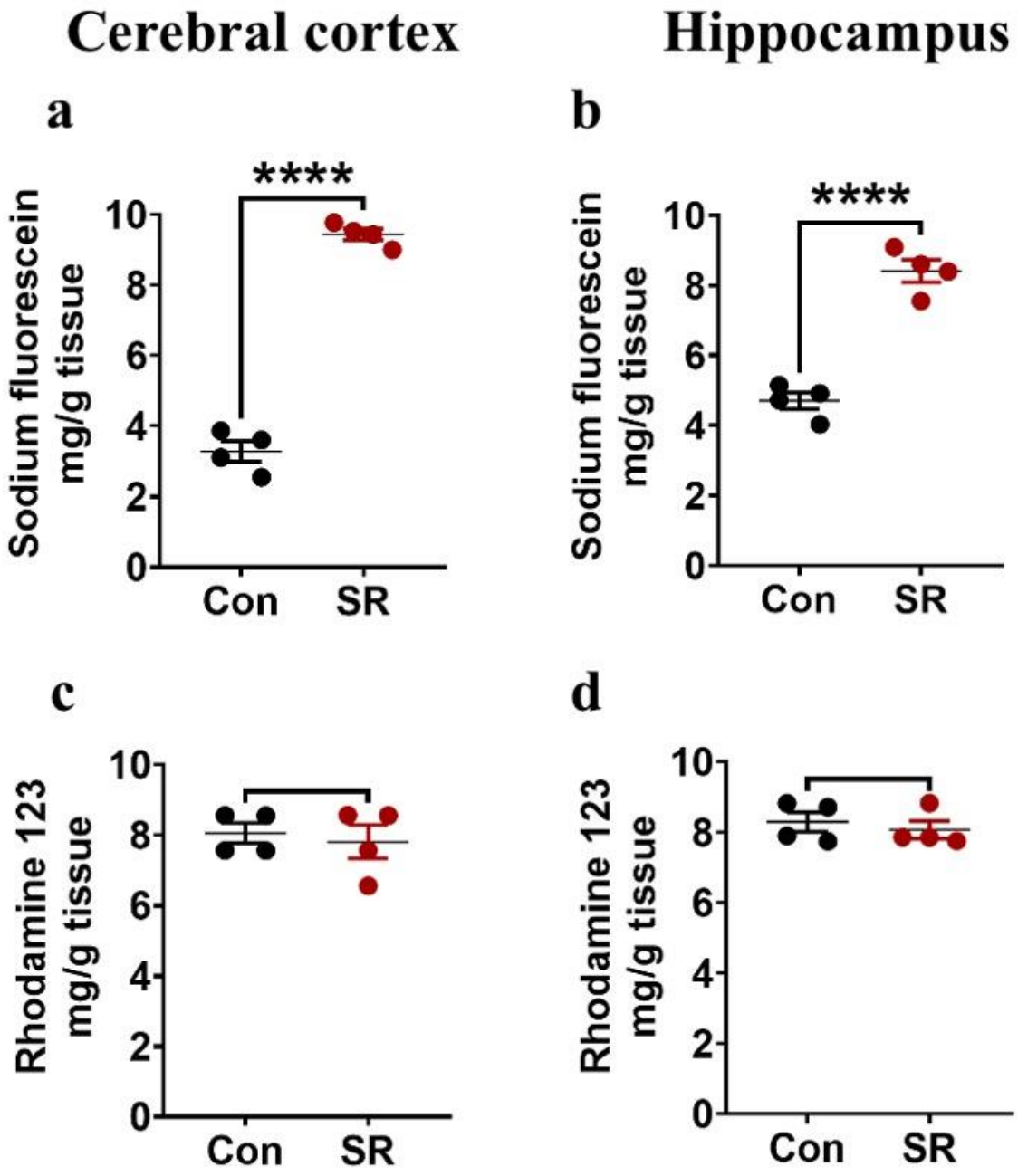

Figure 3. Sleep restriction for 5 days increased the blood-brain barrier permeability to small molecules. Graphs depict the BBB permeability to sodium fluorescein and rhodamine 123 in the cerebral cortex (a,c) and hippocampus (b,d) of the control (Con) and sleep-restricted (SR) groups. Quantification was performed by duplicate for each sample. Mean ± standard error of the mean. ****p<0.0001 as compared to the control.

Pericyte-endothelial cell disruption occurred during early periods of sleep restriction

To determine the early effects of sleep restriction on pericyte-endothelial cell interactions, PDGFR-β, and connexin-43 protein expression were evaluated in isolated microvessels from the cerebral cortex and hippocampus. Five days of sleep restriction reduced the expression of PDGFR-β ($t=6.396$, $p<0.0001$) and connexin-43 ($t=19.60$, $p<0.0001$) proteins in isolated microvessels of the cerebral cortex as compared to the control group (Figure 4a). In the hippocampus, sleep restriction reduced the expression of connexin-43 ($t=5.197$, $p<0.0001$), and no changes were observed in the PDGFR-β protein expression ($t=1.294$, $p=0.3599$) as compared to the control group (Figure 4b).

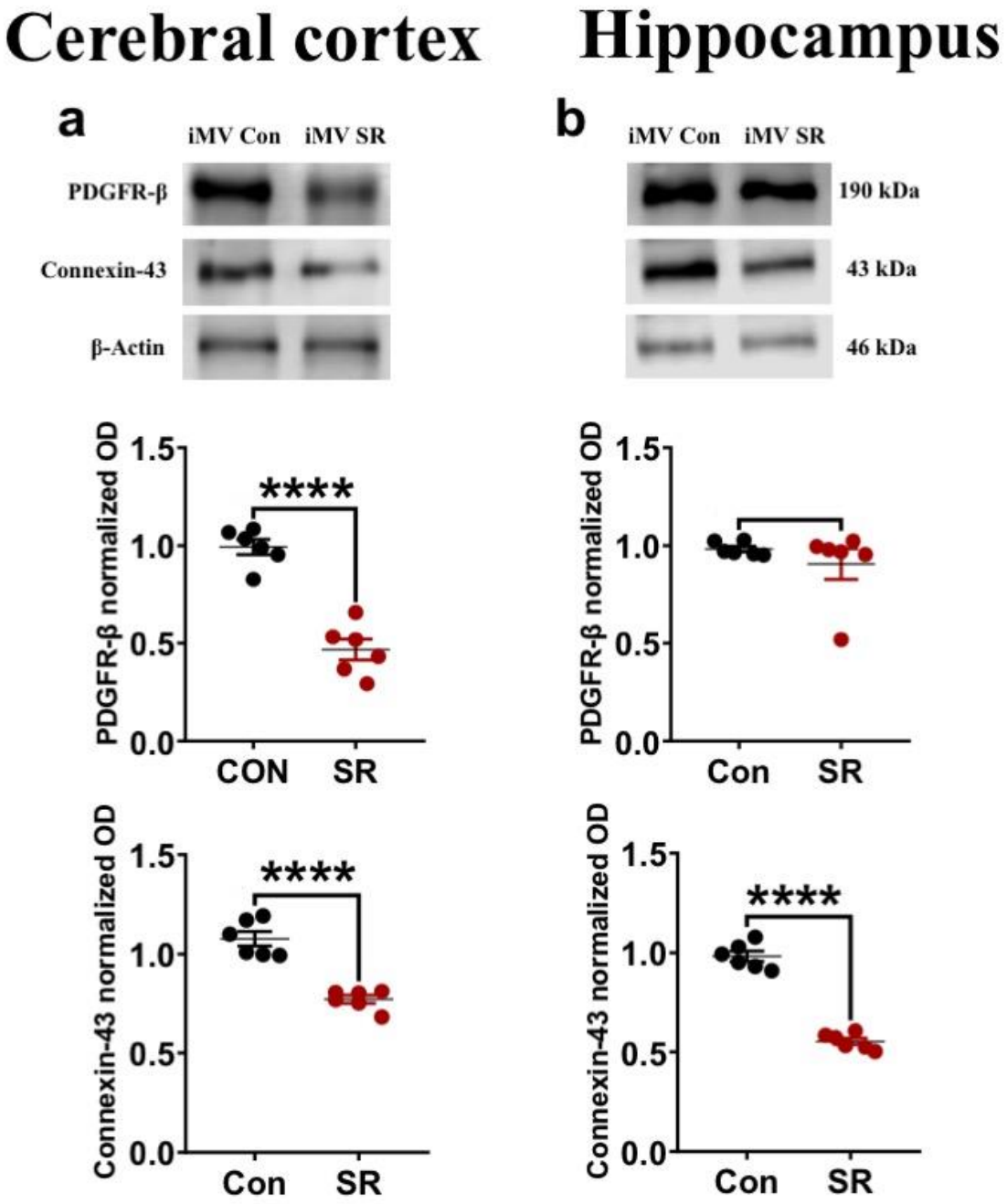

Figure 4. Sleep restriction for 5 days decreased protein expression at the pericyte-endothelial cell interface. Representative western blot images illustrate PDGFR-β and connexin-43 expression in isolated brain microvessels (iMV) from sleep-restricted (SR) and control (Con) groups. SR decreased connexin-43 expression in iMV of the cerebral cortex (a) and hippocampus (b) compared to the intact control group. SR decreased PDGFR-β expression only in iMV of the cerebral cortex compared to the animals sleeping *ad libitum*. The graphs depict the semi-quantitative analysis of the duplicates using β-actin as a reference. Mean ±standard error of the mean. ****p<0.0001 as compared to the control group.

Five days of sleep restriction promoted local accumulation of TNF-α and other pro-inflammatory mediators in the cerebral cortex and hippocampus

To determine if pericyte detachment from the capillary wall was associated with systemic or central TNF-α release during early sleep loss, we evaluated TNF-α concentration in serum, hippocampus, cerebral cortex, and isolated microvessels from the cerebral cortex and hippocampus. The control group presented low serum TNF-α levels and also in the total tissue of the hippocampus and cerebral cortex (Figure 5). Sleep-restricted rats presented the same serum TNF-α levels as the control group ($t=0.9423$, $p=0.3824$; Figure 5a). ELISA results showed that in the cerebral cortex, there was a significant increase in TNF-α levels in the sleep-restricted rats ($t=3.283$, $p=0.0168$) compared to the control group (Figure 5b). Sleep restriction increased the hippocampal concentration of TNF-α compared to the control group ($t=5.243$, $p=0.0019$; Figure 3c). As shown in Figure 5d, sleep restriction for 5 days increased TNF-α expression in isolated microvessels of the cerebral cortex ($t=13.29$, $p<0.0001$) and hippocampus ($t=17.55$, $p<0.0001$) as compared to the control group (Figure 5e). Therefore, local release of TNF-α in brain microvessels may regulate pericyte-endothelial cell interactions and, consequently, BBB function in the early phase of sleep loss.

In addition, 5 days of sleep restriction promoted the accumulation of other pro-inflammatory mediators in the isolated microvessels of the cerebral cortex and hippocampus. Sleep restriction for 5 days increased the expression of the A_{2A} adenosine receptor ($t=2.826$, $p=0.0036$) and MMP-9 ($t=5.515$, $p<0.0001$) in the isolated hippocampal microvessels in comparison with the control group (Figure 6b). In the cerebral cortex, there was an increase in MMP-9 expression ($t=4.657$,

p=0.0025) in isolated microvessels from sleep-restricted animals compared to the intact control group (Figure 6a). A_{2A} adenosine receptor expression in the cerebral cortex remained unchanged (t=0.04890, p=0.2207) in the sleep-restricted and the intact control groups (Figure 6a).

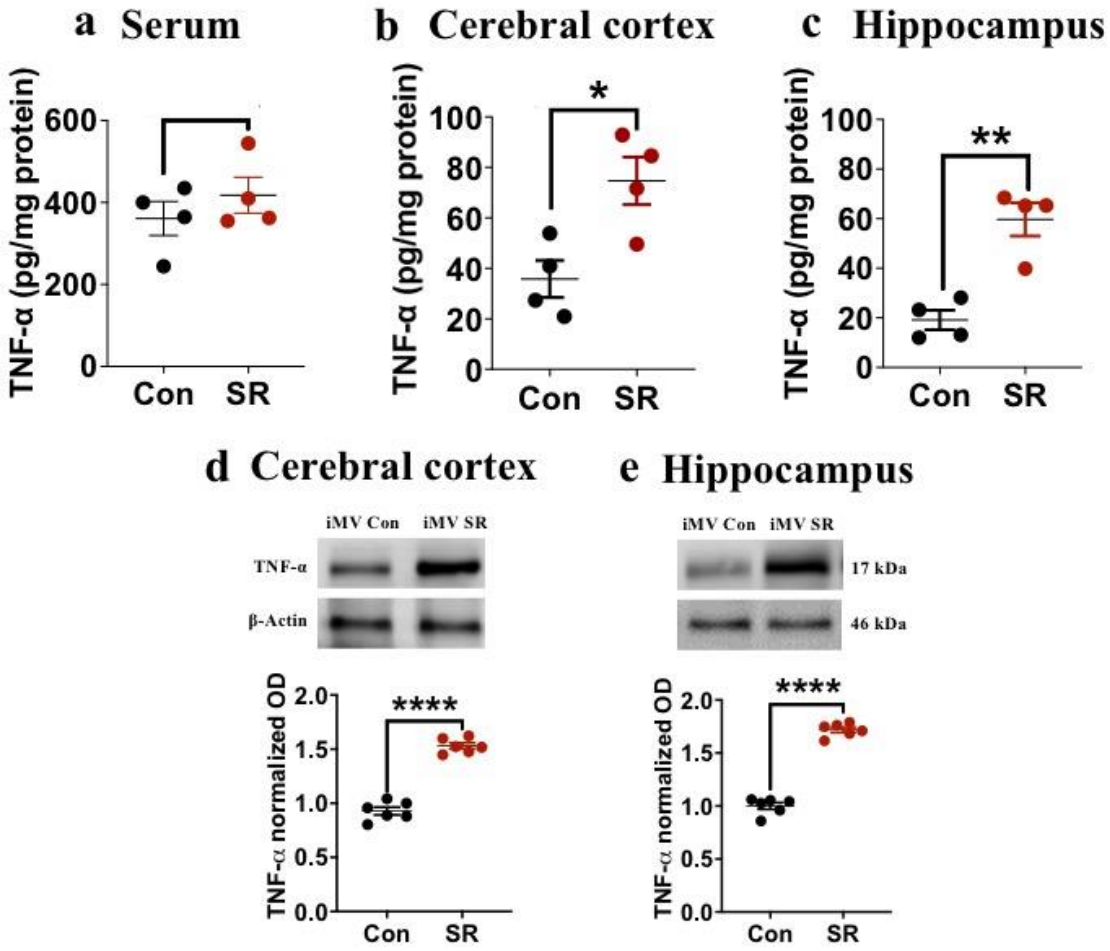

Figure 5. Sleep restriction for 5 days increased TNF-α levels in total brain tissue and isolated brain microvessels. Graphs represent TNF-α concentrations in serum (a), total tissue of cerebral cortex (b), and hippocampus (c) from 5-day sleep-restricted groups as compared to control groups. Western blot images show TNF-α expression in isolated microvessels (iMV) from the cerebral cortex (d) and hippocampus (e) of sleep-restricted rats compared with rats that slept *ad libitum*. Mean ± standard error of the mean. *p<0.05, **p=0.01, ****p<0.0001 as compared to the control group.

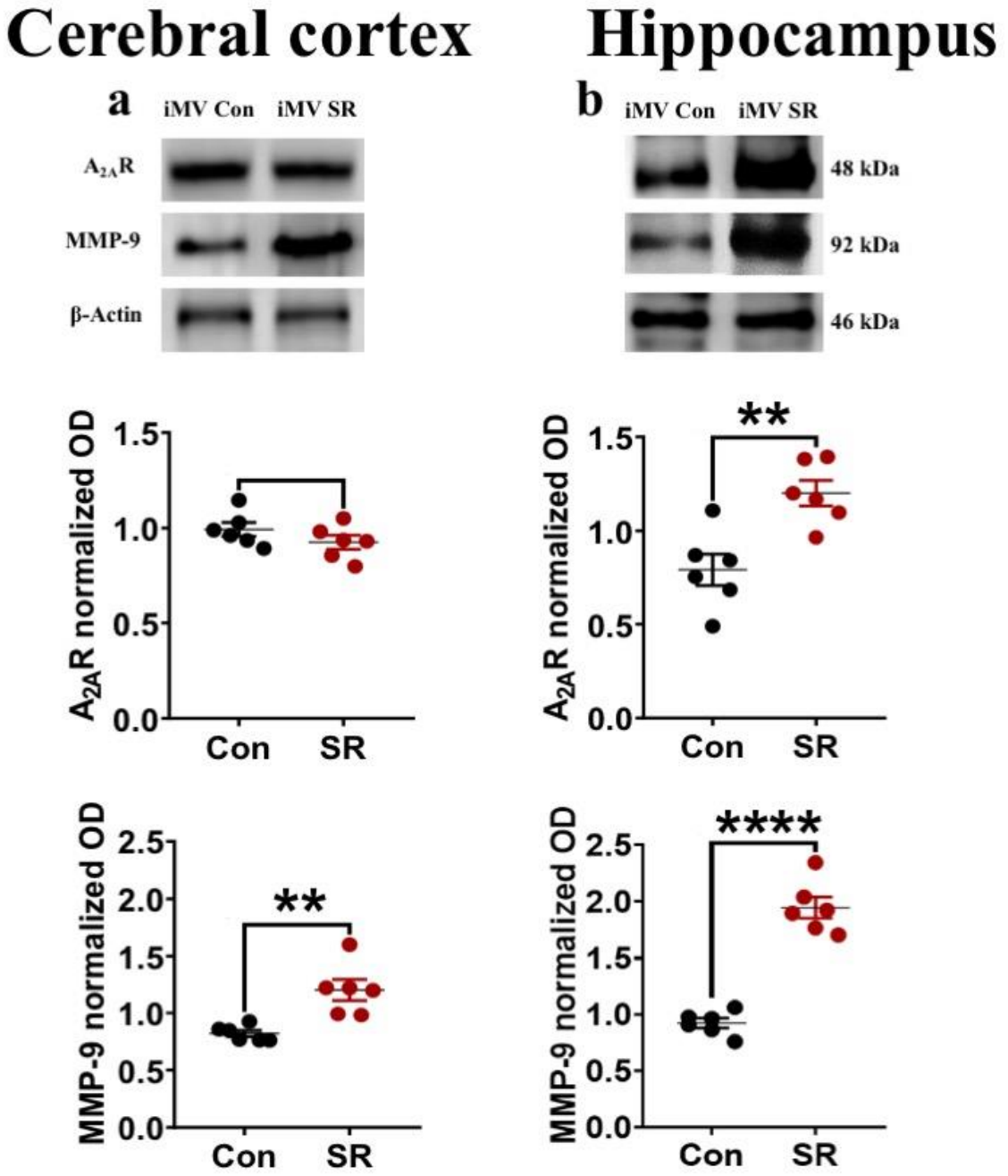

Figure 6. Sleep restriction for 5 days induced the expression of pro-inflammatory mediators in isolated brain microvessels. Western blot analysis of A_{2A} adenosine receptor and MMP-9 of isolated microvessels (iMV) from the cerebral cortex (a) and hippocampus (b) after 5 days of sleep restriction (SR) compared to the control group (Con). SR increased MMP-9 expression in isolated microvessels of the cerebral cortex and hippocampus (a, b) and increased A_{2A} adenosine receptor expression only in isolated microvessels of the hippocampus (b). Mean ± standard error of the mean. **p=0.01, ****p<0.0001 as compared to the control group.

Sleep restriction for 5 days increased microglia and astrocytes density in the hippocampus and cerebral cortex

We evaluated whether 5 days of sleep restriction modified the density of microglia, astrocytes, and neurons in brain slices. Representative immunofluorescent confocal images showed that in the CA1, CA3, hilus, and dentate gyrus of the hippocampus and the cerebral cortex of a 5-day sleep-restricted rat, there was increased immunoreactivity for IBA-1, a specific marker of microglia (Figure 7a). As it is shown in the insert (Figure 7b), in all the brain regions, microglia from the rat sleeping *ad libitum* (control group) presented a thin soma with long ramified processes, characteristic morphology of resting microglia; meanwhile, microglia from the sleep-restricted rat presented a large soma with short ramifications, consistent with microglial activation. The cerebral cortex of the 5-day sleep-restricted animal showed an increase in reactive astrocytes, as depicted by increased GFAP immunoreactivity compared to the control rat. However, this difference was not observed in any region of the hippocampus among the experimental groups (Figure 7a). The hippocampus and the cerebral cortex of the 5-day sleep-restricted animal and the control animal did not show apparent changes in the immunoreactivity of NeuN, suggesting that in short periods of sleep restriction, there were no changes in neuronal density (Figure 7a).

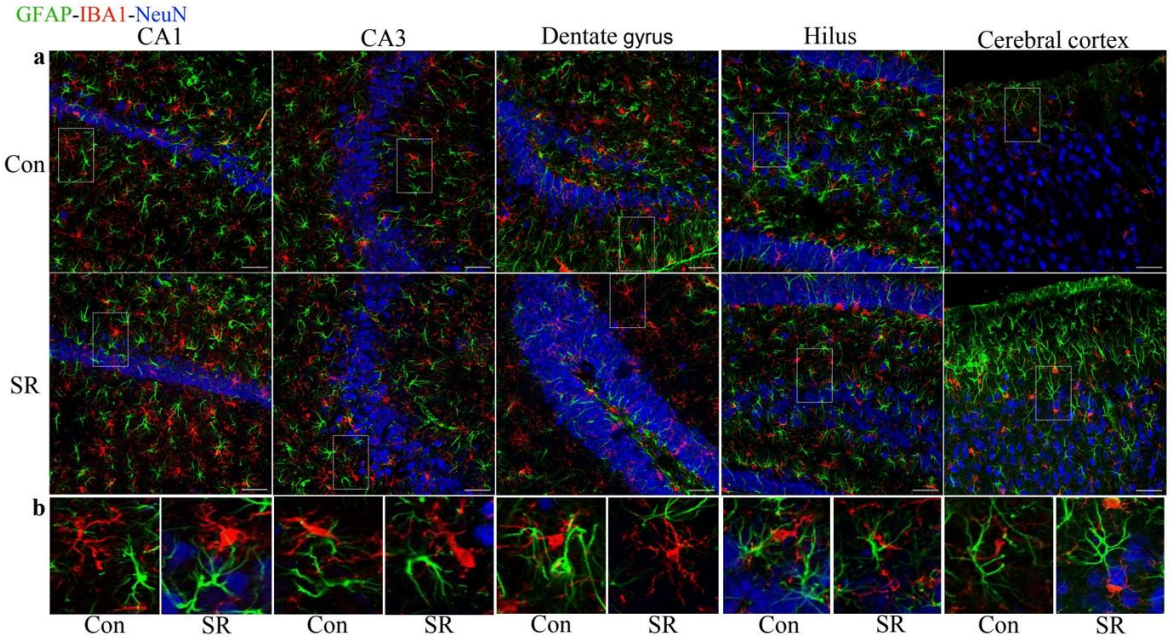

Figure 7. Five days of sleep restriction increased the density of astrocytes and microglia in the hippocampus and cerebral cortex. a) Representative immunofluorescence images show that after 5 days of sleep restriction, there was an increase in immunoreactivity for IBA1 in the hippocampal regions CA1, CA2, dentate gyrus, and hilus, as well as in the cerebral cortex. In the cerebral cortex an increase in GFAP immunoreactivity was observed, with no apparent changes in astroglial number in any of the hippocampal regions. There were no changes in immunoreactivity for NeuN between the 5-day sleep-restricted and the control animal. The insert (**b**) shows an amplification of astroglial and microglial cells. The insert shows microglial activation after five days of sleep restriction in the hippocampal regions CA1 and CA3 and the cerebral cortex, as shown by the increased volume of their soma and an apparent reduction in the number of ramifications.

TNF-α receptor antagonist reverted the blood-brain barrier hyperpermeability induced by 5 days of sleep restriction

As shown in Figure 8, DMSO-treated rats sleeping *ad libitum* presented low extravasation of sodium fluorescein and rhodamine 123 in the cerebral cortex and hippocampus. Moreover, TNF-α receptor antagonism under normal sleep conditions did not affect BBB permeability, since same low concentrations of fluorescein and rhodamine 123 were measured in the cerebral cortex and hippocampus as in control groups treated with the vehicle (Figure 8). Sleep

restriction in rats treated with DMSO increased BBB permeability to fluorescein in the cerebral cortex ($p=0.0253$) and hippocampus ($p<0.0001$) as compared to rats sleeping *ad libitum* and treated with DMSO. A single dose of R-7050 (6 mg/kg) on the fifth day of sleep restriction reverted the BBB hyperpermeability to fluorescein only in the hippocampus ($F_{1,8}=59.35$, $p<0.0001$; Figure 8b). In the cerebral cortex, fluorescein levels in sleep-restricted rats treated with R-7050 were similar to those observed in vehicle-treated sleep restricted rats ($F_{1,8}=5.5541$, $p=0.1093$; Figure 8a). Neither sleep restriction nor the TNF-α receptor antagonist altered P-glycoprotein function, as low BBB permeability to rhodamine 123 was detected in all the groups from both regions (cerebral cortex, $F_{1,8}=0.0006554$, $p=0.9802$; hippocampus, $F_{1,8}=0.002215$, $p=0.9636$) (Figure 8 c-d).

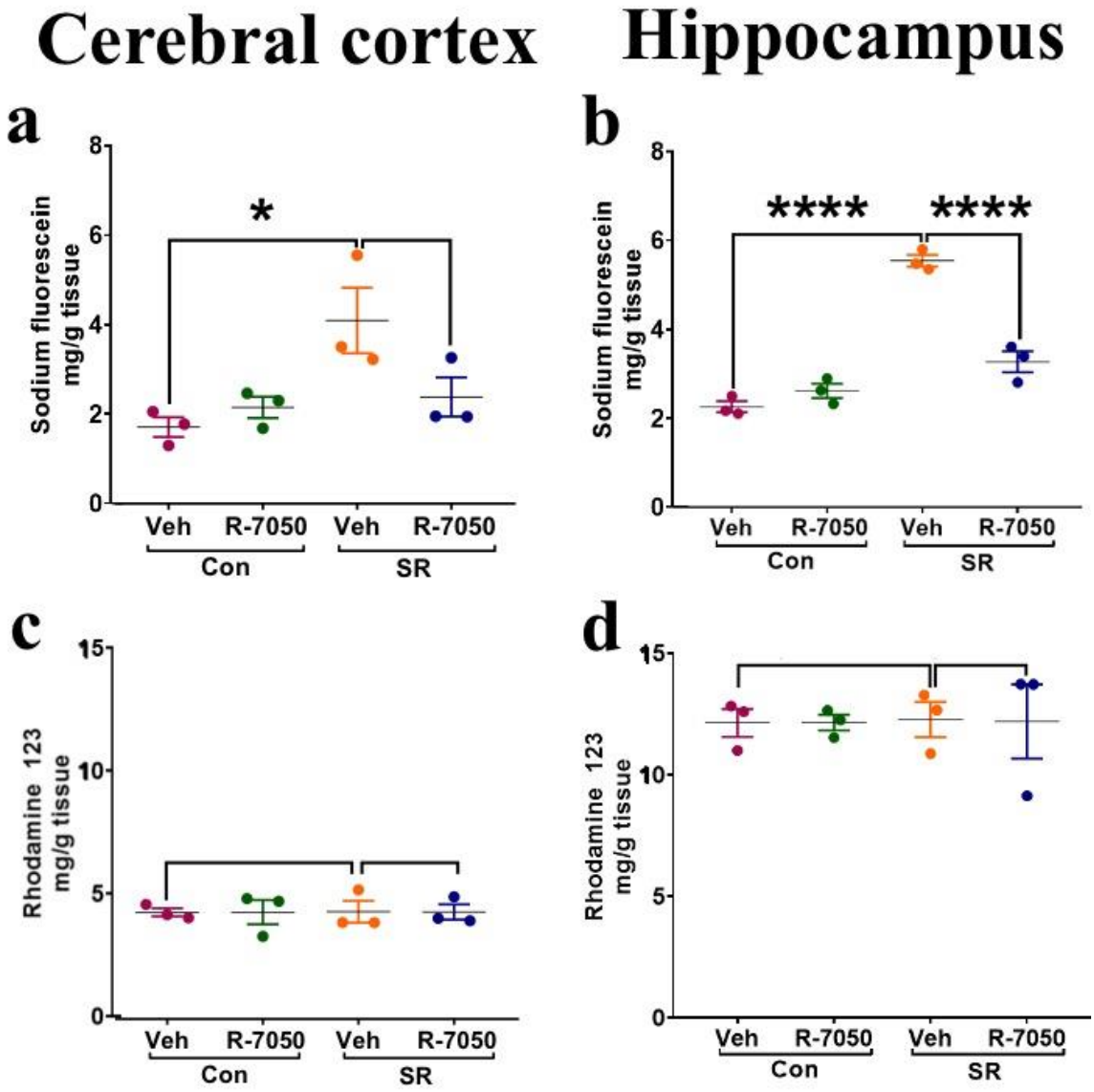

Figure 8. TNF-α receptor antagonist restored normal blood-brain barrier function in sleep-restricted animals. The effect of a single dose of R-7050 (6 mg/kg) reverted the hyperpermeability to sodium fluorescein in the cerebral cortex (a) and hippocampus (b) associated to 5 days of sleep restriction (SR) as compared with the control vehicle group (Veh). No changes were observed in rhodamine 123 permeability as compared to the control vehicle group (c,d). Mean ± standard error of the mean. *p<0.05, ****p<0.0001 as depicted in the graphs.

TNF-α receptor antagonist reverted the tight junction protein downregulation induced by 5 days of sleep restriction

Since the changes in the paracellular permeability of the BBB are related to the maintenance of the tight junctions, we evaluated the effect of five days of sleep restriction on the expression levels of the tight junction proteins. As shown in Figure 9, DMSO and R-7050 administration did not modify claudin-5 protein expression in the hippocampus and cerebral cortex of animals sleeping *ad libitum*. Sleep restriction for 5 days decreased claudin-5 expression in the isolated microvessels from the hippocampus ($p<0.0001$; Figure 9b), but did not modify claudin-5 expression in the cerebral cortex ($F_{2,30}=0.1881$, $p=0.8905$; Figure 9a) compared to the controls. TNF-α receptor antagonism partially reverted the claudin-5 downregulation induced by 5 days of sleep restriction ($F_{2,30}=8.732$, $p=0.003$), but the expression levels were still lower than in the vehicle control groups (Figure 9b). Sleep restriction decreased ZO-1 expression in isolated microvessels from the hippocampus ($p<0.0001$; Figure 9a) and cerebral cortex ($p<0.0001$; Figure 9b), compared to control groups. The treatment with R-7050 in sleep-restricted rats reversed ZO-1 expression only in the isolated microvessels from the cerebral cortex ($F_{2,30}=22.10$, $p<0.0001$), while its expression remained reduced in the isolated microvessels from the hippocampus ($F_{2,30}=0.3412$, $p=0.7323$). In addition, the sleep-restricted animals presented a decrease in the expression of occludin in the isolated microvessels from the cerebral cortex ($p<0.0001$; Figure 9a) and hippocampus ($p=0.0054$; Figure 9b) as compared with the animals that slept normally. R-7050 did not restore occludin expression in isolated microvessels from

the cerebral cortex ($F_{2,30}$=2.595, p=0.7993) nor from the hippocampus ($F_{2,30}$=2.818, p=0.4907).

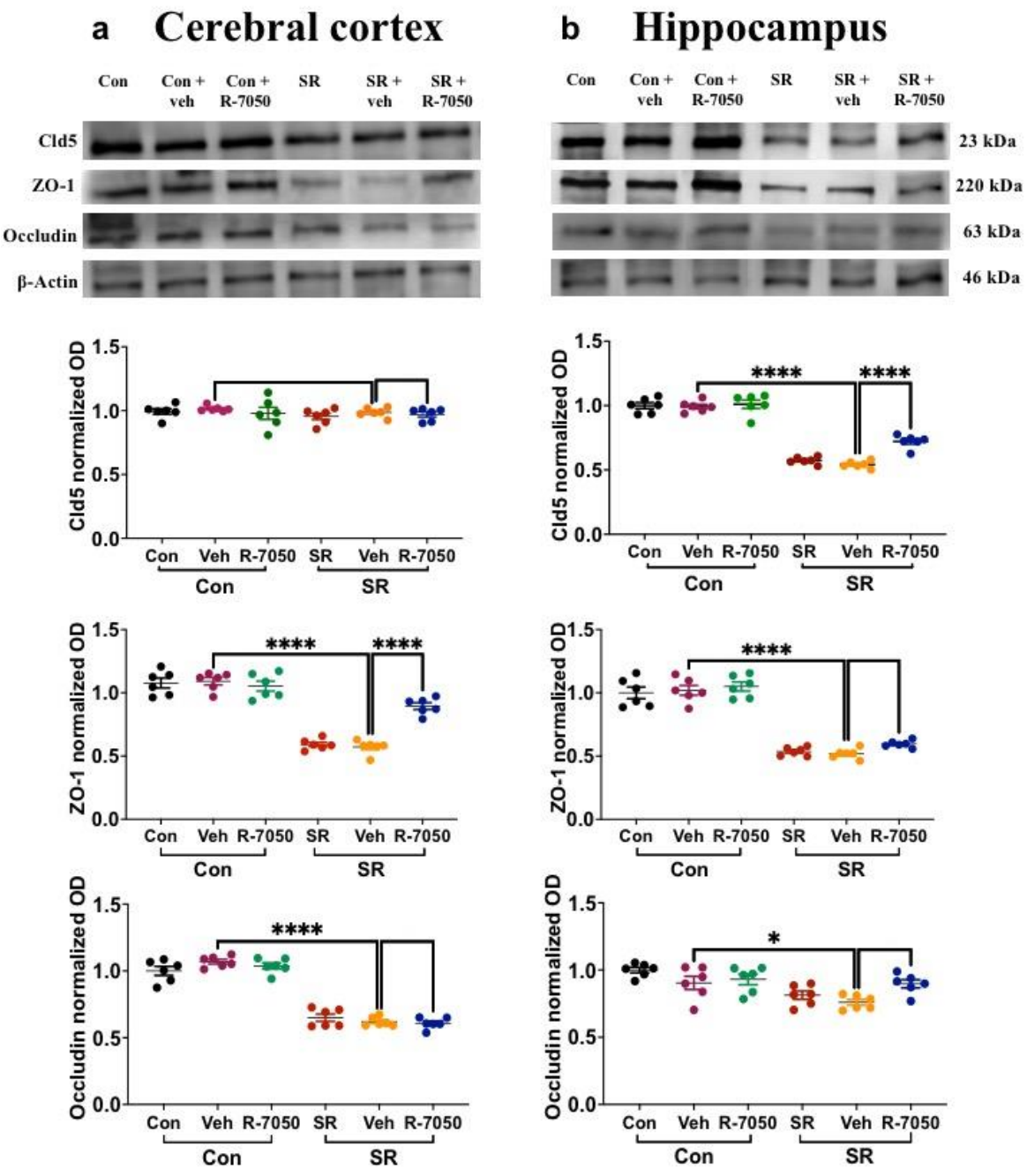

Figure 9. TNF-α receptor antagonist partially restored claudin-5 and ZO-1 expression in sleep-restricted animals. Representative western blot images show that sleep restriction for 5 days decreased claudin-5 expression in isolated hippocampal blood microvessels (b) but not in the cerebral cortex (a) as compared to the control group (Con). ZO-1 and occludin expression were reduced in isolated

microvessels from the cerebral cortex (a) and hippocampus (b) after 5 days of sleep restriction. The administration of R-7050 in sleep-restricted animals improved the expression of ZO-1 in the cerebral cortex (a) and claudin-5 in the hippocampus (b). In contrast, occludin expression remained decreased compared to the control groups. Mean ± standard error of the mean. *p<0.05, ****p<0.0001 as depicted in the graphs.

R-7050 treatment restored pericyte-endothelial cell interactions after sleep restriction

The TNF-α receptor antagonist did not modify the expression of PDGFR-β in the isolated microvessels from the cerebral cortex and hippocampus of animals sleeping normally (Figure 10). Five days of sleep restriction altered the interactions between pericytes and endothelial cells, as shown by a decrease in the expression of PDGFR-β in isolated microvessels of the cerebral cortex ($p<0.0001$) compared with the vehicle control group. A single dose of R-7050 reversed the effect of sleep restriction and restored normal PDGFR-β expression in isolated microvessels from the cerebral cortex of sleep-restricted rats ($F_{2,30}=39.81$, $p<0.0001$; Figure 10a). In isolated hippocampal microvessels, neither sleep restriction nor R-7050 modified the expression of PDGFR-β as compared to the vehicle control group ($F_{2,30}=0.8002$, $p>0.05$; Figure 10b).

All the animals sleeping *ad libitum* showed the same levels of connexin-43 in the isolated microvessels from the cerebral cortex and hippocampus, despite of DMSO or R-7050 treatment (Figure 10). A decrease in the expression of connexin-43 was observed after 5 days of sleep restriction in the cerebral cortex ($p<0.0001$; Figure 10a) and hippocampus ($p<0.0001$; Figure 10b) compared to the control group that slept normally. In sleep-restricted rats receiving DMSO, the same low levels of connexin-43 expression were found in the cerebral cortex and hippocampus as in

the non-treated sleep-restricted rats. The administration of R-7050 restored connexin-43 expression in the cerebral cortex ($F_{2,30}$=20.19, p<0.0001) and hippocampus ($F_{2,30}$=108.7, p<0.0001) of sleep-restricted rats, as compared to the sleep-restricted animals that did not receive the antagonist (Figure 10a-b).

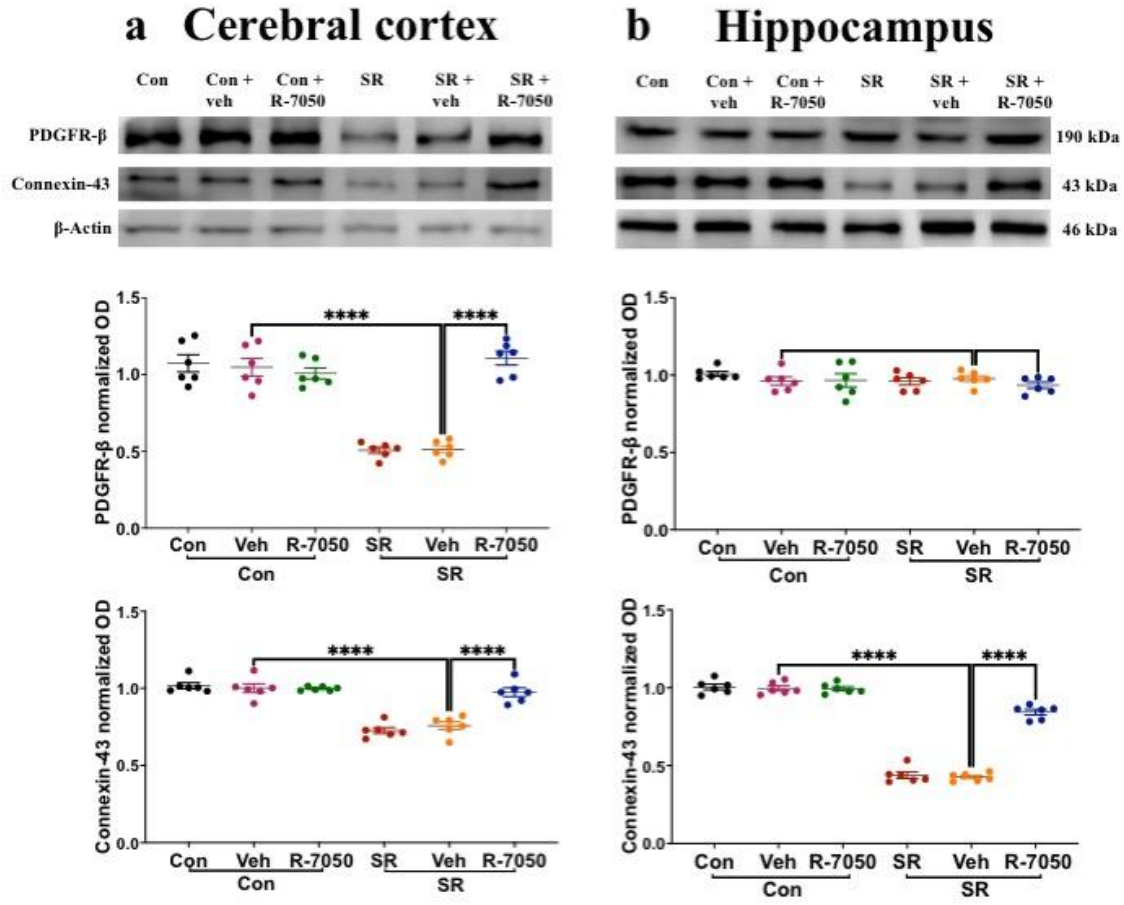

Figure 10. TNF-α receptor antagonist restored protein expression at the pericyte-endothelial cell interface after sleep restriction. Western blot analysis showed that 5 days of sleep restriction (SR) reduced the expression of PDGFR-β in isolated brain microvessels from the cerebral cortex, and the administration of R-7050 restored its expression (a). PDGFR-β expression in the hippocampus remained unchanged among the experimental groups (b). Sleep restriction reduced connexin-43 expression, and R-7050 restored its expression in the cerebral cortex (a) and hippocampus (b) compared with the control group. Mean ± standard error of the mean. ****p<0.0001 as depicted in the graphs.

Sleep loss increased local expression of TNF-α in the cerebral cortex and hippocampus without changing serum TNF-α concentration

Low serum levels of TNF-α were found in the control groups (Figure 11a). In sleep-restricted DMSO- or R-7050-treated rats similarly low serum TNF-α levels were found as in the control groups ($F_{2,18}$=0.2336, p=0.2716). In the cerebral cortex, TNF-α levels were significantly increased in the DMSO-treated sleep-restricted rats compared to the control group ($F_{2,18}$=5.733, p=0.0106). Remarkably, R-7050 significantly reduced TNF-α levels in the cerebral cortex of sleep-restricted animals compared to those that did not receive the TNF-α antagonist (p=0.0034; Figure 11b). Sleep restriction also increased the concentration of TNF-α in the hippocampus as compared to the vehicle control group (p=0.0462), but R-7050 administration did not affect TNF-α concentration ($F_{2,18}$=0.6539, p=0.9742; Figure 11c).

In isolated microvessels from the cerebral cortex, a low TNF-α expression was observed in all the control groups, which was elevated by sleep restriction (p=0.0056). This increased expression of TNF-α was maintained in the isolated microvessels from the cerebral cortex despite the administration of R-7050 ($F_{2,30}$=0.8895, p=0.8743; Figure 11d). Likewise, in isolated hippocampal microvessels, sleep restriction increased TNF-α expression (p<0.0001) as compared to the vehicle control group and was not modified by R-7050 ($F_{2,30}$=0.8146, p=0.9712; Figure 11e).

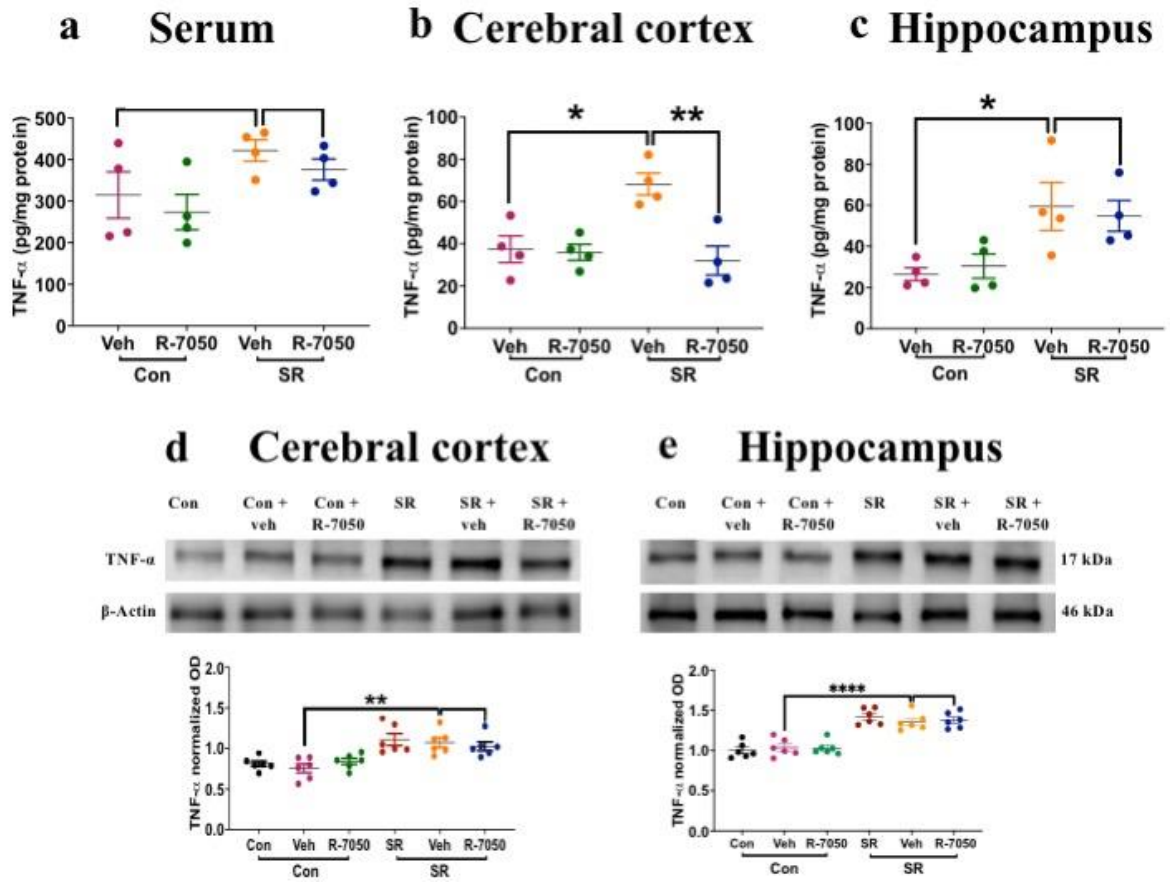

Figure 11. Five days of sleep restriction increased TNF-α levels in the central nervous system but not in the periphery. The data obtained by the ELISA assay showed that in serum, (a) TNF-α levels were unchanged between sleep-restricted groups compared to the DMSO control group (Veh). The sleep-restricted animals (SR) have higher levels of TNF-α in the cerebral cortex (b) and hippocampus (c) as compared to the DMSO control group (Veh). R-7050, administered as a single dose on the fifth day of sleep restriction, decreased the levels of TNF-α in the cerebral cortex. Western blot images showed that sleep loss (SR) increased TNF-α expression in isolated microvessels of the cerebral cortex (d) and hippocampus (e) as compared to the control group (Con). In isolated brain microvessels of sleep-restricted groups, TNF-α levels remained elevated despite administering R-7050 compared to the control groups. Mean ±standard error of the mean. *p<0.05, **p=0.01, ****p<0.0001 as depicted in the graphs.

Local inflammation in the hippocampus and cerebral cortex was maintained despite TNF-α receptor antagonism in sleep-restricted rats

Five days of sleep restriction increased the expression of pro-inflammatory mediators in isolated microvessels from the hippocampus and cerebral cortex (Figure 12). In the hippocampal isolated microvessels, the A_{2A} adenosine receptor expression was increased in sleep-restricted rats compared to the intact control group ($p<0.0001$; Figure 12b). A_{2A} adenosine receptor expression in the sleep-restricted groups remained high despite R-7050 administration ($F_{2,30}=0.6193$, $p=0.7852$; Figure 12b). In the isolated microvessels from the cerebral cortex, the A_{2A} adenosine receptor did not show any change in its expression in sleep-restricted rats as compared to the vehicle control group ($p=0.9940$), and its expression also remained unchanged despite the administration of R-7050 ($F_{2,30}=0.4283$, $p>0.9999$; Figure 12a). Sleep restriction increased MMP-9 expression in isolated microvessels from the cerebral cortex ($p<0.0001$) and hippocampus ($p<0.0001$), and R-7050 treatment did not modify MMP-9 expression in these samples (cerebral cortex, $F2,30=0.2502$, $p=0.9755$; hippocampus, $F_{2,30}=0.1174$, $p=0.8524$) as compared to the sleep-restricted group (Figure 12a-b). Sleep restriction increased the expression of phosphorylated NFκB in the isolated microvessels from the cerebral cortex ($p=0.0346$) but not from the hippocampus ($p=0.7703$) as compared to the intact control group (Figure 13a-b). The administration of R-7050 to sleep restricted rats did not modify the expression of phosphorylated NFκB in the cerebral cortex ($F_{2,30}=0.08222$, $p>0.9999$) or hippocampus ($F_{2,30}=0.2909$, $p>0.6212$) as compared to sleep-restricted rats (Figure 13a-b).

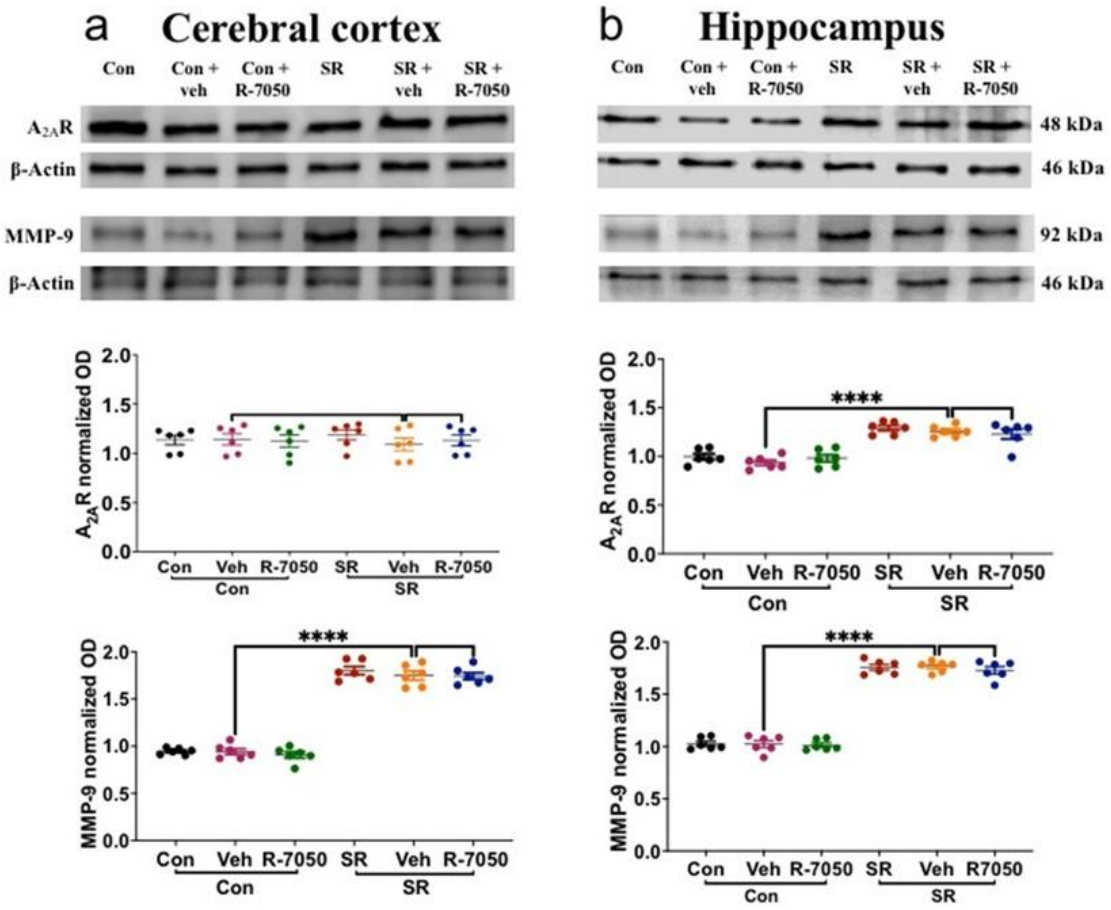

Figure 12. TNF-α receptor antagonist did not modify the expression of pro-inflammatory mediators in isolated brain microvessels. Sleep loss increased A_{2A} adenosine receptor expression in the hippocampus (b) but not in the cerebral cortex (a). R-7050 administration to controls did not modify the expression of the A_{2A} adenosine receptor. Five days of sleep restriction increased the expression of MMP-9 in isolated microvessels of the cerebral cortex (a) and hippocampus (b), but R-7050 did not decrease its expression in any experimental group. The graph shows the mean ±standard error of the mean. ****p<0.0001 as depicted in the graphs.

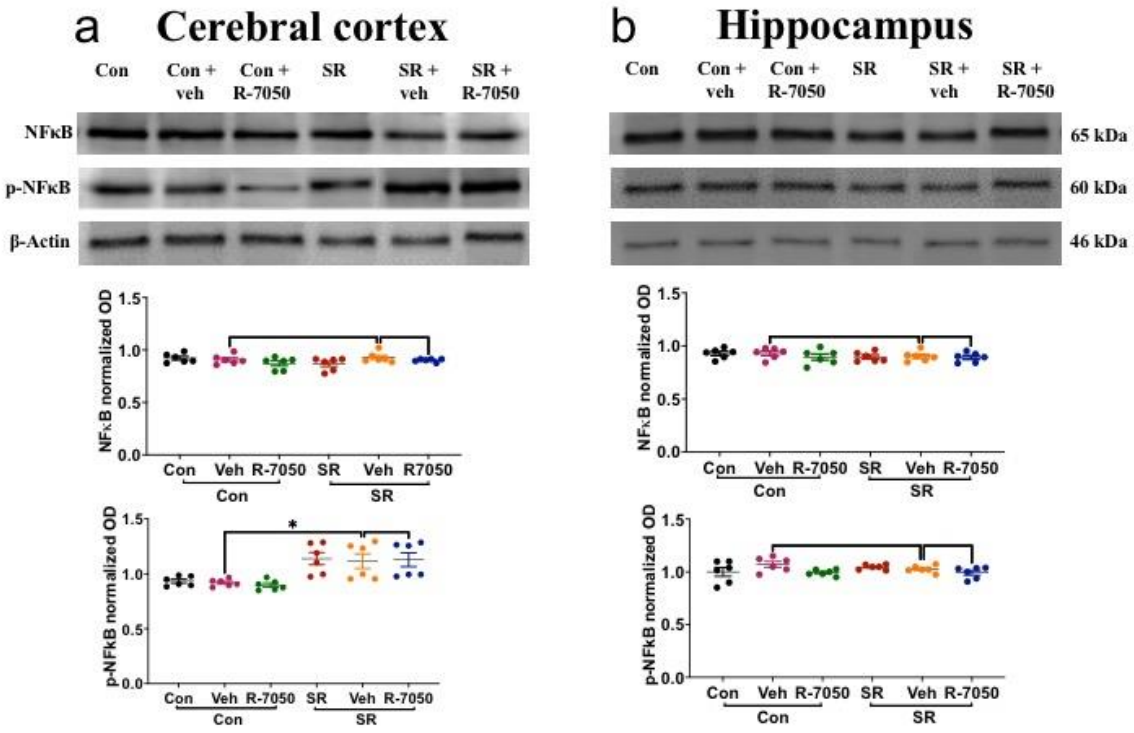

Figure 13. Sleep loss induced the expression of phosphorylated NFκB in isolated brain microvessels. The representative western blot images show that the expression of p-NFκB after 5 days of sleep restriction (SR) was increased only in the cerebral cortex, and this remained elevated after the administration of R-7050 (a). In hippocampal microvessels, there was no change in p-NFκB expression in sleep-restricted groups compared to the vehicle control group (Con) (b). Mean ± standard error of the mean. *p<0.05 as depicted in the graphs.

TNFR1 receptor antagonism reverted astroglial reactivity associated to 5-day sleep restriction

To evaluate whether TNFR1 receptor inhibition modified the expression of markers of microglia, astrocytes, and neurons in 5-day sleep-restricted rats, the expression of IBA-1, GFAP, and NeuN proteins from total brain tissue were evaluated by western blot. Representative western blot images show that in total brain tissue, sleep restriction for 5 days significantly increased IBA-1 protein expression as compared to the DMSO-treated control group (t=0.9586, p<0.0001). The expression of IBA-1 remained elevated in the sleep-restriction group after the single

administration of R-7050 (t=0.9586, p=0.9975; Figure 14). Five days of sleep restriction increased GFAP expression in the total brain tissue compared to the control group treated with DMSO (t=0.9107, p<0.0001). A single dose of R-7050 reversed the effect of sleep restriction on astroglial reactivity as shown by decreased GFAP expression in the sleep restricted group plus R-7050 as compared with sleep restricted rats treated with DMSO (0=0.9107, p<0.0001; Figure 14). Short periods of sleep loss or R-7050 administration did not modify the expression of NeuN (t=0.9107, p=0.9036, Figure 14).

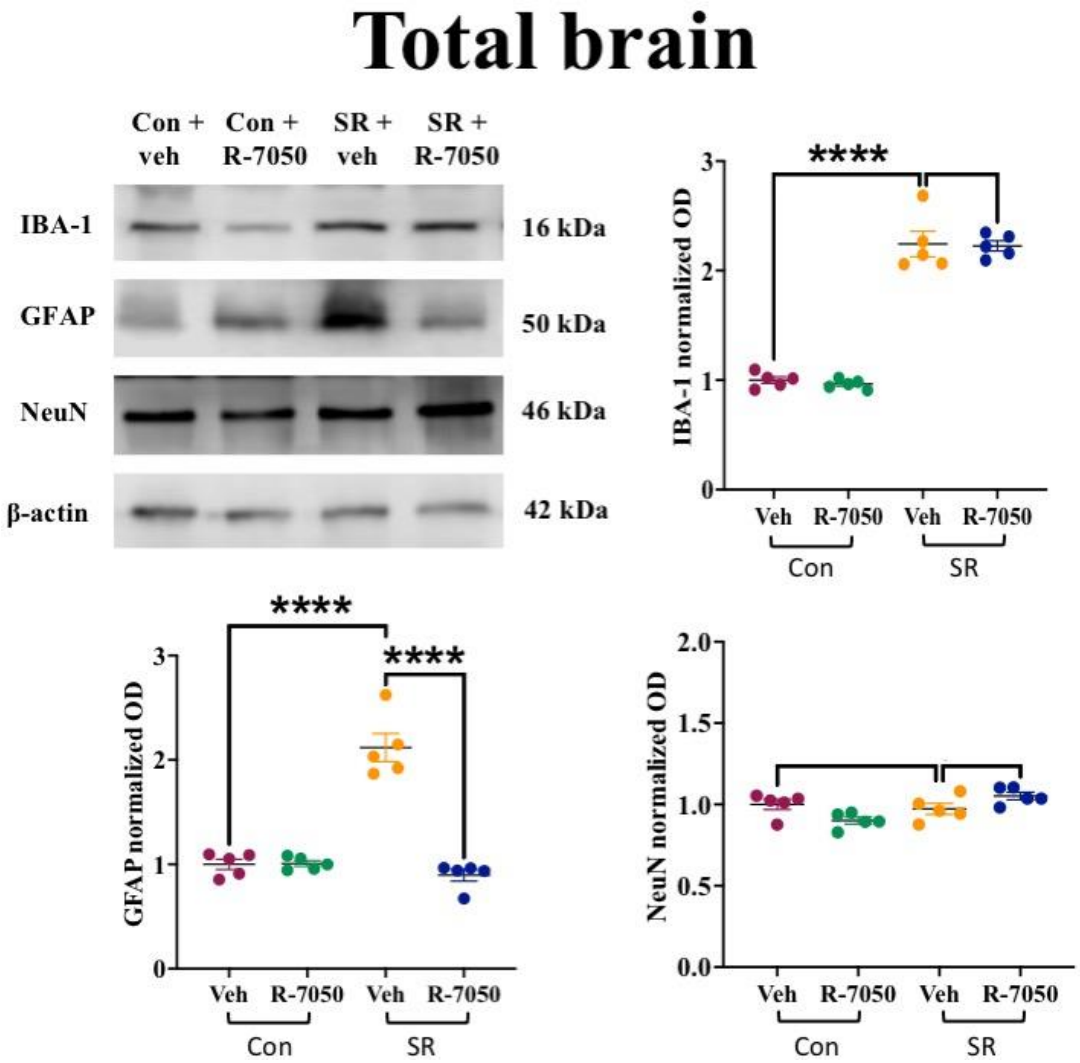

Figure 14. TNF-α receptor antagonist reverted sleep loss-related astroglial activation but not microglial activation in total brain tissue. Representative western blot images of total brain tissue show that five days of sleep restriction (SR) increased the expression of IBA-1 and GFAP compared to the control groups (Con). The single administration of R-7050 reverted the increased expression of GFAP associated with sleep restriction, while IBA-1 expression was maintained elevated despite R-7050 administration. NeuN protein expression remained unchanged among the experimental groups. The graph shows the mean ±standard error of the mean. ****p<0.0001 as depicted in the graphs.

9. DISCUSSION

Pericytes are essential to maintain BBB stability (Nakagawa et al., 2009; Bell et al., 2010; Rustenhoven et al., 2017). Sleep restriction during short periods of time (5 days) disrupted the pericyte-endothelial cell interactions. Gap junctions were downregulated during the early stages of sleep restriction, as the gap junction protein connexin-43 presented a reduced expression in the isolated microvessels of the hippocampus and cerebral cortex. The cerebral cortex also presented a reduced expression of PDGFR-β in isolated microvessels of sleep-restricted animals. The loss of pericyte-endothelial cell interactions might be involved in the increased BBB permeability to a small molecular weight tracer (sodium fluorescein) but did not alter the efflux transport of a P-glycoprotein substrate (rhodamine 123). We found that sleep restriction increased the local release of TNF-α in the hippocampus and cerebral cortex. Likewise, isolated brain microvessels presented higher TNF-α protein expression in sleep-restricted rats than the animals sleeping normally, underlining the critical role played by TNF-α in regulating BBB physiology. In addition, we found that already in early sleep restriction periods, isolated microvessels from sleep-restricted rats presented an increase in the expression of MMP-9 in the hippocampus and cerebral cortex and elevated expression of the A_{2A} adenosine receptor in the hippocampus and p65 NFκB in the cerebral cortex. Furthermore, five days of sleep restriction are sufficient to induce microglial and astroglial activation in the cerebral cortex and hippocampus, which contribute to maintaining the pro-inflammatory state in the central nervous system. The antagonism of the TNFR1 normalized BBB permeability to sodium fluorescein in sleep-restricted animals partially reverted hippocampal claudin-5, and ZO-1

downregulation in the cerebral cortex fully reverted the downregulation of connexin-43 in isolated microvessels of the hippocampus and cerebral cortex, and normalized PDGFR-β expression in isolated microvessels of the cerebral cortex, accompanied by a decrease in astroglial activation in the total brain tissue. Despite restoring BBB function by TNF-α receptor antagonism, local neuroinflammation persisted in both brain regions, as R-7050 did not modify MMP-9, A_{2A} adenosine receptor, or p65 NFκB expression levels in isolated brain microvessels of sleep-restricted rats, these results may be related to the continued microglial activation despite the administration of R-7050. Interestingly, TNF-α receptor inhibition decreased TNF-α levels in total cerebral cortex tissue but not in the hippocampus. R-7050 did not modify TNF-α levels in the hippocampus and cerebral cortex isolated microvessels. The antagonist R-7050 is a small molecule that disrupts TNFR1-mediated downstream signaling pathways and potentially reduces its apoptotic effects by inhibiting the association of TNFR1 with its intracellular adaptor molecules (King et al., 2013; Gururaja et al., 2007; Balzano et al., 2020). TNFR1 is widely expressed in neurons, oligodendrocytes, macrophages, microglia, and astrocytes (Dopp et al., 1997). During neuroinflammation, microglia and astrocytes can produce and release high concentrations of TNF-α (Liddelow et al., 2017). However, this can be reversed by TNFR1 antagonism, which reduces microglial and astroglial activation and increases apoptosis of cytokine-producing cells, leading to the downregulation of TNF-α production (Charles at al., 1999; Gururaja et al., 2007; Diallo et al., 2021); this phenomenon may explain why we found a decrease in TNF-α levels in the brain regions, which is related to a decrease in astroglial reactivity.

Previously, we showed that chronic sleep restriction promotes pericyte detachment from the capillary wall (Hurtado-Alvarado et al., 2017; Medina-Flores et al., 2020a). In those studies, decreased expression of connexin-43 and PDGFR-β were found in isolated microvessels of animals with 10 days of sleep restriction. The present findings suggest that pericyte detachment from the capillary wall begins at short periods of sleep restriction (5 days). The initial event could be the downregulation of connexin-43 followed by the loss of PDGFR-β. Gap junctions are essential to maintain tissue homeostasis, and their alterations can compromise intercellular communication (De Maio, et al., 2002). In biological barriers, gap junctions regulate tight junction dynamics (Nagasawa et al., 2006; Li et al., 2012). Gap junction proteins co-immunoprecipitate with the tight junction proteins claudin-5 and occludin (Nagasawa et al., 2006). The close association of connexin-43 with the tight junction proteins claudin-5, occludin, and ZO-1 in brain endothelial cells (Nagasawa et al., 2006) and retinal endothelial cells (Tien et al., 2013) is regulated by the last 19 amino acid residues of the C-terminus of connexin-43 and the second PDZ domain of ZO-1 (Sorgen et al., 2004). Connexin blockers have also been shown to decrease transendothelial electric resistance and increase barrier permeability in an *in vitro* model of porcine brain endothelial cells (Nagasawa et al., 2006). In addition, a decrease in the expression levels of connexin-43 is related to a reduction of tight junction protein expression, such as occludin, claudin-1, and ZO-1 (Lee et al., 2007; Bekheet and Stahlmann, 2009) and increased vascular permeability (Tien et al., 2013). In our study, sleep restriction downregulated connexin-43 expression in isolated brain microvessels from the cerebral cortex and hippocampus suggesting a disruption of cell-cell communication, which may contribute to pericyte detachment

from the capillary wall and subsequent BBB disruption. This condition has been previously reported in pathological conditions, such as diabetic retinopathy, where connexin-43 downregulation in the retina disrupts the crosstalk between endothelial cells and promotes vascular permeability (Tien et al., 2014).

Loss of junctions between pericytes and endothelial cells results in increased blood-brain barrier permeability to exogenous circulating tracers of diverse molecular weight, ranging from 365 Da to 70 kDa (Hurtado-Alvarado et al., 2017; Medina-Flores et al., 2020a). In agreement with our findings, He et al. (2014) showed that sleep loss for 6 days increased BBB permeability to sodium fluorescein in C57/BL6 mice. At 10 days of sleep loss, P-glycoprotein dysfunction was also shown, as increased permeability to rhodamine 123 was observed in the hippocampus and cerebral cortex (Medina-Flores et al., 2020a). The present results show that the initial disruption in the blood-brain barrier occurs for sodium fluorescein, which mainly crosses via the paracellular route (Kaya and Ahishali, 2011), and no change in rhodamine 123 permeability was observed, indicating that the P-glycoprotein efflux transport system is affected during long-term sleep restriction periods (Medina-Flores et al., 2020a) but not in shorter ones.

Pericyte detachment from the capillary wall was accompanied by local inflammation in both brain regions after a 5-day sleep restriction, similar to what was reported for chronic (10 days) sleep restriction (Medina-Flores et al., 2020a). Here, we showed that 5 days of sleep restriction increased MMP-9 expression in isolated microvessels from both brain regions, similar to what was reported for 10 days of sleep restriction (Medina-Flores et al., 2020a). Five days of sleep restriction also induced the expression of other pro-inflammatory factors: A_{2A} adenosine receptor expression

was increased in the hippocampus, and p65 NFκB expression was elevated in the cerebral cortex. These results are consistent with our previously reported experiments in which 10 days of sleep restriction increased A_{2A} receptor expression in the hippocampus but not in the cerebral cortex (Hurtado-Alvarado et al., 2016b; Medina-Flores et al., 2020a) and increased p65 NFκB expression only in the cerebral cortex (Medina-Flores et al., 2020a). Others have also shown that sleep restriction effects on BBB function are related to the accumulation of inflammatory mediators, both centrally and peripherally (He et al., 2004; Hurtado-Alvarado et al., 2016a; 2018). He et al. (2004) demonstrated that sleep loss promoted brain accumulation of cyclooxygenase-2 (COX-2) and increased serum concentration of C-reactive protein. Hurtado-Alvarado et al. (2018) showed that BBB hyperpermeability during chronic sleep loss was related to increased TNF-α and interferon-γ (IFN-γ) plasma concentration, as sleep-restricted animals that did not show plasma changes in pro-inflammatory cytokines presented normal BBB function. Our study shows that at 5 days of sleep restriction, elevated levels of TNF-α are found only in the central nervous system but not in serum, suggesting that the sleep-related inflammatory event begins in the brain and not in the peripheral system.

Previous studies report that brain levels of TNF or TNF mRNA correlate with sleep propensity. Within the hypothalamus, concentrations of TNF (Floy and Krueger, 1997) and TNF mRNA (Bredow et al., 1997; Taishi et al., 1999) fluctuate diurnally. These levels are highest at day break, correlating with the beginning of the rat sleep period. TNF mRNA concentrations double during this same time frame, indicating the presence of post-transcriptional upregulation of TNF underlying this process.

TNF levels are also influenced by sleep deprivation. Sleep deprivation increases hypothalamic TNF and TNFR mRNA concentration (Floy and Krueger, 1997).

TNF-α is an inflammatory mediator produced and released by endothelial cells, microglia, astrocytes, and neurons (Gahring et al., 1996; Ranta et al., 1999); different studies have shown that its up-regulation hinders the physiological functions of the BBB in several brain diseases (Petty and Lo, 2002; Muhammad, 2020). TNF-α binds to TNFR1 and TNFR2, which are differently regulated on various cell types (Al-Lamki et al., 2001) and activate the NFκB mediated pro-inflammatory pathway (Gough & Myles, 2020). Pericytes express both TNF-α receptors, with higher expression levels of TNFR2 in pericytes as compared to astroglia and endothelial cells (Takata et al., 2011). TNF-α stimulation promotes higher levels of MMP-9 expression in pericytes compared to endothelial cells or astrocytes (Takata et al., 2011; Thanabalasundaram et al., 2010; Underly et al., 2017). TNF-α promotes MMP-9 transcription through the transcription factors AP-1 and NFκB (Moon et al., 2004). In pericytes, MMP-9 up-regulation induced by TNF-α involves the activation of mitogen-activated protein kinases (p42/p44 MAPK, p38 MAPK, and JNK) and phosphoinositide 3 kinase (PI3K)/protein kinase B (Akt) pathways (Takata et al., 2011; Cheng et al., 2009). However, for brain pericytes, it is unclear to which receptor TNF-α binds to activate the signaling pathways leading to elevated MMP-9 expression (Takata et al., 2011).

Here, we used the TNF-α antagonist R-7050, which is a cell-permeable triazoloquinoxaline compound that selectively inhibits TNFR1 signaling by blocking its association with intracellular adaptor molecules, such as TRADD and RIP, stopping receptor internalization and preventing subsequent NFκB and MAPK

signaling pathway activation (Gururaja et al., 2007), thus attenuating the neuroinflammatory environment. Nevertheless, our results showed that R-7050 did not revert the local inflammation induced by sleep loss in the hippocampus and cerebral cortex, as shown by the high levels of MMP-9, p65 NFκB, and A_{2A} adenosine receptor observed in isolated brain microvessels of 5-day sleep-restricted rats. These results indicate the potential role of TNFR2 and other inflammatory modulators in regulating sleep loss-related neuroinflammation.

Despite the lack of effect on local inflammatory mediators, TNR-α receptor antagonism did modify pericyte-endothelial cell interaction under sleep restriction conditions. A single dose of the TNF-α receptor antagonist R-7050 restored PDGFR-β expression in the cerebral cortex and connexin-43 in both brain regions as well as improved the function of the BBB by restoring the expression of tight junction proteins in isolated brain microvessels and inhibiting elevated BBB permeability to sodium-fluorescein in both brain regions. TNF-α can downregulate connexin-43 expression on the vascular smooth muscle cells through JNK and p38 MAPK kinase pathways (Wu et al., 2013; Kimura et al., 2013). TNF-α may also increase the tyrosine (Tyr) kinase c-Src portion, which is physically associated with connexin-43, and induces phosphorylation of Tyr-247 and Try-265, preventing connexin-43 channel closure by Src (Huang et al., 2003). TNF-α plays an important role in the regulation of gap junctions; particularly, it modulates the expression levels of connexin-43 protein, which is restored after R-7050 administration, thus improving the cellular communication between the pericyte and the endothelial cell, as our results suggest.

Previous studies reported that a single dose of R-7050 (6 mg/kg) reduced Evans blue extravasation when administered 0.5 h or 2 h after inducing intracerebral hemorrhage (King et al., 2013). In addition, in an *in vitro* model of cerebral ischemia, the pretreatment with R-7050 restored blood-brain barrier integrity by decreasing the permeability to dextran-FITC and improving ZO-1 expression (Lin et al., 2021). Our results are consistent with those since, after the administration of R-7050, the BBB dysfunction was attenuated, accompanied by an increase in the expression of claudin-5 and ZO-1 after 5 days of sleep restriction. Therefore, our results suggest that TNF-α plays a key role in modulating pericyte-endothelial cell junctions during short periods of sleep restriction.

10. CONCLUSION

Pericytes are essential cells that maintain the function and stability of the BBB through their close relationship with endothelial cells. Pericyte-endothelial cell communication can be altered with neuropathological and non-pathological conditions, such as sleep restriction. Pericytes are cells highly vulnerable to the inflammatory environment induced by sleep restriction. Here, we showed that alterations in cell communication between pericytes and endothelial cells are due primarily to a reduction in the expression of gap junction proteins. These alterations in pericyte-endothelial cell interaction depended on a pro-inflammatory local environment characterized by the early upregulation of TNF-α in the CNS. High levels of TNF-α induced by sleep restriction were accompanied by the activation of a pro-inflammatory signaling cascade involving MMP-9, NFκB, and A_{2A} adenosine receptor, ensuing hyperpermeability of the blood-brain barrier to low molecular

weight molecules in the cerebral cortex and hippocampus. A single dose of R-7050, a selective antagonist of TNF-α receptor 1, restored pericyte-endothelial cell interactions by increasing the expression of connexin-43 and restored normal BBB permeability by enhancing claudin-5 and ZO-1 expression, despite the lack of changes in local inflammation in both brain regions. Our study suggests that in the early stages of sleep restriction, TNF-α is an important inflammatory mediator that modulates pericyte-endothelial cell interactions, which are necessary to maintain the integrity of the blood-brain barrier, and TNF-α up-regulation compromises homeostasis of the central nervous system.

11. PERSPECTIVES

According to the results obtained in this research project, new questions arise and their study will contribute to understanding the mechanisms by which sleep regulates the function of the blood-brain barrier, in particular the interactions between the pericyte and the endothelial cell. Therefore, the following perspectives are suggested:

1.- What happens to pericytes when their interactions with endothelial cells are disrupted?

2.- How will other proinflammatory cytokines be expressed and how will they modify pericyte-endothelial cell interactions after 5 days of sleep restriction?

3.- Evaluation of the microglia morphology after five days of sleep restriction

4.- Will sleep recovery after five days of sleep restriction restore the interactions between the pericyte and the endothelial cell?

5.- How does sleep regulate the expression and function of gap junctions between pericytes and endothelial cells?

12. REFERENCES

Aasen, T., Johnstone, S., Vidal-Brime, L., Lynn, K. S., & Koval, M. (2018). Connexins: Synthesis, Post-Translational Modifications, and Trafficking in Health and Disease. *International Journal of Molecular Sciences*, *19*(5), 1296. https://doi.org/10.3390/ijms19051296

Al-Lamki, R. S., Wang, J., Skepper, J. N., Thiru, S., Pober, J. S., & Bradley, J. R. (2001). Expression of Tumor Necrosis Factor Receptors in Normal Kidney and Rejecting Renal Transplants. *Laboratory Investigation*, *81*(11), 1503-1515. https://doi.org/10.1038/labinvest.3780364

Alonso A, Reinz E, Jenne JW, Fatar M, Schmidt-Glenewinkel H, Hennerici MG, Meairs S. Reorganization of gap junctions after focused ultrasound blood-brain barrier opening in the rat brain. J Cereb Blood Flow Metab. 2010 Jul;30(7):1394-402. doi: 10.1038/jcbfm.2010.41

Alvarez, J. I., Cayrol, R., & Prat, A. (2011). Disruption of central nervous system barriers in multiple sclerosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1812*(2), 252-264. https://doi.org/10.1016/j.bbadis.2010.06.017

Armulik, A., Abramsson, A., & Betsholtz, C. (2005). Endothelial/Pericyte Interactions. *Circulation Research*, *97*(6), 512-523. https://doi.org/10.1161/01.RES.0000182903.16652.d7

Armulik, A., Genové, G., & Betsholtz, C. (2011). Pericytes: Developmental, Physiological, and Pathological Perspectives, Problems, and Promises.

Developmental Cell, *21*(2), 193-215. https://doi.org/10.1016/j.devcel.2011.07.001

Attwell, D., Mishra, A., Hall, C. N., O'Farrell, F. M., & Dalkara, T. (2016). What is a pericyte? *Journal of Cerebral Blood Flow & Metabolism*, *36*(2), 451-455. https://doi.org/10.1177/0271678X15610340

Balzano, T., Arenas, Y. M., Dadsetan, S., Forteza, J., Gil-Perotin, S., Cubas-Nuñez, L., Casanova, B., Gracià, F., Varela-Andrés, N., Montoliu, C., Llansola, M., & Felipo, V. (2020). Sustained hyperammonemia induces TNF-a IN Purkinje neurons by activating the TNFR1-NF-κB pathway. *Journal of Neuroinflammation*, *17*(1), 70. https://doi.org/10.1186/s12974-020-01746-z

Barakat, S., Turcotte, S., Demeule, M., Lachambre, M.-P., Régina, A., Baggetto, L. G., & Béliveau, R. (2008). Regulation of brain endothelial cells migration and angiogenesis by P-glycoprotein/caveolin-1 interaction. *Biochemical and Biophysical Research Communications*, *372*(3), 440-446. https://doi.org/10.1016/j.bbrc.2008.05.012

Bazzoni, F., & Beutler, B. (1996). The Tumor Necrosis Factor Ligand and Receptor Families. *New England Journal of Medicine*, *334*(26), 1717-1725. https://doi.org/10.1056/NEJM199606273342607

Bazzoni, G., & Dejana, E. (2004). Endothelial Cell-to-Cell Junctions: Molecular Organization and Role in Vascular Homeostasis. *Physiological Reviews*, *84*(3), 869-901. https://doi.org/10.1152/physrev.00035.2003

Bazzoni, G. (2011). Pathobiology of Junctional Adhesion Molecules. *Antioxidants & Redox Signaling*, *15*(5), 1221-1234. https://doi.org/10.1089/ars.2010.3867

Begley, D. J., & Brightman, M. W. (2003). Structural and functional aspects of the blood-brain barrier. En L. Prokai & K. Prokai-Tatrai (Eds.), *Peptide Transport and Delivery into the Central Nervous System* (pp. 39-78). Birkhäuser Basel. https://doi.org/10.1007/978-3-0348-8049-7_2

Bekheet, Souad. H. M., & Stahlmann, R. (2009). Disruption of gap junctional intercellular communication by antibiotic gentamicin is associated with aberrant localization of occludin, N-cadherin, connexin 43, and vimentin in SerW3 Sertoli cells in vitro. *Environmental Toxicology and Pharmacology*, *28*(2), 155-160. https://doi.org/10.1016/j.etap.2009.03.002

Bell, R. D., Winkler, E. A., Sagare, A. P., Singh, I., LaRue, B., Deane, R., & Zlokovic, B. V. (2010). Pericytes Control Key Neurovascular Functions and Neuronal Phenotype in the Adult Brain and during Brain Aging. *Neuron*, *68*(3), 409-427. https://doi.org/10.1016/j.neuron.2010.09.043

Breder, C. D., Tsujimoto, M., Terano, Y., Scott, D. W., & Saper, C. B. (1993). Distribution and characterization of tumor necrosis factor-α-like immunoreactivity in the murine central nervous system. *Journal of Comparative Neurology*, *337*(4), 543-567. https://doi.org/10.1002/cne.903370403

Bredow, S., Guha-Thakurta, N., Taishi, P., Obál, F., Jr, & Krueger, J. M. (1997). Diurnal variations of tumor necrosis factor alpha mRNA and alpha-tubulin mRNA in rat brain. *Neuroimmunomodulation*, *4*(2), 84–90. https://doi.org/10.1159/000097325

Bruzzone, R., White, T. W., & Paul, D. L. (1996). Connections with Connexins: The Molecular Basis of Direct Intercellular Signaling. *European Journal of*

Biochemistry, *238*(1), 1-27. https://doi.org/10.1111/j.1432-1033.1996.0001q.x

Cheng, G., Wei, L., Xiurong, W., Xiangzhen, L., Shiguang, Z., & Songbin, F. (2009). IL-17 Stimulates Migration of Carotid Artery Vascular Smooth Muscle Cells in an MMP-9 Dependent Manner via p38 MAPK and ERK1/2-Dependent NF-κB and AP-1 Activation. *Cellular and Molecular Neurobiology*, *29*(8), 1161-1168. https://doi.org/10.1007/s10571-009-9409-z

Cheng, Y., Desse, S., Martinez, A., Worthen, R. J., Jope, R. S., & Beurel, E. (2018). TNFα disrupts blood brain barrier integrity to maintain prolonged depressive-like behavior in mice. *Brain, Behavior, and Immunity*, *69*, 556-567. https://doi.org/10.1016/j.bbi.2018.02.003

De Maio, A., Vega, V. L., & Contreras, J. E. (2002). Gap junctions, homeostasis, and injury. *Journal of Cellular Physiology*, *191*(3), 269-282. https://doi.org/10.1002/jcp.10108

Demeule, M., Jodoin, J., Gingras, D., & Béliveau, R. (2000). P-glycoprotein is localized in caveolae in resistant cells and in brain capillaries. *FEBS Letters*, *466*(2-3), 219-224. https://doi.org/10.1016/S0014-5793(00)01087-5

Diallo, K., Simons, N., Sayegh, S., Baron, M., Degboé, Y., Boyer, J.-F., Kruglov, A., Nedospasov, S., Novarino, J., Aloulou, M., Fazilleau, N., Constantin, A., Cantagrel, A., Davignon, J.-L., & Rauwel, B. (2021). Evidence for tmTNF reverse signaling in vivo: Implications for an arginase-1-mediated therapeutic effect of TNF inhibitors during inflammation. *iScience*, *24*(4), 102331. https://doi.org/10.1016/j.isci.2021.102331

Díaz-Flores L. Jr, Gutiérrez R., Madrid J.F., Sáez F.J., Valladares F., Villar J. and Díaz-Flores L. (2011). Peg-and-socket junctions between smooth muscle cells and endothelial cells in femoral veins are stimulated to angiogenesis by prostaglandin E_2 and glycerols. Histol. Histopathol. 26, 623-630.

Díaz-Flores, L., Gutiérrez, R., Madrid, J. F., Varela, H., Valladares, F., Acosta, E., Martín-Vasallo, P., & Díaz-Flores, L. (2009). Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. *Histology and Histopathology*, *24*(7), 909-969. https://doi.org/10.14670/HH-24.909

Díaz-Flores, L., Gutiérrez, R., Varela, H., Rancel, N., & Valladares, F. (1991). Microvascular pericytes: A review of their morphological and functional characteristics. *Histology and Histopathology*, *6*(2), 269-286.

Dobrowolski, R., & Willecke, K. (2009). Connexin-Caused Genetic Diseases and Corresponding Mouse Models. *Antioxidants & Redox Signaling*, *11*(2), 283-296. https://doi.org/10.1089/ars.2008.2128

Dopp, J. M., Mackenzie-Graham, A., Otero, G. C., & Merrill, J. E. (1997). Differential expression, cytokine modulation, and specific functions of type-1 and type-2 tumor necrosis factor receptors in rat glia. *Journal of Neuroimmunology*, *75*(1-2), 104-112. https://doi.org/10.1016/S0165-5728(97)00009-X

Dwyer, D. S., Vannucci, S. J., & Simpson, I. A. (2002). Expression, regulation, and functional role of glucose transporters (GLUTs) in brain. En *International Review of Neurobiology* (Vol. 51, pp. 159-188). Elsevier. https://doi.org/10.1016/S0074-7742(02)51005-9

Eberth, C. J. (1871). Handbuch der Lehre von der Gewegen des Menschen und der Tiere. *Leipzig: W. Engelman*.

Ehrlich, P. (1885). *Das Sauerstoff-Bedürfniss des Organismus: eine farbenanalytische Studie*. A. Hirschwald.

Floyd, R. A., & Krueger, J. M. (1997). Diurnal variation of TNFα in the rat brain: *NeuroReport*, *8*(4), 915-918. https://doi.org/10.1097/00001756-199703030-00020

Fujimoto K. (1995). Pericyte-endothelial gap junctions in developing rat cerebral capillaries: a fine structural study. *The Anatomical record*, *242*(4), 562–565. https://doi.org/10.1002/ar.1092420412

Gahring, L. C., Carlson, N. G., Kulmer, R. A., & Rogers, S. W. (1996). Neuronal Expression of Tumor Necrosis Factor Alpha in the OVOUJI fme Brain. *Neuroimmunomodulation*, *3*(5), 289-303. https://doi.org/10.1159/000097283

Goldberg, G. S., Lampe, P. D., & Nicholson, B. J. (1999). Selective transfer of endogenous metabolites through gap junctions composed of different connexins. *Nature cell biology*, *1*(7), 457–459. https://doi.org/10.1038/15693

Gómez-González, B., Domínguez-Salazar, E., Hurtado-Alvarado, G., Esqueda-Leon, E., Santana-Miranda, R., Rojas-Zamorano, J. A., & Velázquez-Moctezuma, J. (2012). Role of sleep in the regulation of the immune system and the pituitary hormones. *Annals of the New York Academy of Sciences*, *1261*(1), 97-106. https://doi.org/10.1111/j.1749-6632.2012.06616.x

Gómez-González, B., Hurtado-Alvarado, G., Esqueda-Leon, E., Santana- Miranda, R., Rojas-Zamorano, J., & Velazquez-Moctezuma, J. (2013). REM Sleep Loss and Recovery Regulates Blood-Brain Barrier Function. *Current*

Neurovascular Research, *10*(3), 197-207. https://doi.org/10.2174/1567202613109990002

Gough, P., & Myles, I. A. (2020). Tumor Necrosis Factor Receptors: Pleiotropic Signaling Complexes and Their Differential Effects. *Frontiers in Immunology*, *11*, 585880. https://doi.org/10.3389/fimmu.2020.585880

Grant, R. I., Hartmann, D. A., Underly, R. G., Berthiaume, A.-A., Bhat, N. R., & Shih, A. Y. (2019). Organizational hierarchy and structural diversity of microvascular pericytes in adult mouse cortex. *Journal of Cerebral Blood Flow & Metabolism*, *39*(3), 411-425. https://doi.org/10.1177/0271678X17732229

Guo, Z., Zhu, J., Zhao, L., Luo, Q., & Jin, X. (2010). Expression and clinical significance of multidrug resistance proteins in brain tumors. *Journal of Experimental & Clinical Cancer Research*, *29*(1), 122. https://doi.org/10.1186/1756-9966-29-122

Gururaja, T. L., Yung, S., Ding, R., Huang, J., Zhou, X., McLaughlin, J., Daniel-Issakani, S., Singh, R., Cooper, R. D. G., Payan, D. G., Masuda, E. S., & Kinoshita, T. (2007). A Class of Small Molecules that Inhibit TNFα-Induced Survival and Death Pathways via Prevention of Interactions between TNFαRI, TRADD, and RIP1. *Chemistry & Biology*, *14*(10), 1105-1118. https://doi.org/10.1016/j.chembiol.2007.08.012

Guzman-Marin, R., Suntsova, N., Bashir, T., Nienhuis, R., Szymusiak, R., & McGinty, D. (2008). Rapid Eye Movement Sleep Deprivation Contributes to Reduction of Neurogenesis in the Hippocampal Dentate Gyrus of the Adult Rat. *Sleep*, *31*(2), 167-175. https://doi.org/10.1093/sleep/31.2.167

Hamel, E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. *Journal of Applied Physiology*, *100*(3), 1059-1064. https://doi.org/10.1152/japplphysiol.00954.2005

Hariharan, A., Weir, N., Robertson, C., He, L., Betsholtz, C., & Longden, T. A. (2020). The Ion Channel and GPCR Toolkit of Brain Capillary Pericytes. *Frontiers in Cellular Neuroscience*, *14*, 601324. https://doi.org/10.3389/fncel.2020.601324

Harris, A. L. (2007). Connexin channel permeability to cytoplasmic molecules. *Progress in Biophysics and Molecular Biology*, *94*(1-2), 120-143. https://doi.org/10.1016/j.pbiomolbio.2007.03.011

Hartmann, D. A., Berthiaume, A.-A., Grant, R. I., Harrill, S. A., Koski, T., Tieu, T., McDowell, K. P., Faino, A. V., Kelly, A. L., & Shih, A. Y. (2021). Brain capillary pericytes exert a substantial but slow influence on blood flow. *Nature Neuroscience*, *24*(5), 633-645. https://doi.org/10.1038/s41593-020-00793-2

Hartmann, D. A., Underly, R. G., Grant, R. I., Watson, A. N., Lindner, V., & Shih, A. Y. (2015). Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. *Neurophotonics*, *2*(4), 041402. https://doi.org/10.1117/1.NPh.2.4.041402

Hautefort, A., Pfenniger, A., & Kwak, B. R. (2019). Endothelial connexins in vascular function. *Vascular Biology*, *1*(1), H117-H124. https://doi.org/10.1530/VB-19-0015

He, J., Hsuchou, H., He, Y., Kastin, A. J., Wang, Y., & Pan, W. (2014). Sleep Restriction Impairs Blood–Brain Barrier Function. *The Journal of*

Neuroscience, *34*(44), 14697-14706. https://doi.org/10.1523/JNEUROSCI.2111-14.2014

Heldin, C.-H., & Westermark, B. (1999). Mechanism of Action and In Vivo Role of Platelet-Derived Growth Factor. *Physiological Reviews*, *79*(4), 1283-1316. https://doi.org/10.1152/physrev.1999.79.4.1283

Hu, J., Chen, Z., Gorczynski, C. P., Gorczynski, L. Y., Kai, Y., Lee, L., Manuel, J., & Gorczynski, R. M. (2003). Sleep-deprived mice show altered cytokine production manifest by perturbations in serum IL-1ra, TNFa, and IL-6 levels. *Brain, Behavior, and Immunity*, *17*(6), 498-504. https://doi.org/10.1016/j.bbi.2003.03.001

Huang, S., Dudez, T., Scerri, I., Thomas, M. A., Giepmans, B. N. G., Suter, S., & Chanson, M. (2003). Defective Activation of c-Src in Cystic Fibrosis Airway Epithelial Cells Results in Loss of Tumor Necrosis Factor-α-induced Gap Junction Regulation. *Journal of Biological Chemistry*, *278*(10), 8326-8332. https://doi.org/10.1074/jbc.M208264200

Huang, W., Ramsey, K. M., Marcheva, B., & Bass, J. (2011). Circadian rhythms, sleep, and metabolism. *Journal of Clinical Investigation*, *121*(6), 2133-2141. https://doi.org/10.1172/JCI46043

Hurtado-Alvarado, G., Becerril-Villanueva, E., Contis-Montes De Oca, A., Domínguez-Salazar, E., Salinas-Jazmín, N., Pérez-Tapia, S. M., Pavon, L., Velázquez-Moctezuma, J., & Gómez-González, B. (2018). The yin/yang of inflammatory status: Blood-brain barrier regulation during sleep. *Brain, Behavior, and Immunity*, *69*, 154-166. https://doi.org/10.1016/j.bbi.2017.11.009

Hurtado-Alvarado, G., Domínguez-Salazar, E., Pavon, L., Velázquez-Moctezuma, J., & Gómez-González, B. (2016). Blood-Brain Barrier Disruption Induced by Chronic Sleep Loss: Low-Grade Inflammation May Be the Link. *Journal of Immunology Research*, *2016*, 1-15. https://doi.org/10.1155/2016/4576012

Hurtado-Alvarado, G., Domínguez-Salazar, E., Velázquez-Moctezuma, J., & Gómez-González, B. (2016). A2A Adenosine Receptor Antagonism Reverts the Blood-Brain Barrier Dysfunction Induced by Sleep Restriction. *PLOS ONE*, *11*(11), e0167236. https://doi.org/10.1371/journal.pone.0167236

Hurtado-Alvarado, G., Velázquez-Moctezuma, J., & Gómez-González, B. (2017). Chronic sleep restriction disrupts interendothelial junctions in the hippocampus and increases blood–brain barrier permeability. *Journal of Microscopy*, *268*(1), 28-38. https://doi.org/10.1111/jmi.12583

Hyder, F., Rothman, D. L., & Bennett, M. R. (2013). Cortical energy demands of signaling and nonsignaling components in brain are conserved across mammalian species and activity levels. *Proceedings of the National Academy of Sciences*, *110*(9), 3549-3554. https://doi.org/10.1073/pnas.1214912110

Iadecola, C. (2017). The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. *Neuron*, *96*(1), 17-42. https://doi.org/10.1016/j.neuron.2017.07.030

Jodoin, J., Demeule, M., Fenart, L., Cecchelli, R., Farmer, S., Linton, K. J., Higgins, C. F., & Béliveau, R. (2003). P-glycoprotein in blood–brain barrier endothelial cells: Interaction and oligomerization with caveolins. *Journal of*

Neurochemistry, *87*(4), 1010-1023. https://doi.org/10.1046/j.1471-4159.2003.02081.x

Jones, E. G. (1970). On the mode of entry of blood vessels into the cerebral cortex. *Journal of Anatomy*, *106*(Pt 3), 507-520.

Kaya, M., & Ahishali, B. (2011). Assessment of Permeability in Barrier Type of Endothelium in Brain Using Tracers: Evans Blue, Sodium Fluorescein, and Horseradish Peroxidase. En K. Turksen (Ed.), *Permeability Barrier* (Vol. 763, pp. 369-382). Humana Press. https://doi.org/10.1007/978-1-61779-191-8_25

Kimura, K., Orita, T., Morishige, N., Nishida, T., & Sonoda, K.-H. (2013). Role of the JNK Signaling PathWay in Downregulation of Connexin43 by TNF-α in Human Corneal Fibroblasts. *Current Eye Research*, *38*(9), 926-932. https://doi.org/10.3109/02713683.2013.798419

King, M. D., Alleyne, C. H., & Dhandapani, K. M. (2013). TNF-alpha receptor antagonist, R-7050, improves neurological outcomes following intracerebral hemorrhage in mice. *Neuroscience Letters*, *542*, 92-96. https://doi.org/10.1016/j.neulet.2013.02.051

Kovacs-Oller, T., Ivanova, E., Bianchimano, P., & Sagdullaev, B. T. (2020). The pericyte connectome: Spatial precision of neurovascular coupling is driven by selective connectivity maps of pericytes and endothelial cells and is disrupted in diabetes. *Cell Discovery*, *6*(1), 39. https://doi.org/10.1038/s41421-020-0180-0

Krueger, J. M., Frank, M. G., Wisor, J. P., & Roy, S. (2016). Sleep function: Toward elucidating an enigma. *Sleep Medicine Reviews*, *28*, 46-54. https://doi.org/10.1016/j.smrv.2015.08.005

Kumar, N. M., & Gilula, N. B. (1996). The Gap Junction Communication Channel. *Cell*, *84*(3), 381-388. https://doi.org/10.1016/S0092-8674(00)81282-9

Laird, D. W. (2006). Life cycle of connexins in health and disease. *Biochemical Journal*, *394*(3), 527-543. https://doi.org/10.1042/BJ20051922

Lebrin, F., Srun, S., Raymond, K., Martin, S., Van Den Brink, S., Freitas, C., Bréant, C., Mathivet, T., Larrivée, B., Thomas, J.-L., Arthur, H. M., Westermann, C. J. J., Disch, F., Mager, J. J., Snijder, R. J., Eichmann, A., & Mummery, C. L. (2010). Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. *Nature Medicine*, *16*(4), 420-428. https://doi.org/10.1038/nm.2131

Lee, N. P., Y. (2007). Junction interaction in the seminiferous epithelium: Regulatory roles of connexin-based gap junction. *Frontiers in Bioscience*, *12*(1), 1552. https://doi.org/10.2741/2168

Levéen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E., & Betsholtz, C. (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes & development*, *8*(16), 1875–1887. https://doi.org/10.1101/gad.8.16.1875

Li, M. W. M., Mruk, D. D., & Cheng, C. Y. (2013). Gap Junctions and Blood-Tissue Barriers. En C. Y. Cheng (Ed.), *Biology and Regulation of Blood-Tissue Barriers* (Vol. 763, pp. 260-280). Springer New York. https://doi.org/10.1007/978-1-4614-4711-5_13

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., Bennett, M. L., Münch, A. E., Chung, W.-S., Peterson, T. C., Wilton, D. K., Frouin, A., Napier, B. A., Panicker, N., Kumar, M., Buckwalter,

M. S., Rowitch, D. H., Dawson, V. L., Dawson, T. M., … Barres, B. A. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*, *541*(7638), 481-487. https://doi.org/10.1038/nature21029

Liebner, S., Kniesel, U., Kalbacher, H., & Wolburg, H. (2000). Correlation of tight junction morphology with the expression of tight junction proteins in blood-brain barrier endothelial cells. *European Journal of Cell Biology*, *79*(10), 707-717. https://doi.org/10.1078/0171-9335-00101

Lin, S.-Y., Wang, Y.-Y., Chang, C.-Y., Wu, C.-C., Chen, W.-Y., Liao, S.-L., & Chen, C.-J. (2021). TNF-α Receptor Inhibitor Alleviates Metabolic and Inflammatory Changes in a Rat Model of Ischemic Stroke. *Antioxidants*, *10*(6), 851. https://doi.org/10.3390/antiox10060851

Madge, L. A., & Pober, J. S. (2001). TNF Signaling in Vascular Endothelial Cells. *Experimental and Molecular Pathology*, *70*(3), 317-325. https://doi.org/10.1006/exmp.2001.2368

Mäe, M. A., He, L., Nordling, S., Vazquez-Liebanas, E., Nahar, K., Jung, B., Li, X., Tan, B. C., Chin Foo, J., Cazenave-Gassiot, A., Wenk, M. R., Zarb, Y., Lavina, B., Quaggin, S. E., Jeansson, M., Gu, C., Silver, D. L., Vanlandewijck, M., Butcher, E. C., … Betsholtz, C. (2021). Single-Cell Analysis of Blood-Brain Barrier Response to Pericyte Loss. *Circulation Research*, *128*(4). https://doi.org/10.1161/CIRCRESAHA.120.317473

Medic, G., Wille, M., & Hemels, M. (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, *Volume 9*, 151-161. https://doi.org/10.2147/NSS.S134864

Medina-Flores, F., Hurtado-Alvarado, G., Contis-Montes De Oca, A., López-Cervantes, S. P., Konigsberg, M., Deli, M. A., & Gómez-González, B. (2020). Sleep loss disrupts pericyte-brain endothelial cell interactions impairing blood-brain barrier function. *Brain, Behavior, and Immunity*, *89*, 118-132. https://doi.org/10.1016/j.bbi.2020.05.077

Medina-Flores, F., Hurtado-Alvarado, G., Deli, M. A., & Gómez-González, B. (2023). The Active Role of Pericytes During Neuroinflammation in the Adult Brain. *Cellular and Molecular Neurobiology*, *43*(2), 525-541. https://doi.org/10.1007/s10571-022-01208-5

Medina-Flores, F., Hurtado-Alvarado, G., & Gómez-González, B. (2020). Selective Regional Isolation of Brain Microvessels. En K. Turksen (Ed.), *Permeability Barrier* (Vol. 2367, pp. 37-46). Springer US. https://doi.org/10.1007/7651_2020_313

Meşe, G., Richard, G., & White, T. W. (2007). Gap Junctions: Basic Structure and Function. *Journal of Investigative Dermatology*, *127*(11), 2516-2524. https://doi.org/10.1038/sj.jid.5700770

Mohammed, H. S., Aboul Ezz, H. S., Khadrawy, Y. A., & Noor, N. A. (2011). Neurochemical and electrophysiological changes induced by paradoxical sleep deprivation in rats. *Behavioural Brain Research*, *225*(1), 39-46. https://doi.org/10.1016/j.bbr.2011.06.018

Moon, S., Cha, B., & Kim, C. (2004). ERK1/2 mediates TNF-α-induced matrix metalloproteinase-9 expression in human vascular smooth muscle cells via the regulation of NF-κB and AP-1: Involvement of the ras dependent

pathway. *Journal of Cellular Physiology*, *198*(3), 417-427. https://doi.org/10.1002/jcp.10435

Mori, S., Heldin, C. H., & Claesson-Welsh, L. (1993). Ligand-induced ubiquitination of the platelet-derived growth factor beta-receptor plays a negative regulatory role in its mitogenic signaling. *Journal of Biological Chemistry*, *268*(1), 577-583. https://doi.org/10.1016/S0021-9258(18)54190-9

Muhammad, M. (2020). Tumor Necrosis Factor Alpha: A Major Cytokine of Brain Neuroinflammation. En P. Behzadi (Ed.), *Cytokines*. IntechOpen. https://doi.org/10.5772/intechopen.85476

Nadarajah, B., Jones, A. M., Evans, W. H., & Parnavelas, J. G. (1997). Differential Expression of Connexins during Neocortical Development and Neuronal Circuit Formation. *The Journal of Neuroscience*, *17*(9), 3096-3111. https://doi.org/10.1523/JNEUROSCI.17-09-03096.1997

Nagasawa, K., Chiba, H., Fujita, H., Kojima, T., Saito, T., Endo, T., & Sawada, N. (2006). Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. *Journal of Cellular Physiology*, *208*(1), 123-132. https://doi.org/10.1002/jcp.20647

Nakagawa, S., Deli, M. A., Kawaguchi, H., Shimizudani, T., Shimono, T., Kittel, Á., Tanaka, K., & Niwa, M. (2009). A new blood–brain barrier model using primary rat brain endothelial cells, pericytes and astrocytes. *Neurochemistry International*, *54*(3-4), 253-263. https://doi.org/10.1016/j.neuint.2008.12.002

Nishimura, N., Schaffer, C. B., Friedman, B., Lyden, P. D., & Kleinfeld, D. (2007). Penetrating arterioles are a bottleneck in the perfusion of neocortex.

Proceedings of the National Academy of Sciences, *104*(1), 365-370. https://doi.org/10.1073/pnas.0609551104

Novati, A., Hulshof, H. J., Koolhaas, J. M., Lucassen, P. J., & Meerlo, P. (2011). Chronic sleep restriction causes a decrease in hippocampal volume in adolescent rats, which is not explained by changes in glucocorticoid levels or neurogenesis. *Neuroscience*, *190*, 145-155. https://doi.org/10.1016/j.neuroscience.2011.06.027

Olk, S., Zoidl, G., & Dermietzel, R. (2009). Connexins, cell motility, and the cytoskeleton. *Cell Motility*, *66*(11), 1000-1016. https://doi.org/10.1002/cm.20404

Ornelas, S., Berthiaume, A.-A., Bonney, S. K., Coelho-Santos, V., Underly, R. G., Kremer, A., Guérin, C. J., Lippens, S., & Shih, A. Y. (2021). Three-dimensional ultrastructure of the brain pericyte-endothelial interface. *Journal of Cerebral Blood Flow & Metabolism*, *41*(9), 2185-2200. https://doi.org/10.1177/0271678X211012836

Pardridge, W. M. (1988). Recent Advances in Blood-Brain Barrier Transport. *Annual Review of Pharmacology and Toxicology*, *28*(1), 25-39. https://doi.org/10.1146/annurev.pa.28.040188.000325

Petty, M. A., & Lo, E. H. (2002). Junctional complexes of the blood–brain barrier: Permeability changes in neuroinflammation. *Progress in Neurobiology*, *68*(5), 311-323. https://doi.org/10.1016/S0301-0082(02)00128-4

Porkka-Heiskanen, T., Zitting, K. -M., & Wigren, H. -K. (2013). Sleep, its regulation and possible mechanisms of sleep disturbances. *Acta Physiologica*, *208*(4), 311-328. https://doi.org/10.1111/apha.12134

Potter, G. D. M., Skene, D. J., Arendt, J., Cade, J. E., Grant, P. J., & Hardie, L. J. (2016). Circadian Rhythm and Sleep Disruption: Causes, Metabolic Consequences, and Countermeasures. *Endocrine Reviews*, *37*(6), 584-608. https://doi.org/10.1210/er.2016-1083

Raffaele, S., Lombardi, M., Verderio, C., & Fumagalli, M. (2020). TNF Production and Release from Microglia via Extracellular Vesicles: Impact on Brain Functions. *Cells*, *9*(10), 2145. https://doi.org/10.3390/cells9102145

Ranta, V., Orpana, A., Carpén, O., Turpeinen, U., Ylikorkala, O., & Viinikka, L. (1999). Human vascular endothelial cells produce tumor necrosis factor-alpha in response to proinflammatory cytokine stimulation. *Critical care medicine*, *27*(10), 2184–2187. https://doi.org/10.1097/00003246-199910000-00019

Rasch, B., & Born, J. (2013). About Sleep's Role in Memory. *Physiological Reviews*, *93*(2), 681-766. https://doi.org/10.1152/physrev.00032.2012

Rechtschaffen, A., Bergmann, B. M., Everson, C. A., Kushida, C. A., & Carol, A. E. (1989). Sleep Deprivation in the Rat: I. Conceptual Issues. *Sleep*, *12*(1), 1-4. https://doi.org/10.1093/sleep/12.1.1

Robertson, R. T., Levine, S. T., Haynes, S. M., Gutierrez, P., Baratta, J. L., Tan, Z., & Longmuir, K. J. (2015). Use of labeled tomato lectin for imaging vasculature structures. *Histochemistry and cell biology*, *143*(2), 225–234. https://doi.org/10.1007/s00418-014-1301-3

Rouget C. (1873). Memoire sur le developpement, la structures et les proprieties des capillaires sanguins et lymphatiques. Archs Physiol Norm Pathol, 5:603-633.

Roux, F., & Couraud, P.-O. (2005). Rat Brain Endothelial Cell Lines for the Study of Blood–Brain Barrier Permeability and Transport Functions. *Cellular and Molecular Neurobiology*, *25*(1), 41-57. https://doi.org/10.1007/s10571-004-1376-9

Roy, C. S., & Sherrington, C. S. (1890). On the Regulation of the Blood-supply of the Brain. *The Journal of Physiology*, *11*(1-2), 85-158. https://doi.org/10.1113/jphysiol.1890.sp000321

Rustenhoven, J., Jansson, D., Smyth, L. C., & Dragunow, M. (2017). Brain Pericytes As Mediators of Neuroinflammation. *Trends in Pharmacological Sciences*, *38*(3), 291-304. https://doi.org/10.1016/j.tips.2016.12.001

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep State Switching. *Neuron*, *68*(6), 1023-1042. https://doi.org/10.1016/j.neuron.2010.11.032

Schinkel, A. H. (1999). P-Glycoprotein, a gatekeeper in the blood–brain barrier. *Advanced Drug Delivery Reviews*, *36*(2-3), 179-194. https://doi.org/10.1016/S0169-409X(98)00085-4

Sharma, A., Muresanu, D. F., Lafuente, J. V., Patnaik, R., Tian, Z. R., Buzoianu, A. D., & Sharma, H. S. (2015). Sleep Deprivation-Induced Blood-Brain Barrier Breakdown and Brain Dysfunction are Exacerbated by Size-Related Exposure to Ag and Cu Nanoparticles. Neuroprotective Effects of a 5-HT3 Receptor Antagonist Ondansetron. *Molecular Neurobiology*, *52*(2), 867-881. https://doi.org/10.1007/s12035-015-9236-9

Shepro, D., & Morel, N. M. L. (1993). Pericyte physiology. *The FASEB Journal*, *7*(11), 1031-1038. https://doi.org/10.1096/fasebj.7.11.8370472

Smith, M. W., & Gumbleton, M. (2006). Endocytosis at the blood–brain barrier: From basic understanding to drug delivery strategies. *Journal of Drug Targeting*, *14*(4), 191-214. https://doi.org/10.1080/10611860600650086

Sorgen, P. L., Duffy, H. S., Sahoo, P., Coombs, W., Delmar, M., & Spray, D. C. (2004). Structural Changes in the Carboxyl Terminus of the Gap Junction Protein Connexin43 Indicates Signaling between Binding Domains for c-Src and Zonula Occludens-1. *Journal of Biological Chemistry*, *279*(52), 54695-54701. https://doi.org/10.1074/jbc.M409552200

Staunton, H. (2005). Mammalian sleep. *Naturwissenschaften*, *92*(5), 203-220. https://doi.org/10.1007/s00114-005-0618-0

Sweeney, M. D., Kisler, K., Montagne, A., Toga, A. W., & Zlokovic, B. V. (2018). The role of brain vasculature in neurodegenerative disorders. *Nature Neuroscience*, *21*(10), 1318-1331. https://doi.org/10.1038/s41593-018-0234-x

Sweeney, M. D., Zhao, Z., Montagne, A., Nelson, A. R., & Zlokovic, B. V. (2019). Blood-Brain Barrier: From Physiology to Disease and Back. *Physiological Reviews*, *99*(1), 21-78. https://doi.org/10.1152/physrev.00050.2017

Taishi, P., Bredow, S., Guha-Thakurta, N., Obál, F., & Krueger, J. M. (1997). Diurnal variations of interleukin-1β mRNA and β-actin mRNA in rat brain. *Journal of Neuroimmunology*, *75*(1-2), 69-74. https://doi.org/10.1016/S0165-5728(97)00002-7

Taishi, P., Gardi, J., Chen, Z., Fang, J., & Krueger, J. M. (1999). Sleep deprivation increases the expression of TNF alpha mRNA and TNF 55kD receptor mRNA in rat brain. *The Physiologist*, *42*, A4.

Takata, F., Dohgu, S., Matsumoto, J., Takahashi, H., Machida, T., Wakigawa, T., Harada, E., Miyaji, H., Koga, M., Nishioku, T., Yamauchi, A., & Kataoka, Y. (2011). Brain pericytes among cells constituting the blood-brain barrier are highly sensitive to tumor necrosis factor-α, releasing matrix metalloproteinase-9 and migrating in vitro. *Journal of Neuroinflammation*, *8*(1), 106. https://doi.org/10.1186/1742-2094-8-106

Tartaglia, L. A., & Goeddel, D. V. (1992). Two TNF receptors. *Immunology Today*, *13*(5), 151-153. https://doi.org/10.1016/0167-5699(92)90116-O

Thanabalasundaram, G., Pieper, C., Lischper, M., & Galla, H.-J. (2010). Regulation of the blood–brain barrier integrity by pericytes via matrix metalloproteinases mediated activation of vascular endothelial growth factor in vitro. *Brain Research*, *1347*, 1-10. https://doi.org/10.1016/j.brainres.2010.05.096

Tien, T., Barrette, K. F., Chronopoulos, A., & Roy, S. (2013). Effects of High Glucose-Induced Cx43 Downregulation on Occludin and ZO-1 Expression and Tight Junction Barrier Function in Retinal Endothelial Cells. *Investigative Opthalmology & Visual Science*, *54*(10), 6518. https://doi.org/10.1167/iovs.13-11763

Tobaldini, E., Costantino, G., Solbiati, M., Cogliati, C., Kara, T., Nobili, L., & Montano, N. (2017). Sleep, sleep deprivation, autonomic nervous system and cardiovascular diseases. *Neuroscience & Biobehavioral Reviews*, *74*, 321-329. https://doi.org/10.1016/j.neubiorev.2016.07.004

Underly, R. G., Levy, M., Hartmann, D. A., Grant, R. I., Watson, A. N., & Shih, A. Y. (2017). Pericytes as Inducers of Rapid, Matrix Metalloproteinase-9-

Dependent Capillary Damage during Ischemia. *The Journal of Neuroscience*, *37*(1), 129-140. https://doi.org/10.1523/JNEUROSCI.2891-16.2016

Urich, E., Lazic, S. E., Molnos, J., Wells, I., & Freskgård, P.-O. (2012). Transcriptional Profiling of Human Brain Endothelial Cells Reveals Key Properties Crucial for Predictive In Vitro Blood-Brain Barrier Models. *PLoS ONE*, *7*(5), e38149. https://doi.org/10.1371/journal.pone.0038149

Valiunas, V., Polosina, Y. Y., Miller, H., Potapova, I. A., Valiuniene, L., Doronin, S., Mathias, R. T., Robinson, R. B., Rosen, M. R., Cohen, I. S., & Brink, P. R. (2005). Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. *The Journal of physiology*, *568*(Pt 2), 459–468. https://doi.org/10.1113/jphysiol.2005.090985

Vandenhaute, E., Dehouck, L., Boucau, M.-C., Sevin, E., Uzbekov, R., Tardivel, M., Gosselet, F., Fenart, L., Cecchelli, R., & Dehouck, M.-P. (2011). Modelling the Neurovascular Unit and the Blood-Brain Barrier with the Unique Function of Pericytes. *Current Neurovascular Research*, *8*(4), 258-269. https://doi.org/10.2174/156720211798121016

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Del Gaudio, F., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., Sun, Y., Raschperger, E., Räsänen, M., Zarb, Y., Mochizuki, N., Keller, A., Lendahl, U., & Betsholtz, C. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature*, *554*(7693), 475-480. https://doi.org/10.1038/nature25739

Villaseñor, R., Kuennecke, B., Ozmen, L., Ammann, M., Kugler, C., Grüninger, F., Loetscher, H., Freskgård, P.-O., & Collin, L. (2017). Region-specific permeability of the blood–brain barrier upon pericyte loss. *Journal of Cerebral*

Blood Flow & Metabolism, *37*(12), 3683-3694. https://doi.org/10.1177/0271678X17697340

Voirin, A.-C., Perek, N., & Roche, F. (2020). Inflammatory stress induced by a combination of cytokines (IL-6, IL-17, TNF-α) leads to a loss of integrity on bEnd.3 endothelial cells in vitro BBB model. *Brain Research*, *1730*, 146647. https://doi.org/10.1016/j.brainres.2020.146647

Vorbrodt, A. W., & Dobrogowska, D. H. (2003). Molecular anatomy of intercellular junctions in brain endothelial and epithelial barriers: Electron microscopist's view. *Brain Research Reviews*, *42*(3), 221-242. https://doi.org/10.1016/S0165-0173(03)00177-2

Walker, M. (2017). Why we sleep: The new science of sleep and dreams. Penguin UK.

Wehr, T. A. (1992). In short photoperiods, human sleep is biphasic. *Journal of Sleep Research*, *1*(2), 103-107. https://doi.org/10.1111/j.1365-2869.1992.tb00019.x

Winkler, E. A., Bell, R. D., & Zlokovic, B. V. (2011a). Central nervous system pericytes in health and disease. *Nature Neuroscience*, *14*(11), 1398-1405. https://doi.org/10.1038/nn.2946

Winkler, E. A., Bell, R. D., & Zlokovic, B. V. (2011b). Central nervous system pericytes in health and disease. *Nature Neuroscience*, *14*(11), 1398-1405. https://doi.org/10.1038/nn.2946

Winkler, E. A., Sengillo, J. D., Bell, R. D., Wang, J., & Zlokovic, B. V. (2012). Blood–Spinal Cord Barrier Pericyte Reductions Contribute to Increased Capillary

Permeability. *Journal of Cerebral Blood Flow & Metabolism*, *32*(10), 1841-1852. https://doi.org/10.1038/jcbfm.2012.113

Wolburg, H., Neuhaus, J., Kniesel, U., Krauß, B., Schmid, E.-M., Öcalan, M., Farrell, C., & Risau, W. (1994). Modulation of tight junction structure in blood-brain barrier endothelial cells Effects of tissue culture, second messengers and cocultured astrocytes. *Journal of Cell Science*, *107*(5), 1347-1357. https://doi.org/10.1242/jcs.107.5.1347

World Health Organization. Regional Office for Europe. (2004). WHO technical meeting on sleep and health: Bonn Germany, 22–24 January 2004. World Health Organization. Regional Office for Europe. https://iris.who.int/handle/10665/349782

Wu, X., Huang, W., Luo, G., & Alain, L. A. (2013). Hypoxia induces connexin 43 dysregulation by modulating matrix metalloproteinases via MAPK signaling. *Molecular and Cellular Biochemistry*, *384*(1-2), 155-162. https://doi.org/10.1007/s11010-013-1793-5

Wu, Y., Li, P., Bhat, N., Fan, H., & Liu, M. (2023). Effects of repeated sleep deprivation on brain pericytes in mice. *Scientific Reports*, *13*(1), 12760. https://doi.org/10.1038/s41598-023-40138-0

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., Iliff, J. J., Takano, T., Deane, R., & Nedergaard, M. (2013). Sleep Drives Metabolite Clearance from the Adult Brain. *Science*, *342*(6156), 373-377. https://doi.org/10.1126/science.1241224

Yang, G.-Y., Gong, C., Qin, Z., Liu, X.-H., & Lorris Betz, A. (1999). Tumor necrosis factor alpha expression produces increased blood–brain barrier permeability following temporary focal cerebral ischemia in mice. *Molecular Brain Research*, *69*(1), 135-143. https://doi.org/10.1016/S0169-328X(99)00007-8

Yehuda, S., Sredni, B., Carasso, R. L., & Kenigsbuch-Sredni, D. (2009). REM Sleep Deprivation in Rats Results in Inflammation and Interleukin-17 Elevation. *Journal of Interferon & Cytokine Research*, *29*(7), 393-398. https://doi.org/10.1089/jir.2008.0080

Zielinski, M. R., Kim, Y., Karpova, S. A., McCarley, R. W., Strecker, R. E., & Gerashchenko, D. (2014). Chronic sleep restriction elevates brain interleukin-1 beta and tumor necrosis factor-alpha and attenuates brain-derived neurotrophic factor expression. *Neuroscience Letters*, *580*, 27-31. https://doi.org/10.1016/j.neulet.2014.07.043

Zielinski, M. R., McKenna, J. T., & McCarley, R. W. (2016). Functions and Mechanisms of Sleep. *AIMS Neuroscience*, *3*(1), 67-104. https://doi.org/10.3934/Neuroscience.2016.1.67

Zimmermann, K. W. (1923). Der feinere bau der blutcapillaren. *Zeitschrift für Anatomie und Entwicklungsgeschichte*, *68*, 29-109.

13. APPENDICES

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Sleep loss disrupts pericyte-brain endothelial cell interactions impairing blood-brain barrier function

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ABSTRACT

Sleep loss in the rat increases blood-brain barrier permeability to circulating molecules by disrupting inter-endothelial tight junctions. Despite the description of the ultrastructure of cerebral microvessels and the evidence of an apparent pericyte detachment from capillary wall in sleep restricted rats the effect of sleep loss on pericytes is unknown. Here we characterized the interactions between pericytes and brain endothelial cells after sleep loss using male Wistar rats. Animals were sleep-restricted 20 h daily with 4 h sleep recovery for 10 days. At the end of the sleep restriction, brain microvessels (MVs) were isolated from cerebral cortex and hippocampus and processed for Western blot and immunocytochemistry to evaluate markers of pericyte-endothelial cell interaction (connexin 43, PDGFR-β), tight junction proteins, and proinflammatory mediator proteins (MMP9, A_{2A} adenosine receptor, CD73, NFκB). Sleep restriction reduced PDGFR-β and connexin 43 expression in MVs; in addition, scanning electron microscopy micrographs showed that pericytes were detached from capillary walls, but did not undergo apoptosis (as depicted by a reduced active caspase-3 expression). Sleep restriction also decreased tight junction protein expression in MVs and increased BBB permeability to low- and high-molecular weight tracers in *in vivo* permeability assays. Those alterations seemed to depend on a low-grade inflammatory status as reflected by the increased expression of phosphorylated NFκB and A_{2A} adenosine receptor in brain endothelial cells from the sleep-restricted rats. Our data show that pericyte-brain endothelial cell interaction is altered by sleep restriction; this evidence is essential to understand the role of sleep in regulating blood-brain barrier function.

1. Introduction

Pericytes are perivascular cells that surround the endothelium and contribute to blood-brain barrier stabilization through the induction of endothelial tight junctions and establishment of gap junctions with brain endothelial cells (Fujimoto, 1995; Armulik et al., 2010). Pericytes regulate capillary diameter, cerebral blood-flow and control the entry of immune cells to the central nervous system during angiogenesis and blood-brain barrier maturation (Armulik et al., 2005; Shimizu et al., 2008; Stark et al., 2013; Hall et al., 2014; Neuhaus et al., 2017). Reduced pericyte coverage induces microvascular defects (Benjamin et al., 1998; Tarallo et al., 2012) and blood-brain barrier dysfunction (Vates et al., 2010; Bell et al., 2010; Rustenhoven et al., 2017).

The platelet-derived growth factor receptor-β (PDGFR-β) is expressed predominantly in pericytes (Armulik et al., 2005) and its endothelium-secreted ligand, PDGF-B, has been proposed as the signaling molecule that modulates pericyte-endothelial cell crosstalk (Lebrin et al., 2010; Armulik et al., 2011). Animal models with impaired PDGFB-PDGFR-β signaling pathway present brain microvascular dysfunction (Villaseñor et al., 2017; Arango-Lievano et al., 2018) and perinatal death (Leveen et al., 1994). In addition, monocultures of endothelial cells present lower transendothelial electrical resistance (TEER) and higher permeability to low- and large-molecular weight tracers than co-cultures of brain endothelial cells and pericytes

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REVIEW PAPER

The Active Role of Pericytes During Neuroinflammation in the Adult Brain

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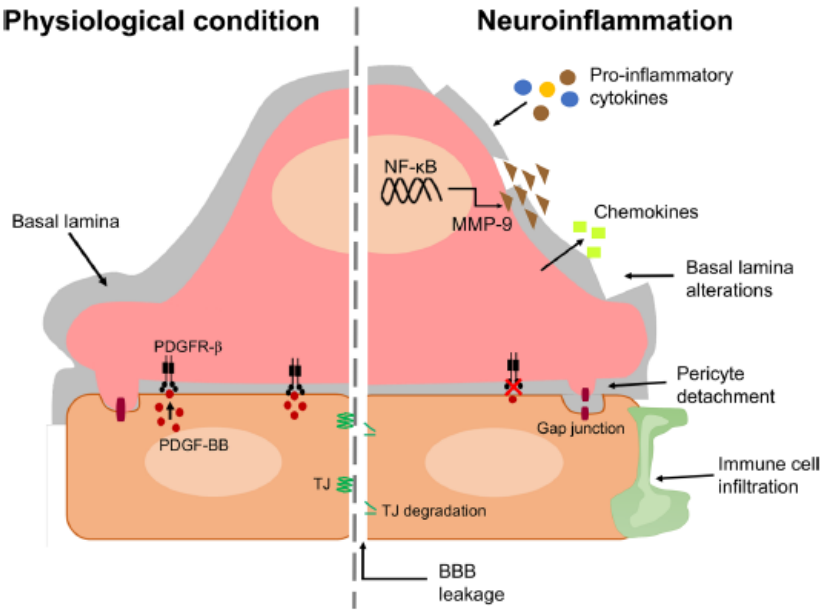
Abstract

Microvessels in the central nervous system (CNS) have one of the highest populations of pericytes, indicating their crucial role in maintaining homeostasis. Pericytes are heterogeneous cells located around brain microvessels; they present three different morphologies along the CNS vascular tree: ensheathing, mesh, and thin-strand pericytes. At the arteriole–capillary transition ensheathing pericytes are found, while mesh and thin-strand pericytes are located at capillary beds. Brain pericytes are essential for the establishment and maintenance of the blood–brain barrier, which restricts the passage of soluble and potentially toxic molecules from the circulatory system to the brain parenchyma. Pericytes play a key role in regulating local inflammation at the CNS. Pericytes can respond differentially, depending on the degree of inflammation, by secreting a set of neurotrophic factors to promote cell survival and regeneration, or by potentiating inflammation through the release of inflammatory mediators (e.g., cytokines and chemokines), and the overexpression of cell adhesion molecules. Under inflammatory conditions, pericytes may regulate immune cell trafficking to the CNS and play a role in perpetuating local inflammation. In this review, we describe pericyte responses during acute and chronic neuroinflammation.

Graphical Abstract



Extended author information available on the last page of the article

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Selective Regional Isolation of Brain Microvessels

Fernanda Medina-Flores, Gabriela Hurtado-Alvarado, and Beatriz Gómez-González

Abstract

The study of the regionalized function of the blood-brain barrier at the level of brain endothelial cells and pericytes is essential to understand the biological properties and molecular mechanisms regulating this biological barrier. The isolation of blood vessels from specific brain regions will allow to understand regional differences in susceptibility to pathological phenomena such as ischemia, traumatic brain injury, and neurodegenerative diseases, such as Alzheimer disease. Here, we propose an efficient and fast method to isolate brain endothelial cells and pericytes from a specific cerebral region. The isolated brain endothelial cells and pericytes are viable to perform conventional molecular and histological techniques such as Western blots, immunocytofluorescence, and scanning electron microscopy.

Keywords Blood-brain barrier, Brain endothelial cells, Brain microvessel isolation, Immunocytofluorescence, Pericytes, Western blot

1 Introduction

The blood-brain barrier selectively regulates the passage of soluble and potentially toxic molecules from the capillary lumen into the brain parenchyma [1, 2]. Those barrier properties depend on endothelial cells, which interact intimately with pericytes [3–5]. Brain endothelial cells and pericytes are crucial for the establishment and maintenance of the blood-brain barrier; thus, it is important to study their molecular profile and morphology under physiological and pathological conditions [6–8]. The study of each cellular type and their interactions will improve the understanding of the molecular mechanisms that modulate blood-brain barrier function at the local level. Previous studies show that the blood-brain barrier permeability to circulating molecules is heterogeneous among cerebral regions, according to their metabolic demand, the local release of inflammatory mediators, and the regional differences in astroglial and microglial density [9–12]. However, the study of the regionalized function of the blood-brain barrier at the level of brain endothelial cells and pericytes is currently infrequent. Here, we describe an easy method to efficiently isolate blood microvessels that contain endothelial cells

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Characterization of cellular and molecular changes in brain pericytes during sleep restriction

En la Ciudad de México, se presentaron a las 13:00 horas del día 29 del mes de enero del año 2024 en la Unidad Iztapalapa de la Universidad Autónoma Metropolitana, los suscritos miembros del jurado:

DR. JAVIER VELAZQUEZ MOCTEZUMA
DRA. MINA KONIGSBERG FAINSTEIN
DRA. JANET MURBARTIAN AGUILAR
DRA. ANAHI CHAVARRIA KRAUSER

Bajo la Presidencia del primero y con carácter de Secretaria la última, se reunieron a la presentación de la Disertación Pública cuya denominación aparece al margen, para la obtención del grado de:

DOCTORA EN BIOLOGÍA EXPERIMENTAL

DE: MARIA FERNANDA MEDINA FLORES

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APROBAR

Acto continuo, el presidente del jurado comunicó a la interesada el resultado de la evaluación y, en caso aprobatorio, le fue tomada la protesta.

Ma. Fernanda M.F.

MARIA FERNANDA MEDINA FLORES
ALUMNA

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