Microglial-Neurovascular Interactions and Neuronal Function at High Altitude

by

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ABSTRACT

Microglial-Neurovascular Interactions and Neuronal Function at High Altitude

Kathleen Anne Guzek Whiting, PhD, 2022

Thesis directed by: Dr. Zygmunt Galdzicki, Professor, Department of Anatomy, Physiology and Genetics, Neuroscience, Molecular and Cell Biology

Millions of people worldwide live at high altitude without evolutionary adaptation, and millions more travel to and stay at high altitudes for work or recreational activities that result in chronic exposure to this austere environment. Chronic high altitude exposure is characterized by hypobaric hypoxia, where a low pressure low oxygen environment reduces the availability of oxygen for respiration and distribution throughout the body. Compromised tissue oxygenation has long lasting effects even after return to normoxic conditions, causing a range of physiological disturbances including cognitive dysfunction. During the acute stages of exposure, the body and the central and peripheral nervous systems undergo short term acclimatization processes relying on increased heart rate, respiration and vasodilation to improve oxygen delivery.

Prolonged stays at high altitude are associated with enhanced brain angiogenesis, blood-brain barrier disruption, and altered metabolic profiles in the brain and periphery, as well as presence of inflammatory markers leading to memory deficits and cognitive decline. Previous preclinical research in the laboratory identified increased phagocytic

activity of microglia in mice after chronic 12 week simulated high altitude exposure, as well as an increase in brain vasculature and evidence of blood-brain barrier leakage. There was also an effect of high altitude on brain glucose metabolism, altered hippocampal transcriptional profiles, and behavioral deficits relating to hippocampal mediated learning and memory. Functional impairments following shorter (3 week) high altitude exposure are hypothesized to be influenced by region-specific neurovascular and inflammatory interactions, and the studies presented here explore those connections.

It is hypothesized that high altitude exposure induces angiogenesis in the brain and disruption of the blood-brain barrier, leading to an altered microenvironment that negatively impacts microglial and neuronal activity. The first part of this dissertation focuses on assessing brain angiogenesis through structural quantification of brain vasculature using high resolution micro-CT imaging, identifying increased whole brain vascular volume and branching. Experiments investigate further the interaction of vasculature and inflammatory response by assessing loss of blood-brain barrier integrity as evidenced by the increased presence of blood components in the extracellular space following chronic high altitude exposure. This leakage demonstrates a compromised blood-brain barrier, leading to inflammation, which may be influenced in part by the activity of microglia. Microglia are known to play a role in maintaining neurovascular integrity. Depletion of microglia to assess their role in blood-brain barrier maintenance revealed increased leakage consistent with existing findings. Remodeling of the neurovasculature after high altitude exposure may increase the magnitude of blood-brain barrier disruption, contributing to a pro-inflammatory microenvironment which may negatively impact microglia activity. 2-photon *ex vivo* imaging of CX3CR1-GFP$^{+/-}$ mice

(a transgenic mouse line expressing GFP in microglia) exposed to high altitude reveals sex and region-specific changes in microglia chemotactic activity in response to vascular and microglial ablation, indicating different mechanisms relating to process tip speed and proliferation play a role in the brain's adaptation to high altitude. These results indicate that high altitude causes an increase in the density of the neurovasculature and leads to blood-brain barrier disruption, creating a unique microenvironment that contributes to the activation of microglia and their response profiles. Alterations in microglia activity may have far-reaching effects on the stability of neuronal circuitry, contributing to functional deficits after high altitude exposure.

It is also hypothesized that cognitive deficits arising from vascular dysfunction are mediated by microglia and their interactions with neuronal circuitry. The second part of this dissertation explores how these changes in vascular and microglial function following 3 week high altitude exposure may influence and contribute to memory deficits. Attenuation of synaptic plasticity as measured by decreased capacity to elicit long-term potentiation in the CA1 hippocampus is identified in male mice after 3 weeks at high altitude; however, the same effect is not identified in females. The presence of microglia may be an important factor in long-term potentiation, since microglia are known to interact with synapses. To understand the signaling cascades that may be involved in long-term acclimatization and potentially play a role in altered microglial and neuronal activity, markers of inflammation, angiogenesis and metabolism are assessed. Region specific changes in brain protein cytokines are identified, indicating differing mechanisms of acclimatization or unique regional characteristics influence cytokine signaling. Since the brain experiences high levels of oxygen and glucose consumption,

glucose metabolism is an important component of normal brain function and may be indicative of hypoxic stress and its effect on cognitive functioning after high altitude exposure. Peripheral glucose levels decrease after high altitude exposure, as well as following microglia depletion, further supporting the role of microglia in regulating glucose homeostasis in the brain and with possible implications for susceptibility to hypoxic stress and inflammation. Future studies will investigate how this disruption of the primary energy source for neurons may impact functional outcomes. The role of microglia in modulating this effect is complex, and additional studies are necessary to fully understand how sex specific mechanisms and microglia function contribute to the degradation of neural circuitry in the inflammatory environment induced by high altitude.

Current available treatments to mitigate the effects of high altitude exposure are only effective in addressing acute symptoms relating to cardiovascular and pulmonary stress. The current studies show that functional deficits associated with long term hypobaric hypoxia involve the interaction of microglia and neurovascular components, in a region and sex specific manner. Future research should investigate how this may inform the development of therapeutics to improve cognitive outcomes after chronic exposure and to address reversibility.

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LIST OF ABBREVIATIONS

aCSF – artificial cerebrospinal fluid
ATP – adenosine triphosphate
BBB – blood-brain barrier
CNS – central nervous system
CT – computed tomography
CXCL-12 - c-x-c motif chemokine ligand 12
CXCR-4 - c-x-c chemokine receptor 4
CXCR-7 - c-x-c chemokine receptor 7
Flt-1 - vascular endothelial growth factor receptor 1
Fn1 - fibronectin-1
HA – high altitude
HIF-1α – hypoxia inducible factor 1 alpha
IFN-γ - interferon gamma
IL-10 - interleukin 10
IL-12p70 - interleukin 12p70
IL-15 - interleukin 15
IL-17A/F - interleukin 17A/F
IL-1β - interleukin 1 beta
IL-2 - interleukin
IL-27p28 - interleukin 27p28
IL-30 - interleukin 30
IL-33 - interleukin 33
IL-4 - interleukin 4
IL-5 - interleukin 5
IL-6 - interleukin
IL-9 - interleukin 9
IP-10 - interferon gamma-induced protein 10
KC/GRO - keratinocyte chemoattractant / human growth-regulated oncogene
LTD – long-term depression
LTP – long-term potentiation
MCP-1 - monocyte chemoattractant protein-1
MIP-1α - macrophage inflammatory protein-1 alpha
MIP-2 - macrophage inflammatory protein-2
P2RY12 – purinergic receptor P2RY12
PET – positron emission tomography
SDF-1α - stromal cell derived factor-1 alpha
SL – sea level
TNF-α - tumour necrosis factor alpha
VEGF – vascular endothelial growth factor
Vtn - vitronectin
Vwf - von Willebrand factor

CHAPTER 1: Introduction

High altitude is characterized by hypobaric hypoxia, reducing oxygen availability for blood and tissue absorption with serious implications for cardiovascular, metabolic and cognitive functioning (23; 54; 134; 135; 161). Substantial research has characterized the physiological role of hypoxia in response to high altitude but the effects on the brain remain to be elucidated (100).

Clinical research has revealed significant changes in cardiovascular and brain glucose metabolism, and persistent cognitive deficits have also been identified in climbers after high altitude exposure (54; 134; 135); (161). Short term high altitude exposure can cause long-term neurophysiological impairments including diminished manual dexterity and hand-eye coordination and evidence suggests individuals living at high altitude suffer from marked deficits in memory functioning and general cognition (380).

Previous investigations in mouse models have demonstrated neurological changes following chronic high altitude exposure, including alterations in myelination, inflammation and vascularization (63; 64). Transcriptional data have identified changes in inflammatory, angiogenic and metabolic pathways following high altitude exposure (63). This may disproportionally affect brain regions that are more sensitive to the impact of hypoxic stress on cell function and survival, or regions requiring additional metabolic demand for vascularization and/or inflammatory activity. Furthermore, behavioral tests have identified distinct hippocampal memory dysfunction following high altitude, suggesting the induction of maladaptive mechanisms affecting neuronal circuitry and detrimental for normal cognitive functioning (63). It is hypothesized that a key

component of this maladaptive shift is related to the dysfunctional chemotactic activity of microglia in their interactions with the neurovasculature. Due to the clear evidence that hypoxia causes serious shifts in inflammatory profiles and neuronal activity in a region dependent manner and the implications this has for the role of metabolism, the neuronal and molecular basis for changes in cognitive functioning after chronic high altitude exposure are investigated here.

The overarching hypothesis of this dissertation is that **chronic hypobaric hypoxia causes angiogenesis and blood-brain barrier disruption, creating an inflammatory microenvironment. This microenvironment is influenced by angiogenic markers, cytokine signaling and altered glucose metabolism, contributing to a maladaptive shift in microglia activity which influences neuronal function leading to cognitive deficits (Fig. 1).** Of particular interest is the investigation into vascular, metabolic, microglial and neuronal mechanisms after 3 weeks high altitude exposure, as the brain has transitioned from acute to chronic acclimatization.

HIGH ALTITUDE

High altitude is considered any elevation over 2500 m above sea level; over 140 million people worldwide live permanently at high altitude, and many of them born at sea level are not genetically adapted, and approximately 40 million additional individuals frequently spend several hours to days at a time in these extreme environments for work or recreation (230; 261). The low pressure low oxygen environment of high altitude has considerable effects on human physiology (38; 277; 311; 358; 359). Although atmospheric oxygen concentrations remain at ~21% regardless of altitude, air density decreases with increased elevation (so O_2 molecules are spaced farther apart and fewer

molecules are present within the same volume of air), requiring increased ventilation to improve the level of oxygen inhalation but still resulting in hypoxia. While acute adaptation to high altitude does lead the body to undergo compensatory mechanisms like increased heart rate and respiration rate (201; 284), failure to acclimatize following ascent can lead to serious complications such as acute mountain sickness, high-altitude cerebral edema and high-altitude pulmonary edema (116; 117; 170; 203). Prolonged reduction in oxygen absorption by blood and tissue has serious implications (54; 161; 253; 380).

Reduced barometric pressure at high altitude results in decreased partial pressure of oxygen and causes low blood oxygen saturation which can lead to adverse acute neurological consequences and the persistence of impaired cognitive function following extended exposure (27; 266; 310; 362). Individuals at high altitude experience a variety of symptoms including sleep disturbance, memory and attention deficits, and challenges with fine motor coordination (28; 37; 148; 161; 169; 245; 277; 287; 380). Depressive symptoms may develop in association with declining mood at high altitude, but the etiology of these emotional changes is unclear (71; 241). These functional impairments are likely associated with maladaptive mechanisms involving inflammation, metabolic changes, oxidative stress, and vascular adaptation (83; 89; 237; 241; 262; 265; 360).

NEUROVASCULATURE

The neurovasculature provides blood to the organs of the body and is the main source of oxygen and nutrients; this vast network of arteries, capillaries and veins maintain cellular homeostasis throughout the body (99; 276). While vessels undergo acute functional changes resulting from constriction and dilation in response to stimuli,

vascular remodeling occurs when blood vessels undergo a structural change as an adaptive process in response to hemodynamic conditions, potentially contributing to pathophysiology of disease (85; 224).

Angiogenesis is the process by which new capillaries sprout from existing vessels, often in response to oxidative stress indicating the need for extension of the vasculature to improve access to oxygenated, nutrient-rich blood (138; 156). It plays a crucial role in adaptive and maladaptive processes, including tissue regeneration and tumor growth (162). Preclinical data shows that high altitude exposure causes increased brain vascularization (63).

Increased systolic pressure of pulmonary arteries at high altitude can contribute to the acute development of diffuse high altitude pulmonary edema, where uneven vasoconstriction causes increased capillary permeability and pressure which results in fluid accumulation in the lungs (212; 298). Headache is the most common complication of high altitude exposure, occurring in 80% of individuals at elevations over 3000 m, and cerebral vasodilation induced by hypoxia is thought to be the primary cause (47). Hypoxia has been shown to increase cerebral blood perfusion, with variations in dynamic cerebrovascular reactivity between regions (62; 307). Hypobaria during high altitude exposure is associated with greater sensitivity of cerebrovascular reactivity to CO_2 compared to normobaric hypoxia, potentially affecting brain oxygen delivery and vasodilation induced by altitude associated hypercapnia (2) . After extended exposure to chronic hypoxia at high altitude, cerebral blood flow can return to baseline levels with the increased capacity of blood to carry oxygen due to higher blood hematocrit and hemoglobin levels (6; 309; 363; 386).

Increased endothelial activation contributes to proinflammatory response at high altitude (43; 95). Increased stress and blood pressure (as experienced at high altitude) can lead to changes in regional vascular remodeling and breakdown of the blood-brain barrier, contributing to cerebral vasculature damage (like arterial atherosclerosis, vessel resistance/stiffness, increased vessel wall thickness, endothelial damage, etc.) and altered hemodynamics (88). Further evidence shows endothelial cell metabolism is a driver of angiogenesis under hypoxic stimulation (366).

Blood-Brain Barrier

The blood-brain barrier is the interface between the central nervous system and peripheral circulation, providing a regulatory avenue for nutrient and waste exchange (128; 320). Integrity and function of the blood-brain barrier is maintained by the neurovascular unit, comprised of endothelium, neurons, astrocytes, microglia and pericytes, with complex tight junction proteins regulating transportation of solutes across the barrier (20; 124; 128; 365). This is in contrast to peripheral vasculature, which is fenestrated and does not exhibit the high level of selectivity that is found in brain vasculature. Disruption of the blood-brain barrier can affect the transport of molecules between the blood and brain, as well as induce aberrant angiogenesis and inflammatory responses leading to progressive neuronal dysfunction (308; 391). Neuronal activity and neurovascular coupling ensures rapid increases in regional blood flow to quickly supply more nutrients and remove metabolic waste when necessary (149). Disruption of the neurovascular unit is associated with pathogenesis of neurodegenerative diseases including dementia and Alzheimer's disease (157; 258; 274; 308; 320; 379; 391). Moreover, hypoxic conditions have been shown to disrupt blood-brain barrier integrity

and activates microglia, contributing to inflammatory profiles which lead further to impaired cognitive and motor function (84; 387).

MICROGLIA

Microglia are the resident macrophage of the central nervous system; these dynamic cells are constantly maintaining homeostasis and surveilling the extracellular environment by extending and retracting their processes (17; 137; 242; 324; 334). During development, microglia help to sculpt neural circuits by targeting synapses for elimination and promoting synapse formation (231; 293). In the adult brain, microglia contribute to synchronization of neuronal activity (4). Microglia contribute to the maintenance of neuronal network stability, providing structural protection of neuronal dendrites following network perturbation from seizure hyperexcitability (92). They participate in the functional regulation of neurons and glial cells through modulating neurotransmitter release and glutamate exchange, excitation/inhibition balance and synaptic pruning/phagocytosis, as well as interacting with vasculature by contributing to blood-brain barrier maintenance and the neurovascular unit (131; 183; 242; 324; 337). Resting microglia make direct contact with neuronal synapses at a rate modulated by neuronal activity levels to contribute to homeostatic maintenance, and microglial calcium signaling responds to alterations in neuronal activity (338; 346). Microglia exhibit region-specific phenotypes, exhibiting a spectrum of distinct functional states, and research has demonstrated that these diverse characteristics are established and maintained by local cues (17; 72). In addition to regional specificity of microglial populations, there are also subsets of microglia which are spatially associated with the vasculature ("juxtavascular microglia"), establishing constant contact with blood vessels and closely communicating

with neighboring astrocytes; these microglia exhibit rapid and unique reactive transformation in response to brain lesions (17; 232).

Microglial Chemotaxis

Depolarization of microglia membrane potential, as well as decreased extracellular calcium concentrations, affects microglia morphology, surveillance activity, and decelerates chemotactic response kinetics (166). Rapid extension of process tips towards sites of damage/injury relies on signal transduction involving ATP-mediated hyperpolarization via P2Y12 receptor activation and THIK-1 channel opening (69; 166; 210; 323). Microglia have been shown to respond to neuronal hyperexcitation, where glutamate binding to NMDA receptors on neurons facilitates calcium influx and ATP release which triggers microglial process extension via P2Y12 binding to provide inhibitory tone to neuronal circuitry (93). In a mouse model of epilepsy, microglial motility and process velocity during basal activity was preserved even after neuronal hyperexcitation, but territory of basal surveillance was increased and directed process tip velocity towards a purinergic agonist source was elevated (18). Activated microglia following injury use similar molecular mechanisms as those used during development to target and displace inhibitory synapses in cortical neurons to promote neuroprotection and mediate presynaptic stripping (57).

Microglia Depletion through CSF1-R Inhibition

Microglia survival depends on signaling through colony-stimulating factor 1 receptor (CSF1-R), which controls the production, differentiation and function of macrophages (178; 188). The use of CSF1-R inhibitors results in depletion of microglia populations, with nearly complete depletion within 1-2 weeks of continuous inhibitor

administration and full repopulation of the brain possible within 2-3 weeks of ceasing CSF1-R inhibitor treatment (67; 108). Microglia depletion does not seem to have a deleterious effect on healthy cognition and behavior (67). When microglia are repopulated in the brain following depletion, their distinct regional phenotypes are reestablished (72). While microglia activity has an initially protective role in CNS injury and disease response, limiting damage and phagocytosing toxins and waste, the development of a chronic inflammatory profile can contribute to maladaptive local brain pathology and neurodegeneration (67; 173; 280; 283; 352; 369). For this reason, studies have explored the benefits of resetting microglia populations using CSF1-R inhibitors to halt runaway inflammation and improve functional outcomes after injury or disease (67). This research has shown that abnormal microglial morphology and activity in disease models associated with impaired cognition and neurophysiogical deficits like decreased spine density and impaired activity can be rescued through microglia depletion (67; 269).

Microglia and Hypoxia

High altitude causes the release of peripheral and central inflammatory cytokines which increase the number of activated microglia in the brain and exacerbate impaired motor and cognitive abilities (63; 176). Microglia and neuroinflammation under hypoxic conditions are also implicated in the pathophysiology of pulmonary hypertension through blunted synaptic activity of sympathetic neuronal circuitry (250). Following transient cerebral ischemia, microglia experience prolonged contact with synapses and facilitate the pruning of synaptic buttons, indicating a role in hypoxia induced turnover of synaptic connection with possible implications for cognitive deficits (346). Microglia respond to damage-associated cues and show metabolic flexibility by shifting to glutamine

consumption when glucose is unavailable, allowing them to maintain critical phagocytic and surveillance activity in energy deprived environments (33). Hypoglycemia has no independent impact on microglia morphology or motility during surveillance of brain parenchyma and damage-sensing response, but may be vulnerable to combined hypoxia and hypoglycemia (33; 206). Indeed, upregulation of HIF-1 genes after hypoxia negatively impacts microglial mitochondrial metabolism, contributing to microglial dysfunction in neurodegenerative diseases like Alzheimer's (219). Of particular relevance, studies have demonstrated that chronic hypoxia induces blood-brain barrier disruption throughout the CNS in a region-specific manner and that microglia play a crucial role in maintaining vascular integrity, with reactive microglia aggregating around leaky vessels (120-122). Depletion of microglia with a CSF-1R inhibitor increased hypoxia induced cerebrovascular leak and loss of endothelial tight junction proteins (120-122). Regions undergoing the greatest degree of angiogenesis after hypoxia also exhibited the most level of blood-brain barrier disruption, indicative of vascular leakage possibly being a byproduct or a driver of angiogenic remodeling (120-122).

Microglia Mediate/Modulate Neuronal Activity

In addition to their role in phagocytosing dying neurons, pruning synapses and producing ligands to promote neuronal health and communication, microglia also modulate neuronal activity primarily by suppressing neuronal over-activation (19; 356). Neuronal intercommunication involves electrochemical events and molecular processes, where the functional properties of neural and glial networks and the release of signaling factors between these cells elicit functional and structural changes in synaptic activity to facilitate cognitive performance (327). The main interface for neuronal communication is

the synapse, where exchange of signaling molecules dictate the activity of neuronal circuitry (314). Synaptic changes can be measured through the induction of long-term potentiation by the delivery of high-frequency tetanic stimulation or long-term depression by delivery of low-frequency stimulation and this has become a standard approach to assess hippocampal circuitry and their synapses (35; 36; 132; 153; 220; 222). The changes in synaptic strength elicited by artificial induction of long-term potentiation and long-term depression in the hippocampus are considered to demonstrate the capacity of the brain for spatial memory formation and erasure (34; 35; 221; 240; 292). The negative feedback control activity of microglia on synaptic activity is region-specific and relies on microglia sensing extracellular ATP and converting it to adenosine, which then acts at neuronal synapses to increase inhibitory tone (19). Microglia also rescue neurons from excitotoxicity by preventing excess depolarization through migration of microglial processes towards swollen axons where microglia-axon contact facilitates removal of debris and membrane repolarization back to resting potential, thus preventing neuronal damage due to hyperactivity (151). Interestingly, while microglia promote synaptic activity and enhance neuronal synchrony during healthy homeostatic surveillance activity, inflammatory activation of microglia actually contributes to impaired network synchronization, suggesting a role of inflammation and immune response in cognitive functioning (4).

Microglia play a protective role in the presence of neuroinflammation by preserving synaptic plasticity through Sirt2 signaling, without which NMDA-mediated long-term potentiation in the hippocampus is impaired (289). However, microglia activation has also been shown to cause deficits in long-term potentiation through the

release of proinflammatory cytokines like TNF-α and IL-1β (191). Under inflammatory conditions, microglia shift to aerobic glycolysis and stabilize HIF-1α, producing proinflammatory cytokines that contribute to inhibition of long-term potentiation (378).

Crosstalk between microglia and neurons can also affect microglial morphology, with the induction of long-term potentiation causing an increase in the number of microglial processes and increased duration of microglial process contact with dendritic spines (264). Neuronal hyperexcitability can lead to excitotoxicity and disruption of local ATP microgradients which in turn negatively impacts microglial motility and phagocytic efficiency, triggering an uncoupling of critical phagocytosis apoptosis mechanisms (1).

MOLECULAR PATHWAYS IN THE BRAIN IMPACTED BY HIGH ALTITUDE

High altitude exposure induces an increase in plasma VEGF levels due to hypoxic regulation, but circulating VEGF is not associated with pathogenesis of acute mountain sickness (77). Inflammation and hypoxia share significant crosstalk in regulation of transcriptional responses (265). Inflammatory mediators like IL-6 contribute to development of high altitude complications like pulmonary edema, but do not seem to be the direct cause of pathophysiology (298). Oxidative stress drives adaptation to high altitude, but oxidative stress damage is shown to be closely related to acute mountain sickness severity and excessive reactive oxygen species generation under hypoxic conditions can contribute to a reduction in capillary perfusion of oxygenated blood, precipitating the development of maladaptive neurological consequences (15; 142). Mechanisms of chronic high altitude adaptation involve many molecular pathways including oxidative stress, inflammation (IL-1β, IL-6, TNF-α, NF-kB), protein kinase activation (ERK5, p38α and PKCα), and hypoxia signaling (HIF-1α and SDF-1) (172;

176; 261; 262; 348; 377). HIF-1α has been shown to modulate inflammatory response and glial activity following ischemic brain damage, controlling the progression of neurological symptoms (10). HIF expression in the carotid bodies and CNS supports ventilatory acclimatization to hypoxia during chronic exposure most likely through autonomic feedback, with activation of VEGF and erythropoietin pathways involved in reducing metabolic oxygen demands and modulating physiological control circuits (236; 271; 272).

Metabolism Pathways Impacting Brain Function at High Altitude

Evolutionary genetic adaptation to high altitude (relevant for Tibetan, Andean and Ethiopian populations) relies on variations in oxygen transport to improve blood flow to vital organs and efficiency of oxygen utilization, so it is reasonable to explore the role of metabolic shift in acclimatization to chronic high altitude exposure (233; 303). Energy metabolism is affected by the hypoxic conditions of high altitude exposure (237-239). Improved biochemical coupling at the mitochondrial inner membrane may enhance oxygen efficiency at high altitudes, and downregulation of electron chain complexes may ameliorate the effects of increased reactive oxygen species production (238). Adaptation of skeletal muscle mitochondria in high-altitude native deer mice as well as in the acclimatization of Everest climbers shows a shift to enhanced aerobic performance contributing to hypoxia resistance (174; 214). Glutamine supplementation may improve mood and cognition after high altitude exposure by mitigating hypoxia-induced inflammation and providing an additional source of energy as the precursor of glucose (78).

Neuronal Activity and Hypoxia

Oxygen is critical for cell survival, and exposure of the brain to hypoxia leads to a number of adverse effects including neuron apoptosis (46). The metabolic stress induced by hypoxia can contribute to neurodegeneration (260). Models of sleep apnea use chronic intermittent hypoxia exposure and demonstrate memory impairment and disruption of adult neurogenesis as well as attenuation of NMDA-dependent long-term potentiation, possibly through activation of adenosine receptors at the synapse (13; 155; 182). During periods of low glucose exposure paired with hypoxia, long-term potentiation is impaired through nitric oxide release and aberrant NMDA receptor activation at the synapse (392). Prenatal hypoxia is also known to produce memory deficits and impair synaptic plasticity (389). Hypoxia has been used as a preconditioning treatment to induce a neuroprotective state following transient ischemic stroke by increasing extracellular levels of adenosine and cerebral blood flow; upregulation of the adenosine transporter ENT1 prevents this neuroprotection (65). Reducing HIF-1α accumulation and inhibiting IL-1β production by microglia rescues long-term potentiation in proinflammatory conditions (378), suggesting how microglia may be involved potentially in cognitive deficits after hypoxia or high altitude exposure.

CURRENT TREATMENTS FOR HIGH ALTITUDE EXPOSURE

Current Treatments for High Altitude Exposure

While slow ascent or pre-acclimatization with staged ascent have been shown to have some benefit in preventing acute mountain sickness (24; 26; 29; 74; 116; 202), any efficacy for reducing the maladaptive consequences of chronic exposure has not been demonstrated. Pharmacological prophylactic and acute treatment interventions primarily

rely on acetazolamide, dexamethasone, and ibuprofen (82; 87; 118; 152; 189; 197; 202; 203; 254).

Carbonic anhydrase inhibitors

Carbonic anhydrase inhibitors

Acetazolamide is a carbonic anhydrase inhibitor, which is thought to prevent/treat acute mountain sickness through metabolic acidosis, improved arterial oxygenation, and opposition of hypocapnic alkalosis from the hypoxic ventilatory response (171; 321). It has been shown to decrease hematocrit and serum erythropoietin, as well as increasing arterial oxygen saturation and cerebral tissue oxygenation (285; 336). Studies have shown that acetazolamide inhibits angiogenesis and expression of aquaporin-1 after hypoxia, and that it can inhibit the expression of aquaporin-4 and prevent its redistribution along astrocytic endfeet after traumatic brain injury and ischemic stroke, thereby improving glymphatic function and sleep (105; 125; 281; 282; 335; 339; 371). Carbonic anhydrase inhibitors have been found to reduce the effects of ischemic stroke and intracerebral hemorrhage by reducing microglia activation, decreasing tissue damage and improving functional outcomes (75; 112). However, research also shows that acetazolamide impairs fear memory consolidation by decreasing amygdalar long-term potentiation (374).

Steroidal anti-inflammatories

Dexamethasone is a glucocorticoid with anti-inflammatory and immunosuppressant effects which contribute to improved arterial oxygenation and enhanced ventilator response, ultimately improving cognition and maximal aerobic capacity as well as suppressing plasma erythropoietin levels (185; 190; 247; 322). The potential role of dexamethasone in regulating angiogenesis is unclear, with studies

demonstrating both inhibition and induction of angiogenic mechanisms (51; 56; 96; 165; 200). Dexamethasone decreases permeability of cerebral vasculature for macromolecules and has a stabilizing effect on blood-brain barrier integrity, with prevention of glucocorticoid receptor degradation in brain endothelial cells having a protective effect after traumatic brain injury (129; 328). There is also some evidence that aquaporin-4 expression may be mediated by glucocorticoid activity (80; 111; 354). The potential implications for glymphatic function may explain certain effects of dexamethasone on decreasing sleep and preventing the elimination of synapses by microglia as associated with alterations in microglia phenotypes and function (60; 257).

Nonsteroidal anti-inflammatories

Ibuprofen is a non-selective cyclooxygenase inhibitor, and while it ameliorates headache symptoms associated with acute mountain sickness and has been shown to prevent overall incidence of the illness partially by blocking increased hypoxic ventilatory response, it does not have a significant effect on blood cytokines or blood and tissue oxygenation relating to altitude exposure (25; 189; 204). Some studies have shown that ibuprofen can reduce angiogenesis and cell proliferation while modulating VEGF levels (5; 248; 326; 361). Ibuprofen administration can prevent some microglia activation in the acute stages of hypoxia acclimatization, but these effects are not maintained during chronic exposure (73). There is also evidence that COX-2 inhibition through ibuprofen administration can help restore memory function in a model of mouse model of Alzheimer's disease by preventing the suppression of hippocampal long-term potentiation by amyloid-beta protein plaque accumulation (160), but high dose

administration of ibuprofen in C57BL/6 mice actually led to decreased long-term potentiation amplitude (106).

SUMMARY

High altitude exposure is characterized by at least a 6% reduction in available oxygen for absorption by blood and tissue through hypobaric hypoxia, with serious implications for cognitive, cardiovascular, and metabolic functioning. The physiological consequences of acute high altitude exposure are well documented, but the persisting effects of extended stay at high altitude are underexplored. People frequently travel to high altitude locations for work and recreation, and every day millions of people fly in airplane cabins which are not pressurized to sea level conditions. Prolonged high altitude exposure is known to cause long-lasting cognitive effects, so the potential impact of high altitude on modern society is quite considerable. Therefore, it is crucial to explore the mechanisms behind these maladaptive processes, in order to mitigate the danger of high altitude to health and wellness.

Microglia play a critical role in neuronal function through synaptic pruning and response to myelin degradation, and microglial-vasculature interactions are implicated in some neurodegenerative conditions. Angiogenesis and vascular remodeling in response to oxidative stress at high altitude increases the opportunity for blood-brain barrier disruption, which may create an extracellular environment promoting inflammation and degeneration. This altered extracellular milieu may impact microglia activity and the nature of their interactions with neurovasculature, contributing to the deterioration of axonal integrity and synaptic transmission associated with persistent cognitive deficits after high altitude. **It is hypothesized here that high altitude disrupts microglial**

function and their pathophysiological interactions with neurovasculature, impacting neuronal pathways associated with cognitive function (Fig. 1). To test this hypothesis, the following specific aims were pursued:

Specific Aim 1: Characterize whole brain and region-specific changes in neurovascular structure and integrity after high altitude. These experiments test the hypothesis that adaptation to high altitude exposure induces structural changes to the neurovasculature including blood-brain barrier disruption. Changes in neurovascular diameter, length and tortuosity are assessed through micro-CT imaging of BriteVu perfused vessels. Immunohistochemistry identifies compromised blood-brain barrier integrity. To determine how microglia may influence vascular response to high altitude, experiments are replicated in mice whose microglia were depleted during high altitude exposure through administration of CSFR1-inhibitor PLX5622 in chow (see Methods).

Specific Aim 2: Identify region-specific changes in dynamic microglial functional interactions with neurovasculature in response to high altitude exposure. These experiments test the hypothesis that microglial chemotaxis is primed by high altitude exposure, differentially affecting the inflammatory response to vascular or cellular damage depending on the brain region. Microglia chemotaxis in response to vessel ablation or cell ablation is recorded using *ex vivo* 2-photon imaging, measuring speed of microglia process response. Analyses focus on the cortex and hippocampus.

Specific Aim 3: Determine the role of microglia in functional neurophysiological hippocampal deficits after high altitude. These experiments test the hypothesis that maladaptive microglial activity after high altitude exposure negatively impacts properties of neuronal circuitry and contributes to spatial memory impairments

found following high altitude exposure. Associative long-term potentiation (LTP) required for spatial memory formation is measured through field excitatory postsynaptic potential (fEPSP) to assess Schaffer collateral synapses in the CA1 hippocampus. To determine how microglia may influence neuronal response to high altitude, experiments are replicated in mice whose microglia were depleted during high altitude exposure through chow administration of CSFR1-inhibitor PLX5622.

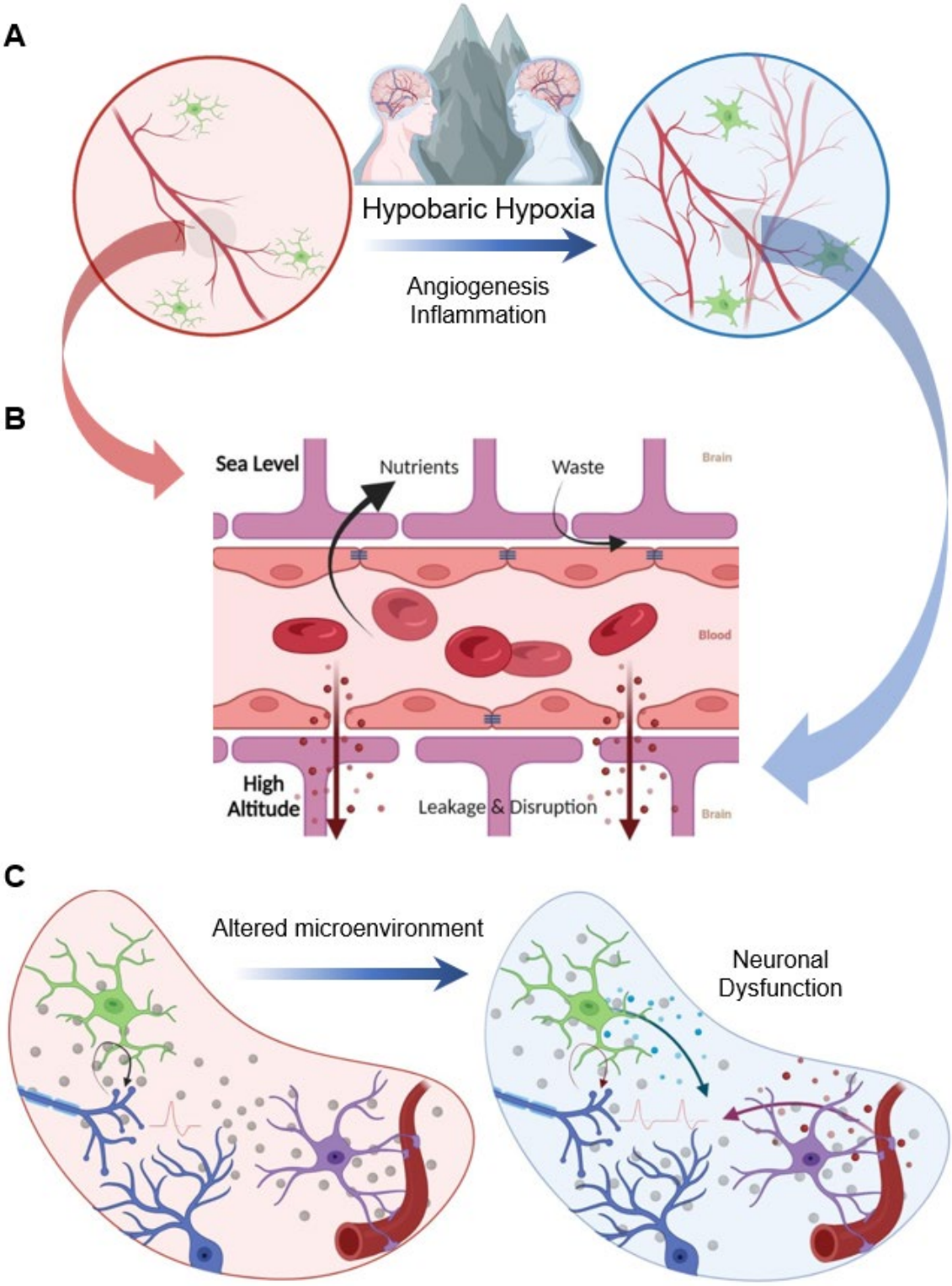

Figure 1. Mechanistic hypothesis of high altitude exposure acclimatization. Hypobaric hypoxia causes increased vascularization and inflammation (A), inducing leakage and disruption of the blood-brain barrier (B) that creates an inflammatory microenvironment which impairs neuronal function (blue) mediated by microglial (green) interactions at the synapse (C) leading to cognitive deficits. Created with BioRender.com

CHAPTER 2: Materials and Methods

General Methods

Animals

Institutional Animal Care and Use Committee (IACUC) approval was obtained under experimental protocol APG-21-847/ APG-19-744/APG-18-847 and breeding protocol APG-20-687. Male and female C57Bl/6J mice were obtained from Jackson Lab (#000664, Jackson Laboratories, Bar Harbor, ME) to arrive at the Uniformed Services University of the Health Sciences (USUHS) at 7 weeks of age, housed separately with 5 littermates per cage in the USUHS animal facility. For experiments requiring heterozygous CX3CR1$^{+/GFP}$ mice, male homozygous B6.129P2(Cg)-Cx3cr1^{tm1Litt}/J breeders (transgenic line of mice on C57Bl/6J background expressing GFP in microglia/immune cells) were acquired from Jackson Lab (#005582, Jackson Laboratories, Bar Harbor, ME) and housed in the USUHS animal facility where they were bred with female C57Bl/6J mice to produce litters of heterozygous CX3CR1$^{+/GFP}$ mice. All experimental mice were housed on a reverse 12 hour light cycle with lights on at 6pm. CX3CR1$^{+/GFP}$ mice entered the high altitude simulation chamber at approximately 20 weeks old (used for 2-photon experiments), while all other experimental C57Bl/6J mice entered the chamber at 8 weeks old. Mice in the high altitude chamber were group housed in their conventional home cages, while mice exposed to sea level conditions were kept in conventional cages in a separate reverse light cycle room to prevent noise interference from the chamber pump. We have previously determined that the noise attenuation within the chamber prevents exposure of high altitude mice to significant noise interference from the chamber pump.

High Altitude Simulation

A modified Vicker's hypobaric chamber altered by Reimers System Inc. (Lorton, VA) reduces atmospheric pressure to ~7.4 psi using a vacuum pump (Welch Model 2585B or 2067B-01) (Fig. 2a). The chamber achieved a simulated altitude of 5000 m (high altitude, equivalent to inspired PO_2 of 78 mmHg, 10-11% O_2, ~60% SpO_2), with ascent and descent procedures at a rate of 200 m per minute. This altitude was used based on previous identification of behavioral phenotypes in the mouse model elicited by this altitude consistent with cognitive deficits observed clinically(63; 64), specifically relating to hippocampal mediated memory impairment(161). The chamber was located in the USUHS animal facility in the common vivarium to ensure consistent environmental parameters across conditions. Chamber altitude was monitored using a digital manometer to ensure differential pressure of 7.4 psi (AZ Instrument Corp., Taichung City, Taiwan). Routine weekly cage maintenance and animal husbandry was performed at sea level, and mice were monitored daily for signs of distress. Mice remain in the chamber for 3-4 weeks high altitude exposure, while sea level mice are housed in the vivarium for the same amount of time (Fig. 2b).

Microglia Depletion

For experiments examining the direct role of microglia, pharmacological depletion of microglia is achieved with relatively new pharmacological tool CSF1R inhibitor PLX5622 obtained under material transfer agreement (Plexxikon, Inc., Berkeley, CA) administered through diet (1200 mg/kg chow). This method of depletion was selected due to ease of administration and brain penetrance, effectiveness in accomplishing robust and sustained depletion in the whole brain over a known time-

course of treatment, and specificity in depleting microglia (305). This dose was established in the literature as sufficient to induce microglia depletion within 1-2 weeks (67; 72). Mice were maintained on the PLX5622 or control AIN-76A diet (Research Diets, Inc., New Brunswick, NJ) throughout high altitude or sea level exposure, so acute adaptation to high altitude occurred with intact microglia populations (Fig. 2b). Preliminary experiments confirmed previous findings in the literature that 2 weeks of PLX5622 diet administration is sufficient to deplete nearly all microglia (Fig. 3) (72). It was previously verified that mice at high altitude eat the same amount of chow on average as those at sea level, and consumption of PLX5622 and control AIN-76A chow is the same. The control diet is nutritionally identical to the treatment diet.

METHODS USED TO ASSESS VASCULATURE

BriteVu and micro-CT Imaging

This method was selected for the stability and high contrast of the perfusion agent and the ability to perform subsequent super high resolution micro-CT imaging of the samples for quantification by a cutting edge vessel tracing software. Mice were euthanized under heavy isoflurane anesthesia by transcardial perfusion with 1x PBS followed by 30 mL of an 18% BriteVu contrast agent solution (1:4.5 dilution) mixed with 1.5% BriteVu Enhancer (Scarlet Imaging, Murray, UT), maintained at 65-70°C. Perfused animals were chilled in ice for at least 1 hour before dissection to ensure solidification of the intravascular contrast agent. Brains were post-fixed in 4% PFA for 48 hours within the skull before craniotomy and transfer to 1x PBS. Brains were scanned at a high resolution 2.98 μm isotropic voxel image size using X-ray acquisition settings of 50 kVp, 201 μA, and 0.5 mm Al filter with exposure time of 2099 ms per frame and 4 frames

averaged at each projection angle, 360° rotation and 0.2° steps, using a Bruker SkyScan 1172 micro-CT (Microphotonics, Allentown, PA). Bruker's CTVox 3D visualization software was used for 3D reconstruction of vasculature image stacks with no smoothing and a 0.54 mm Hamming filter, with 20% beam hardening correction and 10% ring artifact correction applied. Quantification of cerebral vasculature data obtained from micro-CT scanning was performed using Vesselucida 360 software (v2021.1.3, MBF Bioscience, Williston, VT). 3D vascular network reconstructions were automatically traced using identical settings across animals: voxel scooping algorithm with seed sensitivity set to 90 and seed density set to dense with refinement filter for seed validity set to 3, tracing sensitivity set to 90 and maximum gap tolerance allowed.

Parameters measured for morphological assessment were total average length, surface area, and volume, average vessel tortuosity, and average length and volume and tortuosity by binned vessel diameters. These were selected to give insight into potential role of angiogenesis in high altitude acclimatization. Samples were included in analysis based on visual inspection to determine quality of perfusion (conducted on the biological sample and the resulting micro-CT images) as well as to verify successful scanning and reconstruction of the sample (as determined by checking for vessel doubling or poor stitching of micro-CT image stack).

To determine possible region-specific roles in high altitude cerebrovascular adaptation, the cortex, hippocampus and cerebellum of the 3 μm resolution micro-CT datasets was analyzed. Brain regions were isolated by co-registration of the micro-CT image stacks to an MRI mouse brain atlas template, where the micro-CT volume orientation was transformed to the MRI template, and subsequently masked to provide

separate volume datasets for the cortex, hippocampus, and cerebellum. This mapping and masking process was performed using MatLab (Mathworks, Inc., Natick, MA, USA), and then the isolated regions were analyzed using Vesselucida as described above. Successful masking was determined through visual inspection based on our knowledge of the regional vascular architecture.

Immunohistochemistry

Following micro-CT scanning of brains perfused with BriteVu contrast agent, samples were sent for coronal brain section cutting by Histoserv Inc. (Germantown, MD) and stored in the dark at -80° C until staining and imaging. Slide mounted brain slices (20 μm thick) were washed 5 minutes in 4% PFA and twice 10 minutes each in 1x PBS before 1 hour incubation with 3% non-fat dry milk blocking solution and overnight incubation of primary antibodies for labeling extravascular albumin and fibrinogen in 0.3% Triton X-100 in 1x PBS solution containing 3% non-fat dry milk with rabbit polyclonal anti-mouse serum albumin (Cat#ab19196, Abcam, Cambridge, MA) diluted 1:200 and sheep polyclonal IgG anti-human fibrinogen cross reactive with mouse (Cat#4440-8004, Bio-Rad Laboratories, Inc., Hercules, CA) diluted 1:200. After rinsing slides with 1x PBS twice 10 minutes each, slides were incubated with secondary antibodies goat anti-rabbit IgG H&L (AlexaFluor 594) (Cat#A-11037, ThermoFisher Scientific, Inc., Invitrogen/Life Technologies Corporation, Eugene, OR) and donkey anti-sheep IgG H&L (AlexaFluor 488) (Cat#A-11015, ThermoFisher Scientific, Inc., Invitrogen/Life Technologies Corporation, Eugene, OR) both diluted at 1:200. Sections were coverslipped with ProLong Gold antifade reagent with DAPI (P36931; ThermoFisher Scientific, Inc., Life Technologies Corporation, Eugene, OR). Images were

acquired using an Axio Scan.Z1 (Carl Zeiss Microscopy, LLC, Thornwood, NY). Images were analyzed using ImageJ. Quantitative analysis of integrated fluorescent density was performed using ImageJ.

MICROGLIA CHEMOTAXIS AND MOLECULAR METHODS

Ex-vivo 2-photon imaging and novel 4D method for quantitative tip dynamic analysis

This is a live cell imaging modality in intact brain slices that are perfused with aCSF; this method was selected instead of *in vivo* imaging because it facilitates assessment of microglia dynamics in the hippocampus, which exceeds the possible depth of imaging that can be achieved through cranial windows of live mice. $CX3CR1^{+/GFP}$ mice were tail-vein injected with sterile filtered, undiluted DyLight 594 labeled Lycopersicon Esculentum (Tomato) Lectin from Vector Labs (Burlingame CA, Cat# DL-1177) 30 minutes prior to sacrifice by CO2 inhalation (to prevent an interaction of anesthetic on microglial activity). 400-µm thick coronal sections were cut in ice-cold (~4°C) dissection solution (high-sucrose aCSF containing (in mM): KCl, 2; $NaH_2PO_4 \cdot H_2O$, 1.25; $MgSO_4 \cdot 7H_2O$, 2; $MgCl \cdot 6H_2O$, 1; $NaHCO_3$, 26; $CaCl_2 \cdot 2H_2O$, 1; D-glucose, 10; sucrose, 206, bubbled with a mixture of 95% O_2 / 5% CO_2) on a Leica VT1200S Vibratome (Buffalo Grove, IL) and incubated for 30 minutes at 36°C in normal aCSF (containing (in mM): NaCl, 126; KCl, 3, $NaH_2PO_4 \cdot H_2O$, 1.25; $MgSO_4 \cdot 7H_2O$, 2; $NaHCO_3$, 26; $CaCl_2 \cdot 2H_2O$, 2; D-glucose, 10; sucrose, 20, bubbled with a mixture of 95% O_2 / 5% CO_2). Brain slices were kept at room temperature for up to 8 hours. For imaging, the slices were mounted into a tissue slice chamber (Warner Instruments, LLC., Hamden, CT) and perfused with temperature-controlled normal aCSF (~30º C). A ZEISS 7MP microscope was used to perform 2-photon fluorescence microscopy approximately 80 µm

below the surface of the slice. The microscope system used a pulsed infrared laser (Chameleon Vision2, Coherent, Santa Clara, CA) with a tunable wavelength range from 680-1080 nm and a peak power output of 3.3 W at 800 nm. Fluorescence was detected with non-descanned detectors (NDDs) equipped with a 525/50 nm bandpass for green detection (GFP) and a 605/70 nm bandpass for red detection (tomato lectin). Imaging was done using a 40x water immersion objective (NA = 1.0) and controlled with Zeiss ZEN image acquisition software. We recorded activity in the hippocampus in *striatum radiatum* layer beneath CA1, and in the cortex in layers 3-5 of the primary motor and posterior parietal association areas (depending on slice), enabling us to perform experiments in the same slice. For microglia ablation experiments, the cell soma was targeted for maximum laser power exposure in order to destroy the cell (confirmed by loss of GFP fluorescence throughout the cell body and processes). For vessel ablation experiments, a ~10 μm length of target capillary was exposed to maximum laser power until the vessel ruptured. Subsequently, 20-30 frame stacks (1 μm step size) were acquired, and the 4D movement of processes analyzed.

Analysis of the 2-photon imaging datasets of microglia motility and chemotactic response dynamics over time was made possible through the extensive efforts of our collaborators at Virginia Tech, Dr. Guoqiang Yu and his graduate student Mengfan Wang (with significant contributions by former student Dr. Congchao Wang). Compared to individual tip motility data, relational tip motility data not only contain the tip motility information but also include the tip-cell body connectivity information. The complex structures are intractable to be analyzed by the common tip detection approaches. Using a newly-developed algorithm framework, they improved the data quality and quantification

performance (Fig. 4). First, the data are registered and stabilized to uniform noise variances for future analysis. By nonlinear transformation for variance stabilization, weak signals are also enhanced to improve the detection performance. Then the pre-processed data are segmented to foreground and background through an iterative thresholding approach. In each iteration, the most significant regions are segmented as foreground and removed from the data. It was repeated until no more significant regions can be found. Using a novel machine learning algorithm, they have developed a multi-scale microglia tip detection approach, using convex hull analysis rather than local patterns to eliminate the influence of microglia morphology changes on tip detection. This technique has the benefit of not relying on the use of deep neural networks, eliminating the need for time-consuming annotations and instead utilizing an unsupervised approach while maintaining a high degree of accuracy. The algorithm calculates geodesic distance and pixel to convex hull distance, creating a score map for tip detection. This method has a superior precision and recall compared to previously existing methods, demonstrating robust performance even in cases of substantial microglia size and morphology variation. A paper detailing the specifics of the tip detection algorithm has been accepted for publication (351). Compared to their previous algorithm, which prioritized tip detection as isolated signals, the new algorithm maximizes tip detection accuracy and path tracing while maintaining relationship with the parent microglia cell and processes to facilitate more nuanced interpretation of microglia tip populations.

The novelty of this algorithm is that it has the capacity to analyze our 4-dimensional data sets in a fraction of the time required for manual tip detection and without collapsing the image stacks into maximum intensity projections, providing a

more accurate quantification of measures such as speed and distance. The algorithm also exhibits superior performance in precision and recall of tips compared to other peer reviewed methods in the literature (184; 192; 355). In addition to outstanding tip detection, the algorithm maintains the relationship of tips to the parent cell for assessment of cell specific influence on tip dynamics. Furthermore, the algorithm can identify the second wavelength channel for vasculature, allowing analysis of microglia tip dynamics relative to proximity to blood vessels. This will permit future analysis of unique populations of microglia, for instance, comparing the chemotaxis of juxtavascular microglia versus parenchymal microglia.

Cytokine Analysis, Glucose Measurements and Angiogenic Markers

Blood was collected from the submandibular vein or terminally from the heart before perfusion. Glucose readings were taken from the collected blood prior to processing for plasma using FreeStyle Lite Blood Glucose Meter (Abbott, Canada) as well as StatStrip Xpress Glucose Meter (Nova Biomedical, Waltham, MA) to control for hematocrit. Samples were centrifuged for 15 minutes at 4° C and 2000 g/rcf to separate out the plasma, and a 2-fold dilution was used for analysis on Meso Scale Discovery (MSD) plates. For protein homogenate samples, frozen brains were sliced and regions were micropunched (cortex, hippocampus, cerebellum) and put into T-PER (Cat#78510, ThermoFisher) with 1x HALT protease inhibitor (Cat#87785), smashed with a pestle, sonicated, centrifuged for 5 minutes at 10,000 g at 4° C, and supernatant removed. Protein concentrations were determined using a BCA assay and appropriate dilutions were calculated for 25 μg total protein per 50 μL well on the MSD plates.

Plasma and brain tissue cytokine analysis was performed using MSD V-PLEX Plus Mouse Cytokine 19-Plex Kit (Cat#K15255G, pro-inflammatory panel and cytokine panel measuring IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, KC/GRO, IL-10, IL-12p70, IL-15, IL-17A/F, IL-27p28/IL-30, IL-33, IP-10, MCP-1, MIP-1α, MIP-2, TNF-α) and U-PLEX Mouse SDF-1α Assay (Cat#B22VB) run as recommended by the manufacturer and read using MSD Plate Reader model 1201 (MSD, Rockville, Maryland). These assays were selected because of their ability to analyze several targets in the same well (multiplexing) and for their higher sensitivity and broader dynamic range with low background and signal amplification.

Quantitative real time RT-PCR was used to assess changes in mRNA expression of selected angiogenic markers shown to be upregulated after 3 and 12 weeks high altitude exposure in the hippocampus and amygdala through RNA-Sequencing (63). RNA samples from 12 week high altitude exposed mouse hippocampus (previously analyzed through RNA-Seq) were used to create cDNA for qPCR validation of angiogenic transcript expression, specifically of Flt-1, Vtn, Fn1, Vwf and SDF-1. cDNA was quantified on an ABI 7900 real time PCR instrument (Applied Biosystems, Waltham, MA) using specific TaqMan gene expression assays with amplicon lengths 50-150 bp (Cat#4331182, Thermo Fisher Scientific, Waltham, MA). To determine transcript expression, cDNA was diluted 1:2.5 for SDF-1 (Mm00445553_m1) and diluted 1:5 for Flt-1 (Mm00438980_m1), Vtn (Mm00495976_m1), Fn1 (Mm01256744_m1) and Vwf (Mm00550376_m1), normalizing to endogenous control ActB (Mm02619580_g1) and run according to the Taqman Gene Expression Master Mix Protocol (Cat#4369016,

Thermo Fisher Scientific, Waltham, MA). Samples were run in triplicate for analysis of Ct values using the ΔΔCT following guidelines of Applied Biosystems.

FUNCTIONAL METHODS

Hippocampal Long-Term Potentiation

Electrophysiological assessment of long-term potentiation through field excitatory post-synaptic potentials (fEPSPs) is a classic measure of synaptic plasticity that has been used previously in the lab. It involves the persistent strengthening of Schaffer collaterals synapses in the CA1 region of the hippocampus through high frequency stimulation and is considered to be one of the primary mechanisms underlying associative learning and memory. We selected this protocol for its relative simplicity in measuring functional synaptic changes. Mice were euthanized under heavy isoflurane anesthesia by transcardial perfusion with 25 mL of chilled 4° C NMDG-HEPES aCSF containing (in mM): NMDG, 93; HCl, 93; KCl, 2.5; $NaH_2PO_4 \cdot H_2O$, 1.2; NaHCO3, 30, HEPES, 20; D-glucose, 25; sodium ascorbate, 5; thiourea, 2, sodium pyruvate, 3; $MgSO_4 \cdot 7H_2O$, 10; $CaCl_2 \cdot 2H_2O$, 0.5 (329). Perfused animals were decapitated and the brain rapidly removed and placed in ice-cold (4° C) NMDG-HEPES aCSF bubbled with a mixture of 95% O_2 / 5% CO_2. NMDG-aCSF reduces hypoxic damage to the brain tissue, enhancing preservation of neurons and overall brain slice health (16; 329). The hippocampi were dissected from the brain and 400-µm thick transverse slices were cut on a McIlwain tissue chopper (Brinkmann, Westbury, NY, USA) and transferred to a holding chamber for 1 hour incubation in warmed 32° C high magnesium aCSF containing (in mM): NaCl, 124; KCl, 2.5; $NaH_2PO_4 \cdot H2O$, 1.2; $NaHCO_3$, 24; HEPES, 5; D-glucose, 12.5; $MgSO_4$, 4; $CaCl_2 \cdot 2H2O$, 2, bubbled with a mixture of 95% O_2 / 5% CO_2. Slices were then

transferred to a recording chamber (Kerr Scientific Instruments Tissue Recording System, Christchurch, New Zealand) and allowed to equilibrate for at least 1 hour prior to recording. They were constantly superfused (~1-2 mL/min) at ~32° C with standard recording aCSF containing: NaCl, 124; KCl, 2.5; $NaH_2PO_4 \cdot H2O$, 1.2; $NaHCO_3$, 24; HEPES, 5; D-glucose, 12.5; $MgSO_4$, 1.3; $CaCl_2 \cdot 2H2O$, 2, bubbled with a mixture of 95% O_2 / 5% CO_2.

Field excitatory postsynaptic potentials (fEPSPs) adjusted to ~33% of maximal response were recorded with a glass pipette filled with standard recording aCSF, with the pipette tip placed in the CA1 region of the hippocampus and the stimulating electrode (Teflon coated platinum wire) placed in the Schaffer-collateral commissural pathway in the CA3 regions. The fEPSPs were digitized using a digitizer (Model TL-1) and amplifier (Model Axon 200B) from Axon Instruments (Axon Instruments/Molecular Devices, Sunnyvale, CA) and a Universal Imaging PC running WinLTP version 2.30D long-term potentiation acquisition software (Dr. William Anderson, Department of Anatomy, University of Bristol, UK) (11). Following 30 minutes of baseline recordings (1 stimulus every 60 s, with constant stimulus intensity (mA) to evoke approximately one third of maximal response), post-tetanic potentiation was induced with a single train 100 Hz for 1 second high frequency stimulation and evoked responses were measured every 60 s for 1 hour (194; 300).

Recordings were averaged across 5 consecutive waveforms collected at 60 s intervals, with repeated-measures ANOVA used to compare differences in percent fEPSP slope change (mV/ms) between high altitude and sea level mice.

DATA ANALYSIS & STATISTICS

Analysis of vascular density, diameter and tortuosity was processed and analyzed with Vesselucida software (MBF); immunohistochemistry data was processed with ImageJ; microglia chemotaxis was processed using a novel algorithm developed by collaborators from Virginia Tech. Electrophysiology data was processed using WinLTP (11) and Clampfit 10.7 (Axon Instruments, Molecular Devices, San Jose, CA) software. Statistical analyses were performed in GraphPad Prism 9.3.1 and results presented as mean ± standard error of the mean (SEM) unless otherwise noted. In all cases, significance is determined with minimum $p < 0.05$, and individual statistical tests and p values are noted in the results section and figure legends. Most data was analyzed with 3-way or 2-way analysis of variance (ANOVA) followed by Tukey's or Holm-Šídák multiple comparisons post-hoc test. In instances where there are no sex effects, male and female mice are grouped together for analysis. Significance markers are as follows: * = altitude effect, # = microglia depletion effect, $ = sex effect, r = region effect (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, and so forth). Data points were identified and excluded as outliers if they were more than two standard deviations from the mean.

Sex was not a significant factor for whole brain vasculature, so sexes were combined and whole brain vasculature variables were analyzed with 2-way ANOVA for altitude and microglia depletion factors. Sexes were also combined based on whole brain results for regional analyses where multiple unpaired t-tests were performed with correction for multiple comparisons by controlling the false discovery rate using the post-hoc Benjamini, Krieger and Yekutieli method. Integrated density of albumin staining was analyzed by region using 3-way ANOVA followed by Holm-Šídák post-hoc test to

control for multiple comparisons between altitude, sex and depletion factors. Microglia chemotaxis experiments were analyzed with 3-way ANOVA of altitude, sex and region factors followed by Holm-Šídák multiple comparisons test. Glucose readings were analyzed with 2-way ANOVA for altitude and microglia depletion factors followed by Tukey's post hoc test for multiple comparisons. Cytokines and qPCR data were analyzed with Student's t-test. Long-term potentiation data was analyzed with a 3-way ANOVA for percent slope change at the 30 minute timepoint indicating a significant 3-way interaction effect of altitude, sex and microglia depletion, so no groups were consolidated, and an a priori analysis of male control diet long-term potentiation was performed using a 2-way Repeated Measures ANOVA.

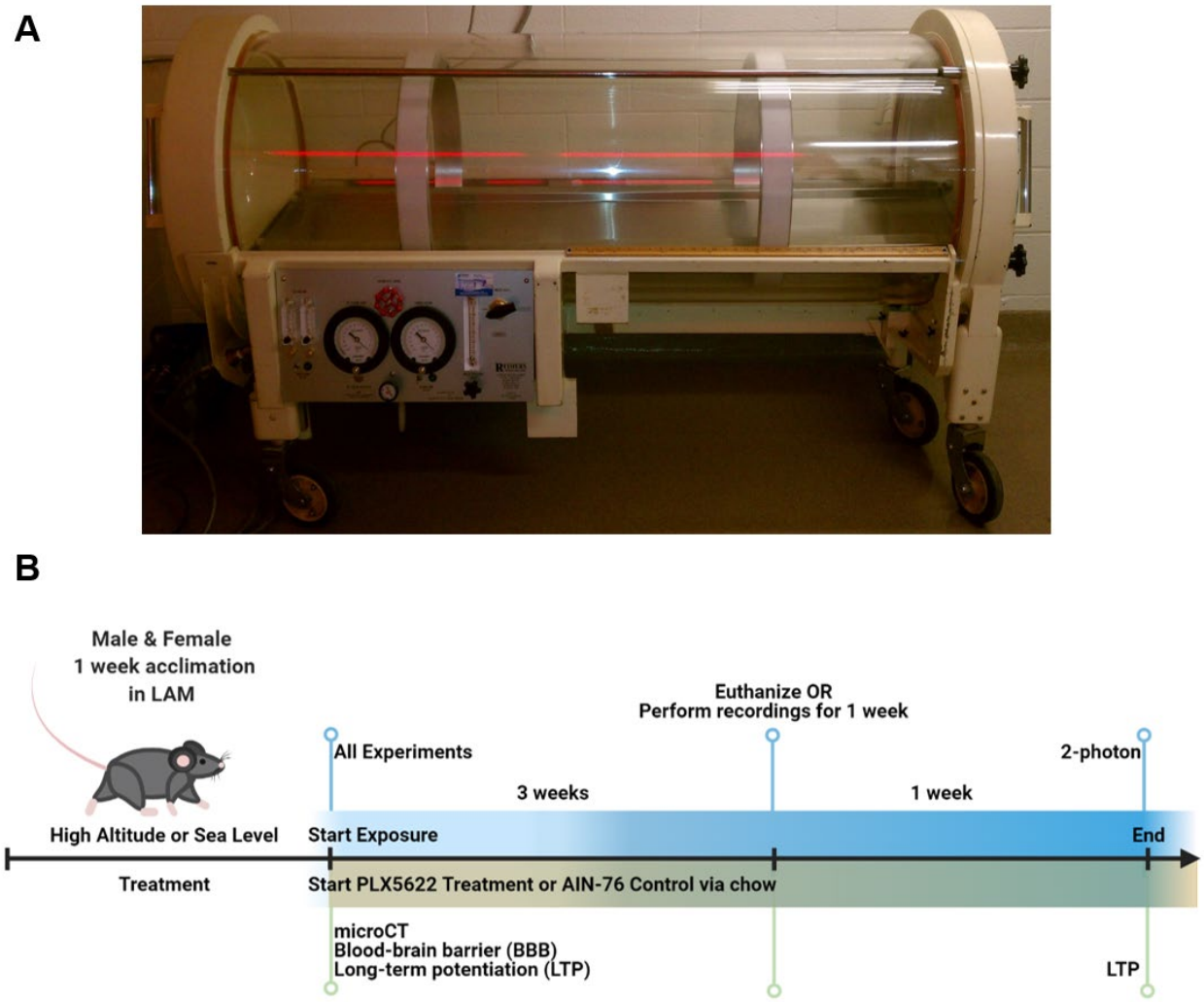

Figure 2. Hypobaric chamber and general experimental timeline.
Converted hypobaric chamber for high altitude simulation, located in the animal facilities at USUHS (A). General experimental timeline: high altitude or sea level exposure lasts 3-4 weeks; for experiments utilizing microglia depletion (PLX5622 treatment), administration begins at the same time as altitude condition exposure and continues throughout (B).

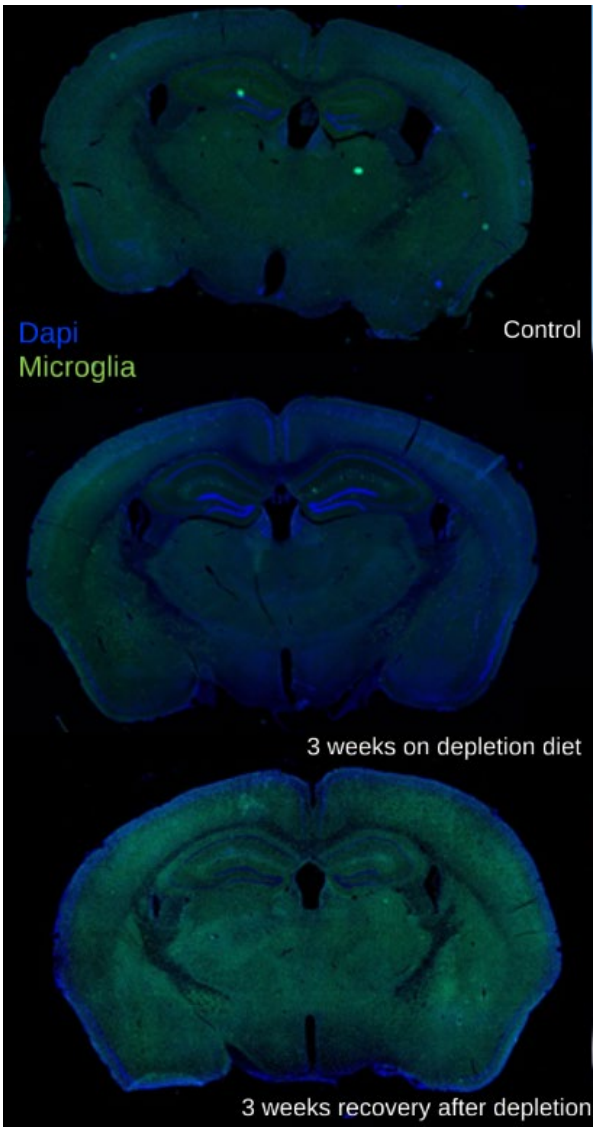

Figure 3. Microglia Depletion.

Representative coronal slices from CX3CR1-GFP$^{+/-}$ mouse brain, demonstrating microglia depletion after 3 weeks PLX5622 diet administration (middle) and 3 weeks microglia repopulation (bottom) compared to control (top).

Algorithm 1 Tip detection algorithm

Input: Foreground F, distance scale set S, smoothness γ

Output: Tip list with scores ($\mathbf{p}$, d)

for every foreground pixel $\mathbf{p}$ **do**
  $Q_{\mathbf{p}}$ = GrassfireTransform($\mathbf{p}$, F)
  for $s \in S$ **do**
    $d_{\mathbf{p},s}$ = GJK($\mathbf{p}$, $Q_{\mathbf{p},s}$)
  end for
end for
$d_{\mathbf{p}}^{max} = \max_s \frac{d_{\mathbf{p},s}}{s}$, $\forall s \in S$, $\forall \mathbf{p}$
$d_{\mathbf{p}}^{avg} = \frac{1}{N} \sum_{\mathbf{q}} d_{\mathbf{q}}^{max}$, $d_G(\mathbf{p}, \mathbf{q}) \leq \gamma$, $\forall \mathbf{p}$
for every $\mathbf{p}$ **do**
  if $d_{\mathbf{p}}^{avg} = \max_{\mathbf{q}} d_{\mathbf{q}}^{avg}$, $d_G(\mathbf{p}, \mathbf{q}) \leq \gamma$ **then**
    Output ($\mathbf{p}$, $d_{\mathbf{p}}^{avg}$)
  end if
end for

Figure 4. Algorithm for 4D analysis of microglia tip detection and movement. As described in Wang et al. (2022). (351)

CHAPTER 3: Results

HIGH ALTITUDE IMPACT ON NEUROVASCULAR STRUCTURE

To determine the effect of chronic high altitude exposure on neurovasculature structure, whole brain and region-specific quantification of vasculature is performed to identify changes in volume and branching as indicators of angiogenesis. Blood-brain barrier integrity is also assessed.

Increased neurovasculature following (3 or 7 week) high altitude exposure revealed by micro-CT

Preliminary analysis of brain vasculature in C57Bl/6J male mice following exposure to simulated high altitude (5000 m) for 7 weeks (normal LAM food diet, no microglia ablation treatment) using 6.7 μm resolutiomn micro-CT imaging revealed significant increase in whole brain vasculature volume (Fig. 5) (Student's t-test, n = 4 mice per group, $f_{(3, 3)} = 28.3$; $p = 0.036$,). Increasing resolution of X-ray micro-CT scans to 3 μm to assess mouse brain vasculature after 3 weeks simulated high altitude exposure reveal a continuation of this chronic vascular pathology as an adaptive response to hypobaric hypoxia, as primarily identified by an increase in whole brain vasculature volume and branching. Sea level and high altitude representative images of micro-CT reconstruction images (Fig. 6A) and subsequent traced vasculature (Fig. 6B) for 3 week exposure show robust visual evidence of increased vasculature. 3-way ANOVA testing was used to determine no significant effect of sex on brain vascularization, so males and females were combined for statistical analysis using 2-way ANOVA with Tukey's post hoc multiple comparisons. High altitude significantly increases whole brain vasculature volume ($f_{(1, 21)} = 30.37$; $p < 0.0001$,) (Fig. 9A), and number of branching nodes ($f_{(1, 22)} =$

13.41, $p = 0.001$) (Fig. 9C) and endings ($f_{(1, 22)} = 11.36$, $p = 0.003$) (Fig. 9D). To assess the role of microglia on vasculature in response to high altitude, microglia were depleted during high altitude exposure. Microglia depletion in sea level mice reduces vascular structural complexity, with significant decrease in vessel length ($f_{(1, 22)} = 3.392$, $p = 0.038$) (Fig. 9B) and number of branching nodes ($f_{(1, 22)} = 4.633$, $p = 0.043$) (Fig. 9C), but mechanisms of high altitude adaptation overcome this effect (n = 5-8 mice per group). The effect of altitude is illustrated in Figure 7A-C (males and females combined, control diet only), while Figure 7D-E show that the effect of altitude may predominantly affect vessels in the 10-40 μm diameter range (arterioles and venules). Increases in the number of branching nodes after high altitude exposure is indicative of angiogenesis to compensate for hypoxic stress in the brain, which is further supported by increases in total number of vessel endings.

Augmented neurovasculature after 3 weeks high altitude particularly affects cortex and hippocampus

To further assess if specific regions drive vascular augmentation following high altitude, 3 week exposure samples were masked to a brain atlas for assessment of the hippocampus, cortex and cerebellum (Fig. 8A). There was a significant effect of high altitude exposure on average total vessel volume in all three regions (Fig. 8B) (hippocampus $p = 0.032$, t = 2.944; cortex $p = 0.006$, t = 4.522; cerebellum $p = 0.042$, t = 2.955). The hippocampus shows increased vessel length ($p = 0.049$, t = 2.587) (Fig. 8C), while the cortex shows a significant increase in tortuosity ($p = 0.035$, t = 0.011) (Fig. 8D), likely reflective of angiogenesis. There was no significant impact of high altitude on vessel diameter or number of endings in the regions assessed. It is important to note that this analysis combined sexes to maximize sample size for altitude effects (multiple

unpaired t-tests, n = 3-4 per group, df = 5; not all brain samples were appropriate for region-specific analysis because the atlas registration is very sensitive to discrepancies in sample orientation). When looking at the degree of change in each region, the hippocampus exhibits the greatest fold difference in vasculature volume (Fig. 8E) and length (Fig. 8F), possibly showing unique adaptive mechanisms in that region.

These results show that even at 3 weeks of high altitude exposure there is significant evidence of brain angiogenesis and vascular remodeling. The impact of high altitude on brain vasculature and previously published finding following 12 week exposure strongly suggest that blood brain barrier might be compromised even at the 3 week time point (63). In order to address this possibility, integrity of the blood-brain barrier was assessed in the brains used for vascular analysis.

Blood-brain barrier integrity compromised by high altitude

Increases in albumin staining are indicative of vascular leakage, since albumin is typically restricted to blood. Therefore, to measure blood-brain barrier integrity, the integrated fluorescent density of albumin staining was evaluated in the hippocampus and cortex. A secondary only control was used to ensure that results are not due to nonspecific fluorescence. Immunohistochemistry found increased extravascular albumin staining after 3 weeks high altitude exposure in the hippocampus ($f_{(1, 51)} = 6.184$, $p = 0.016$) and cortex ($f_{(1, 51)} = 5.512$, $p = 0.023$) (Fig. 10). Albumin staining following microglia depletion was significantly increased in the male cortex ($f_{(1, 51)} = 4.232$, $p = 0.045$), suggesting a sex-specific role for microglia in blood-brain barrier maintenance (3-way ANOVA with Holm-Šídák multiple comparisons test, n = 4-10 slices per group).

These results confirm the initial hypothesis that blood-brain barrier is compromised and may cause exposure of brain neuroparenchyma to peripheral inflammatory factors circulating in the brain or may lead brain produced cytokines or pro-inflammatory factors to become part of blood circulation and affect other peripheral organs.

HIGH ALTITUDE IMPACT ON MICROGLIA, INFLAMMATION AND MOLECULAR MARKERS

Recent studies show that homeostatic microglia may play a critical safe-guarding role in the dynamic protection of BBB and neuronal activity (120-122). In order to address this, microglia movement dynamics and cytokine levels are evaluated, and the impact of microglia on neuronal function is investigated.

To understand how high altitude affects microglia activity and the extracellular environment, microglia chemotaxis is assessed through 2-photon imaging. Peripheral glucose as well as brain cytokine and angiogenic markers are measured to determine possible factors which could influence the microglia movement dynamics after high altitude exposure.

Microglia movement dynamics are influenced by sex, brain region and altitude

Analysis of microglia movement during homeostatic (pre-ablation) surveillance activity and directed (post-ablation) chemotactic response activity in coronal brain slices of transgenic CX3CR1-GFP$^{+/-}$ mice reveals region and sex specific characteristics of microglia activity, some of which are influenced by high altitude exposure. Spontaneous homeostatic surveillance activity is affected by sex and region (Fig. 11C), with females showing a greater number of microglia process tips in the hippocampus at sea level compared to cortex ($f_{(1, 120)} = 12.76$; $p = 0.0003$) or to males ($f_{(1, 120)} = 12.76$; $p = 0.003$)

(3-way ANOVA with Holm-Šídák multiple comparisons test, n = 4-11 slices per group). During directed microglia chemotaxis following laser ablation experiments (Fig. 11A), there is a reduction in microglia tip proliferation observed in male cortex after 3 weeks high altitude exposure ($f_{(1, 112)}$; $p = 0.008$, = 7.286). At sea level, male cortex shows greater tip proliferation than hippocampus ($f_{(3, 112)} = 7.762$; $p = 0.011$), which is sex specific ($f_{(3, 112)} = 7.762$; $p < 0.0001$) (Fig. 12A) (3-way ANOVA with Holm-Šídák multiple comparisons test, n = 4-11 slices per group). When looking at normalized tip speeds at six minutes, there was an interaction of sex, so males and females were analyzed separately, showing that female microglia in the cortex seem to be particularly sensitive to vessel ablation, showing increased tip speed at 6 minutes post ablation (normalized to baseline speeds) (Fig. 12B) (2-way ANOVA with Tukey's multiple comparisons test, n = 8-11 slices per group, $f_{(3, 68)} = 8.045$, $p = 0.0001$).

These findings suggest that some of factors released by "abnormal" microglia may impact proinflammatory cytokines in the brain, and because of blood-brain barrier disruption, these changes in brain cytokine distribution may be impacted by mediators/factors present in peripheral circulation.

Region-specific changes to inflammatory cytokines in brain after 12 weeks high altitude

Analysis of blood serum cytokines does not reveal any significant changes in pro- or anti- inflammatory markers at 3 weeks, although there is a trend towards increased IL-10 and IL-4 and decreased TNF-α, IFN-γ, MCP-1 and IL-33 (Fig. 13) (Unpaired t-tests, n = 5-7 per group, df = 10). Analysis of 12 week high altitude exposed brain homogenate shows increased IL-5 (t = 2.694), IL-6 (t = 2.874), IL-9 (t = 3.263) and IL-10 (t = 6.013) in the cortex, decreased MIP-1a (t = 9.857) in the cortex, and increased IL-4 (t = 3.662)

and MCP-1 (t = 2.895) in the hippocampus (Fig. 14), showing changes in chronic inflammation are affected by brain region (Unpaired t-tests, * $p < 0.05$, n = 3-4 mice per group, df = 6).

Reduced peripheral glucose after high altitude and microglia depletion

Glucose levels measured from peripheral blood samples of female mice show significantly lower blood glucose levels after 3 weeks high altitude exposure and after microglia depletion (Fig. 15). There is a significant main effect of high altitude exposure ($f_{(1, 14)} = 18.51$, $p = 0.030$) as well as microglia depletion ($f_{(1, 14)} = 13.80$, $p = 0.048$) on reducing blood glucose levels in female mice when measured with a traditional blood glucose meter (2-way ANOVA with Tukey's multiple comparisons, n = 4-5 mice per group). Due to the potential interference of increased blood hematocrit in high altitude exposed animals, the same blood samples were tested with another glucose reader that can control for hematocrit levels. The results showed a less robust effect of high altitude and microglia depletion on reduced blood glucose levels. The effect of altitude is no longer significant, but this may be associated with the small sample size.

Increased SDF-1a in brain regions after 3 weeks high altitude

SDF-1α levels were measured in brain homogenate from the cortex, hippocampus and cerebellum. SDF-1 α was significantly increased in the cortex and cerebellum following 3 weeks high altitude exposure (Fig. 16A). Levels in the hippocampus appear elevated, which would be consistent with previous findings showing increased mRNA expression of SDF-1 and other angiogenic markers following 12 weeks high altitude exposure in the hippocampus and amygdala (as assessed by RNA-seq and qPCR; Fig.

16B) (63). (Multiple unpaired t-test, n = 5 mice per group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, df = 8)

HIGH ALTITUDE IMPACT ON NEURONAL FUNCTION

To understand the effect of high altitude on synaptic plasticity, electrophysiological recordings are performed to give a measure of the capacity for learning and memory in the hippocampus.

Reduced hippocampal synaptic plasticity after chronic high altitude

Following the completion of long-term potentiation experiments, a 3-way interaction effect was detected between altitude exposure, sex and microglia depletion, but Tukey's multiple comparisons post hoc test did not determine any main effects (not enough power due to sample size and variability). Therefore, we performed an a priori analysis using 2-way Repeated Measures ANOVA for the male sea level versus high altitude groups (with microglia intact), to test our primary hypothesis that high altitude impairs long-term potentiation in males, consistent with observed cognitive deficits identified previously through behavioral testing in male mice as well as preliminary experiments showing long-term potentiation impairment after 12 weeks high altitude exposure. A significant attenuation of synaptic plasticity is identified after 3 weeks high altitude exposure in the male control mice. While sea level mice demonstrated robust long-term potentiation (as measured by percent slope change of fEPSP after high frequency tetanic stimulation compared to baseline), high altitude mice failed to achieve long-term potentiation. (Fig. 17) ($f_{(1,18)} = 5.134$, $p = 0.036$, n = 6-14 mice)

It has been reported that hippocampal microglia activity although potentially regulated by glucose metabolism exhibits metabolic flexibility through cellular

reprogramming in response to changes in glucose availability (33) and inhibits hippocampal LTP via IL-1β and neuronal interleukin-1 receptor (378). If these mechanisms contribute to the reported outcome remain to be determined with CA1 area IL-1β measurements in the future studies.

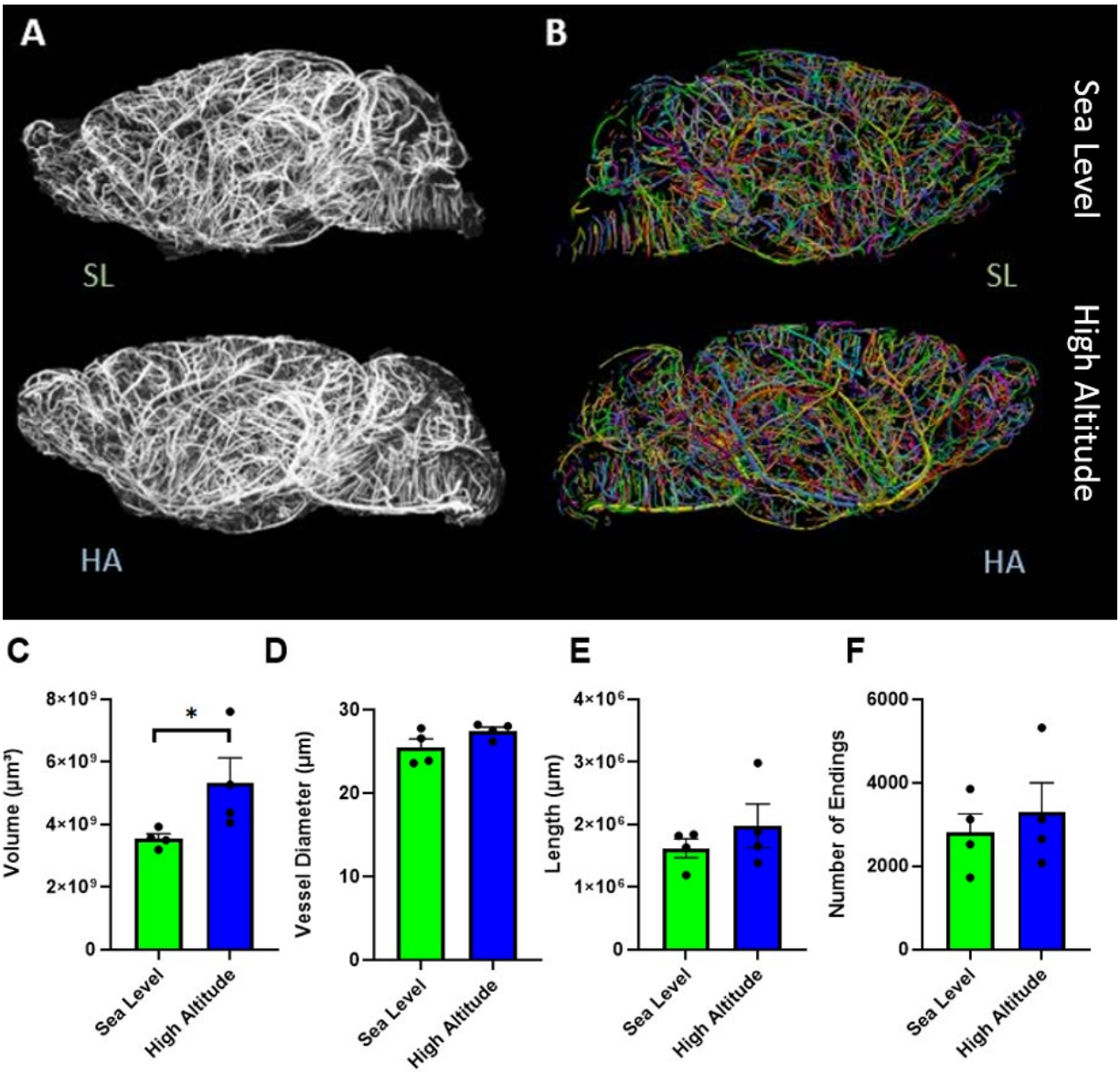

Figure 5. Increased neurovasculature after 7 weeks high altitude.
Representative images of 6.7 µm resolution micro-CT imaging of mouse brain vasculature (A) and subsequent vessel tracing (B) for quantification following 7 weeks high altitude exposure. Significant increase in whole brain vasculature volume is observed (C). Contributions of average vessel diameter (D), whole brain vessel length (E), and total whole brain vessel endings (F) are not significant. Sample includes only male mice on normal LAM chow. Student's t-test, n = 4 mice per group, * $p = 0.036$. Mean ± SEM.

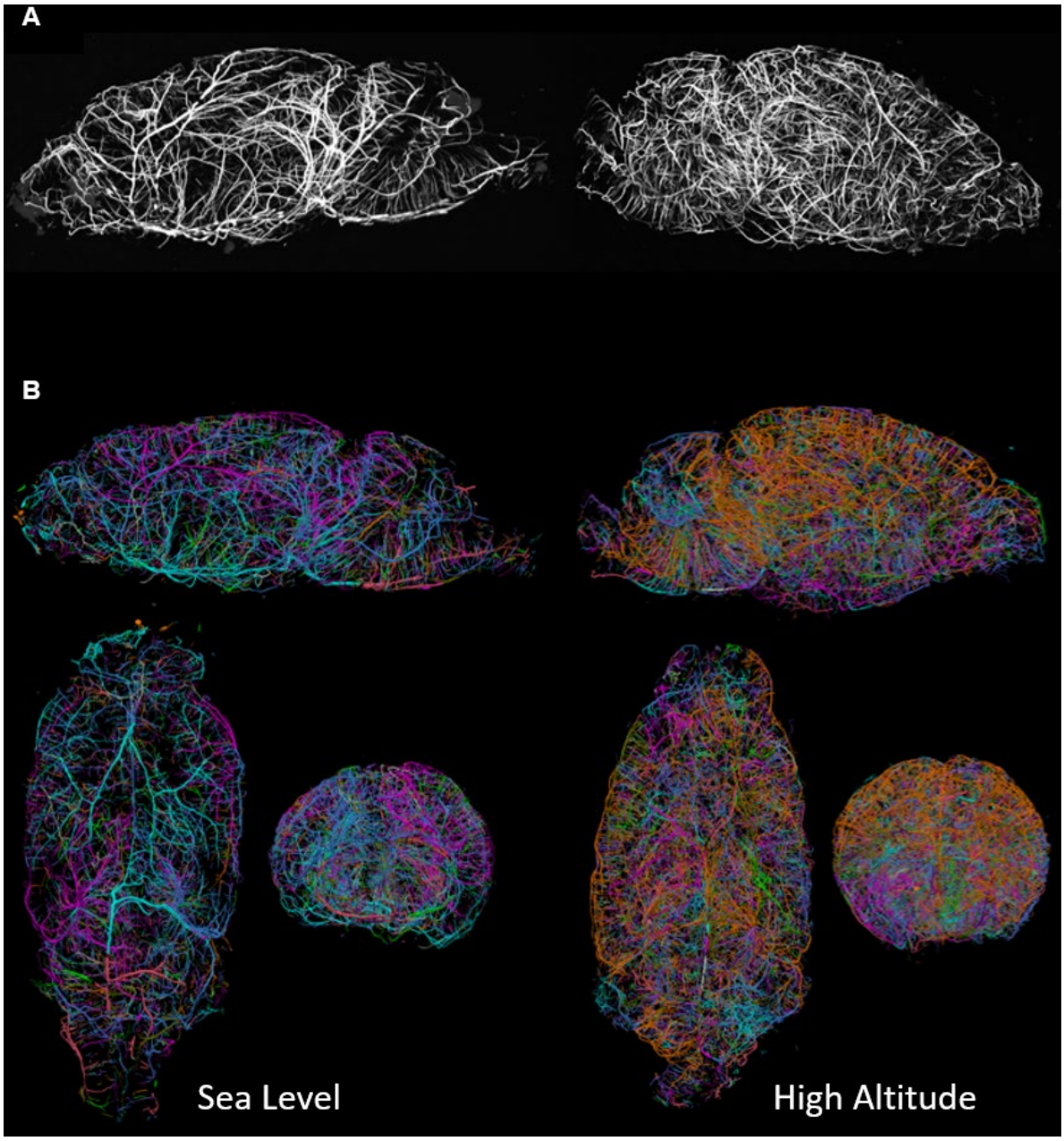

Figure 6. Representative images of whole brain vasculature after 3 weeks high altitude exposure.

Representative images of 3 μm resolution micro-CT imaging of mouse brain vasculature (A) and subsequent vessel tracing (B) for quantification following 3 weeks high altitude exposure. High altitude sample (right) show significant increase in vasculature compared to sea level control (left).

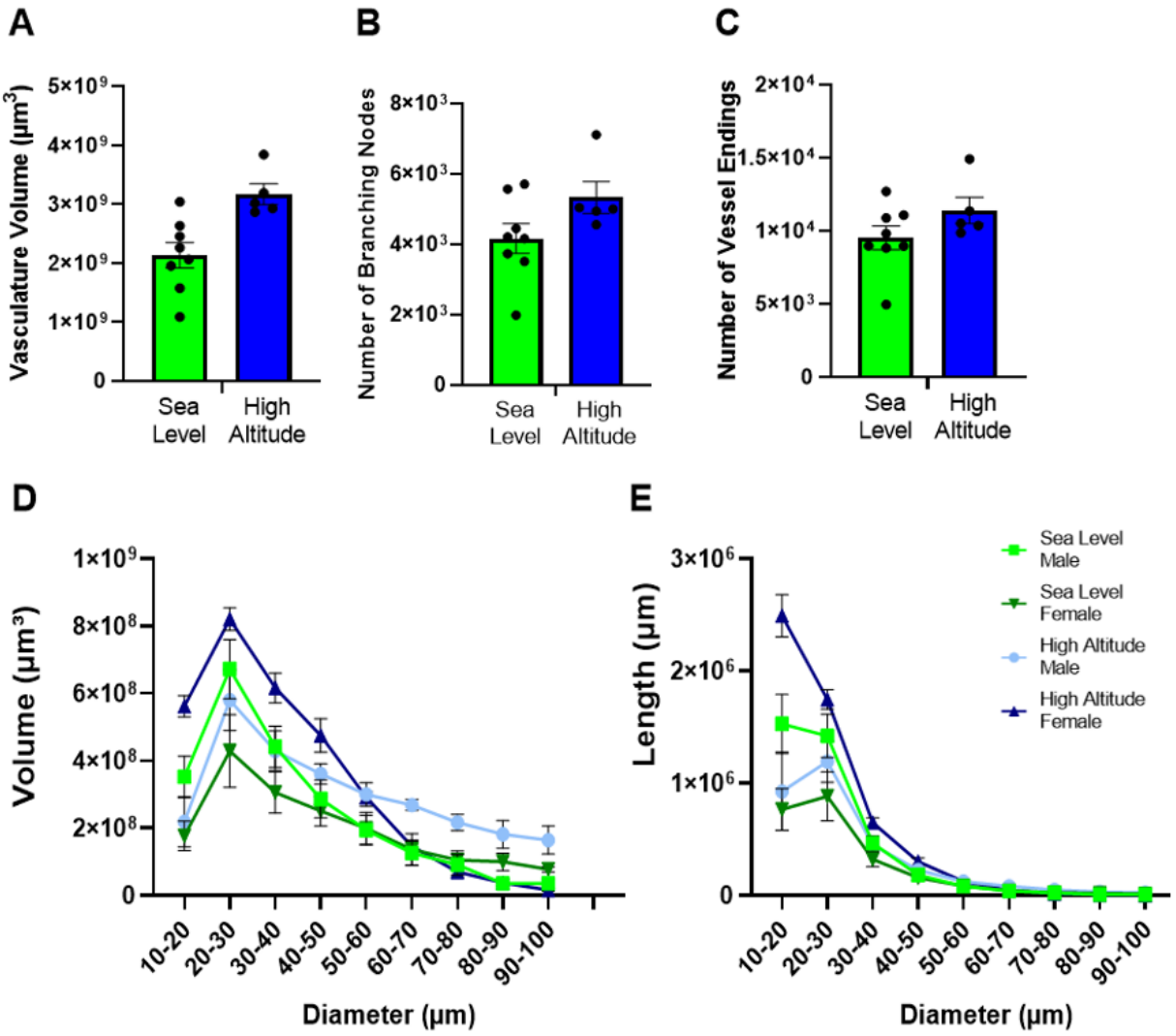

Figure 7. Increased neurovasculature following 3 weeks high altitude exposure. Illustrated altitude effect on increased whole brain vasculature volume (A), and number of vessel branching nodes (B) and endings (C), indicative of vascular remodeling and angiogenesis after 3 weeks high altitude. Vessels in the 10-40 µm diameter range may drive this increase, as shown by binned vessel volume (D) and length (E) graphs. Data shown only from control diet (AIN-76A) mice, graphs A-C showing male and female combined (n = 5-8 mice per group), graphs D and E show male and female separated (n = 2-5 mice per group). Mean ± SEM.

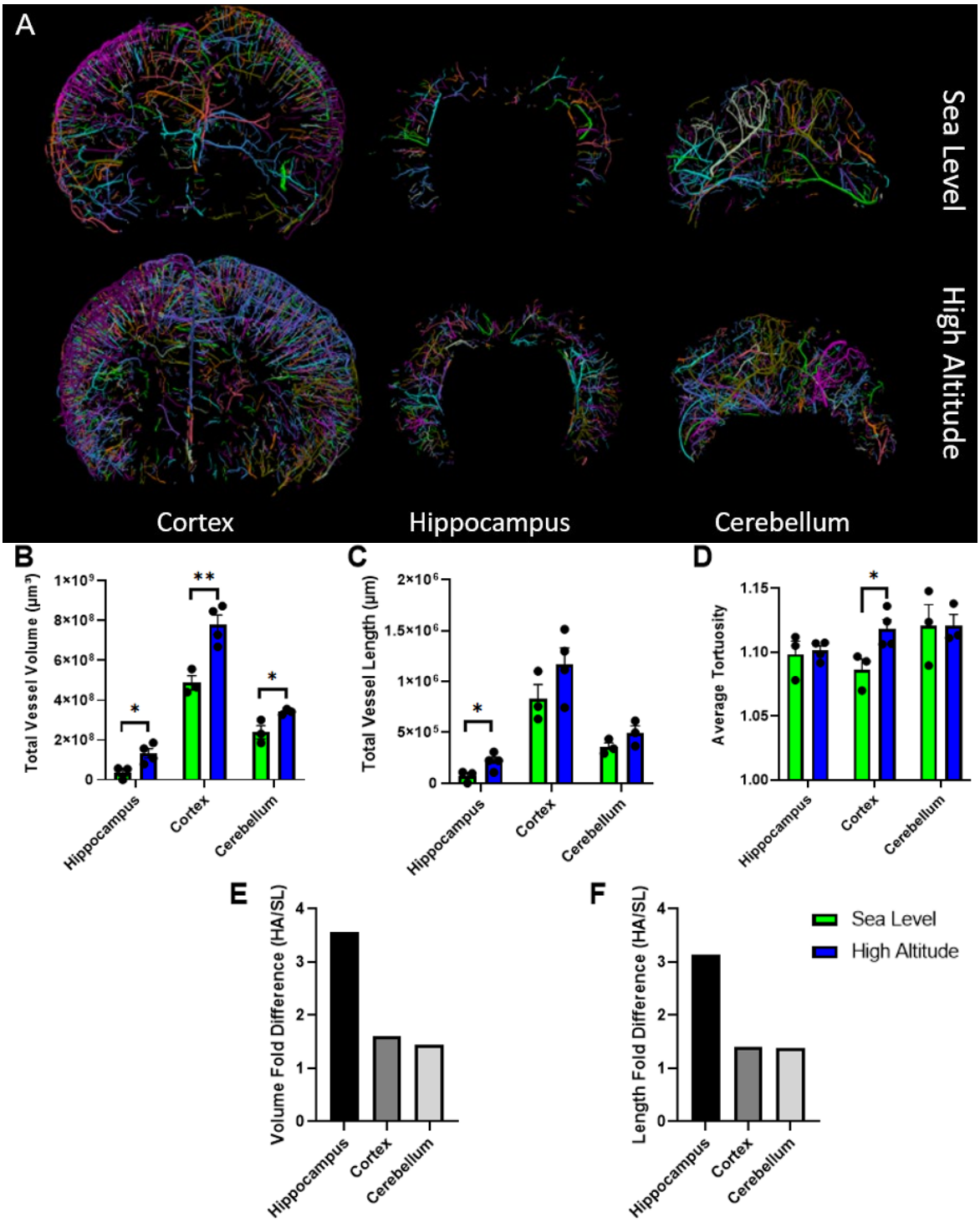

Figure 8. Hippocampus and cortex show unique adaptation to 3 week high altitude. Representative traces (A) for region-specific analysis revealing significant increased vessel volume in the hippocampus, cortex and cerebellum (B), increased length in the hippocampus (C), and increased tortuosity in the cortex (D). Hippocampus experiences the greatest fold difference in volume (E) and length (F). Multiple unpaired t-tests, n = 3-4 mice per group. Mean ± SEM.

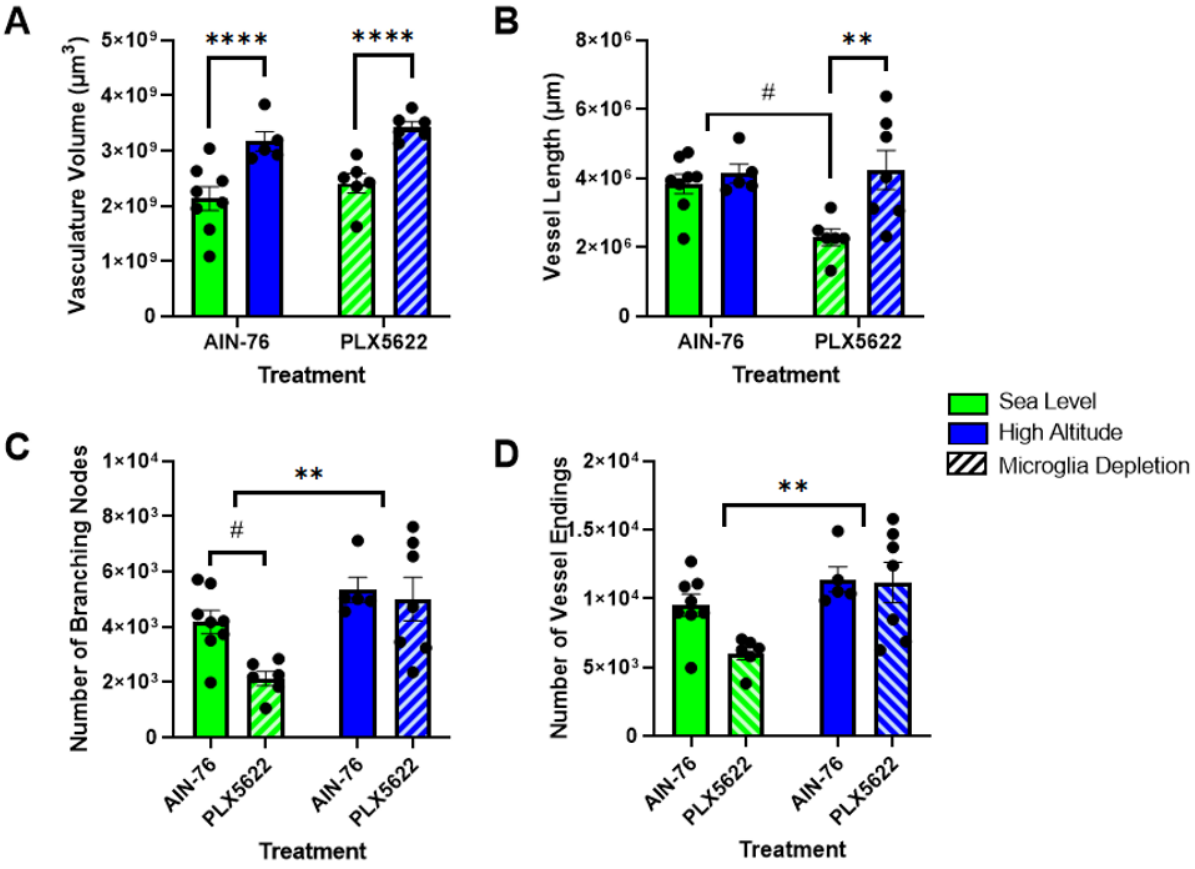

Figure 9. Increased vascular remodeling after 3 weeks high altitude exposure is not initially dependent on microglia.

3-way ANOVA showed no significant effect of sex, so male and female groups were combined for 2-way ANOVA with Tukey's post hoc multiple comparisons. High altitude significantly increases whole brain vasculature volume ($p < 0.0001$) (A), and number of branching nodes ($p = 0.0014$) (C) and endings ($p = 0.0028$) (D). Microglia depletion in sea level mice reduces vascular structural complexity, with significant decrease in vessel length ($p = 0.0376$) (B) and number of branching nodes ($p = 0.0426$) (C), but mechanisms of high altitude adaptation overcome this effect. n = 5-8 mice per group, mean ± SEM.

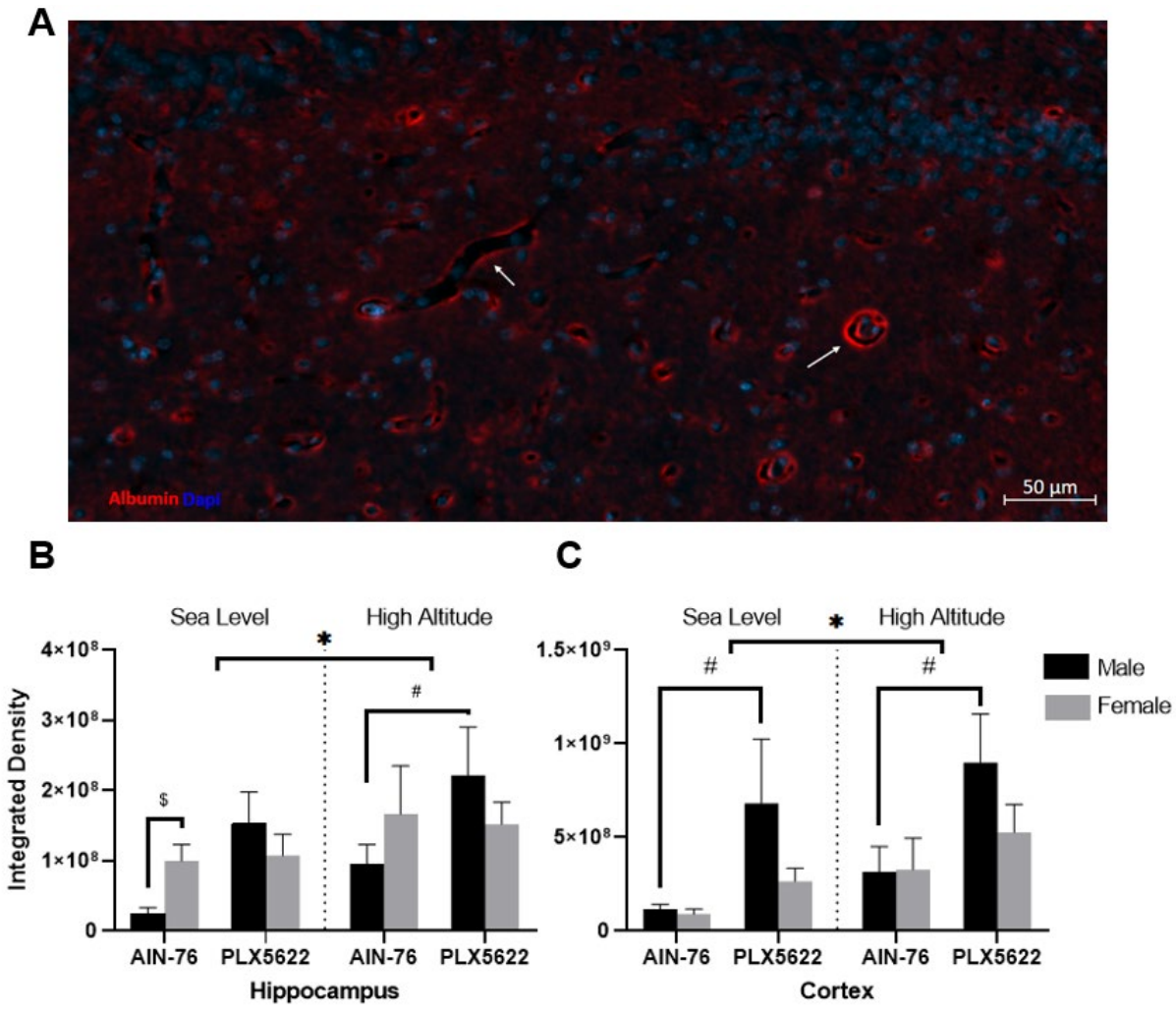

Figure 10. Increased albumin staining after high altitude indicates blood-brain barrier disruption.

Representative image of albumin staining (red) with nuclear marker DAPI (blue) in hippocampus of coronal section of control diet male mouse with microglia intact after 3 weeks high altitude (A), showing extravasation of albumin around vessels and into the extracellular spaces. High altitude significantly increases albumin staining in the hippocampus ($p = 0.0162$) and cortex ($p = 0.0228$). Microglia depletion significantly increases albumin staining in the male cortex ($p = 0.0448$), and females have higher sea level albumin on control diet than males ($p = 0.0181$). 3-way ANOVA with Holm-Šídák multiple comparisons test, n = 4-10 slices per group, mean ± SEM.

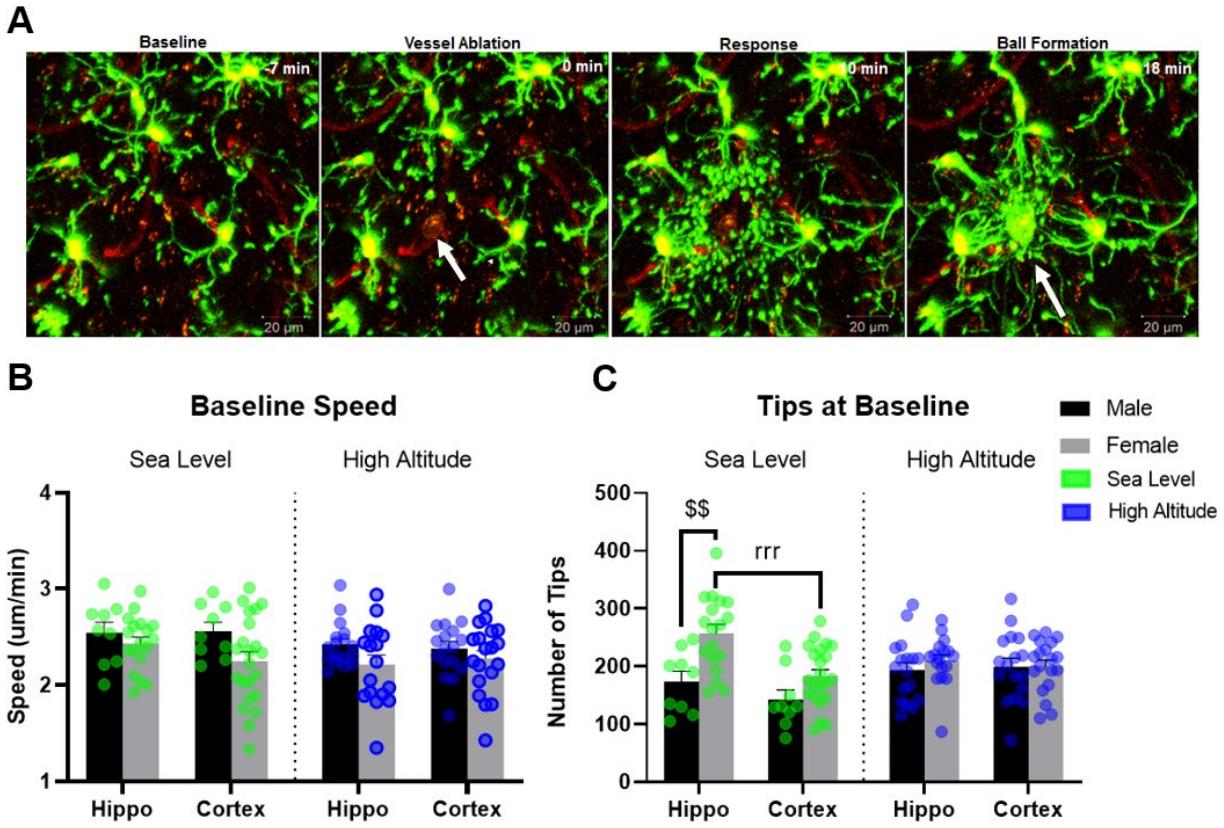

Figure 11. Spontaneous homeostatic microglia activity is affected by sex and region. Laser targeting of micro-vessels or microglia soma for ablation elicits a ball formation response from nearby microglia cells (A), with arrow in second panel indicating site of ablation, and arrow in fourth panel showing completed ball formation. There was no significant effect of altitude on baseline microglia activity. Females overall show a greater degree of variance, and at sea level show increased number of tips present in the hippocampus compared to cortex ($p = 0.0003$) or to males ($p = 0.0025$). 3-way ANOVA with Holm-Šídák multiple comparisons test, n = 4-11 slices per group, mean ± SEM.

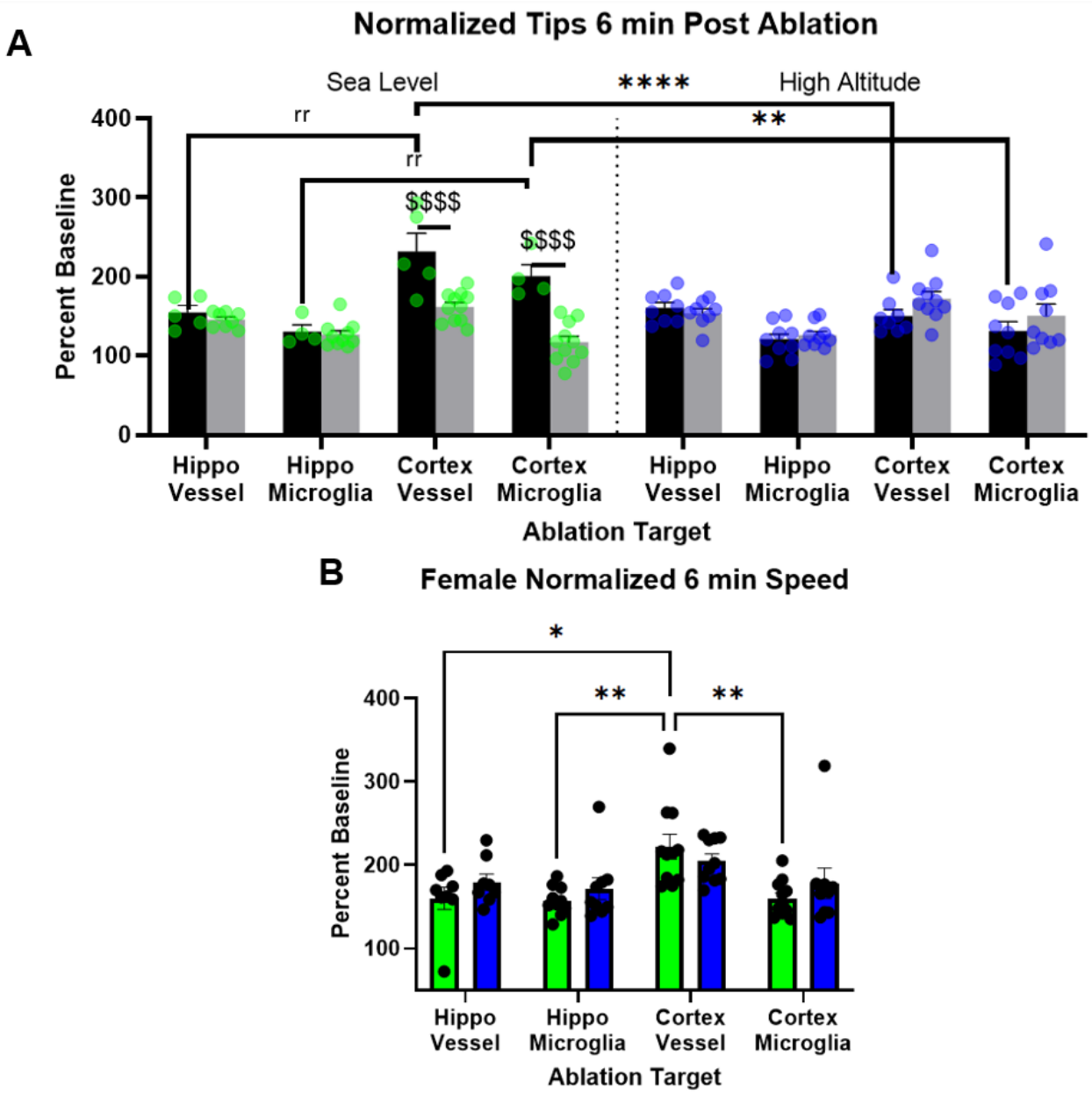

Figure 12. Reduced microglia tip proliferation after 3 weeks high altitude. Analysis of microglia chemotaxis dynamics show high altitude reduces tip proliferation in the male cortex ($p = 0.008$) (A). Male cortex shows greater tip proliferation than hippocampus ($p = 0.011$), which is sex specific ($p < 0.0001$) (A). 3-way ANOVA with Holm-Šídák multiple comparisons test, n = 4-11 slices per group. Female microglia show particular sensitivity to vessel ablation in the cortex, shown by increased tip speed (B). 2-way ANOVA with Tukey's multiple comparisons test, n = 8-11 slices per group, mean ± SEM.

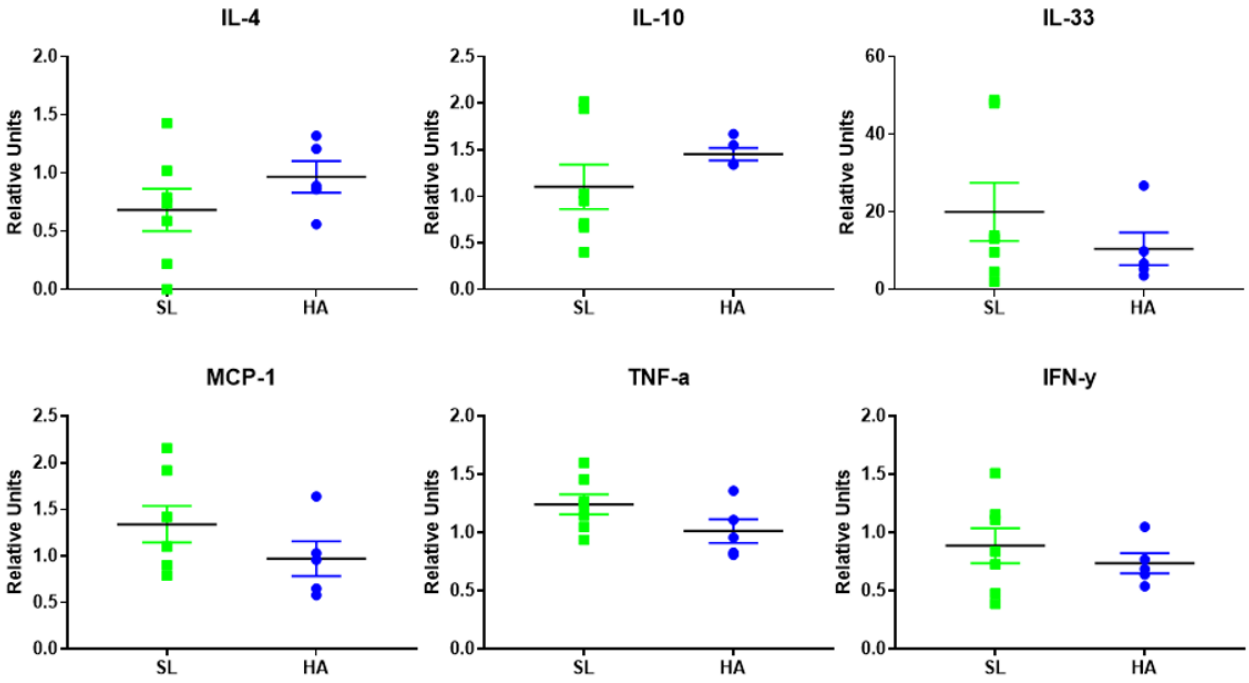

Figure 13. No significant change in peripheral cytokine levels after 3 weeks high altitude.

Cortex (A) and hippocampus (B) significantly altered protein homogenate cytokine levels after 12 weeks high altitude exposure. Increased IL-5, IL-6, IL-9 and IL-10 and decreased MIP-1a is identified in the cortex, while increased IL-4 and MCP-1 is identified in the hippocampus. Unpaired t-tests, n = 5-7 per group, mean ± SEM.

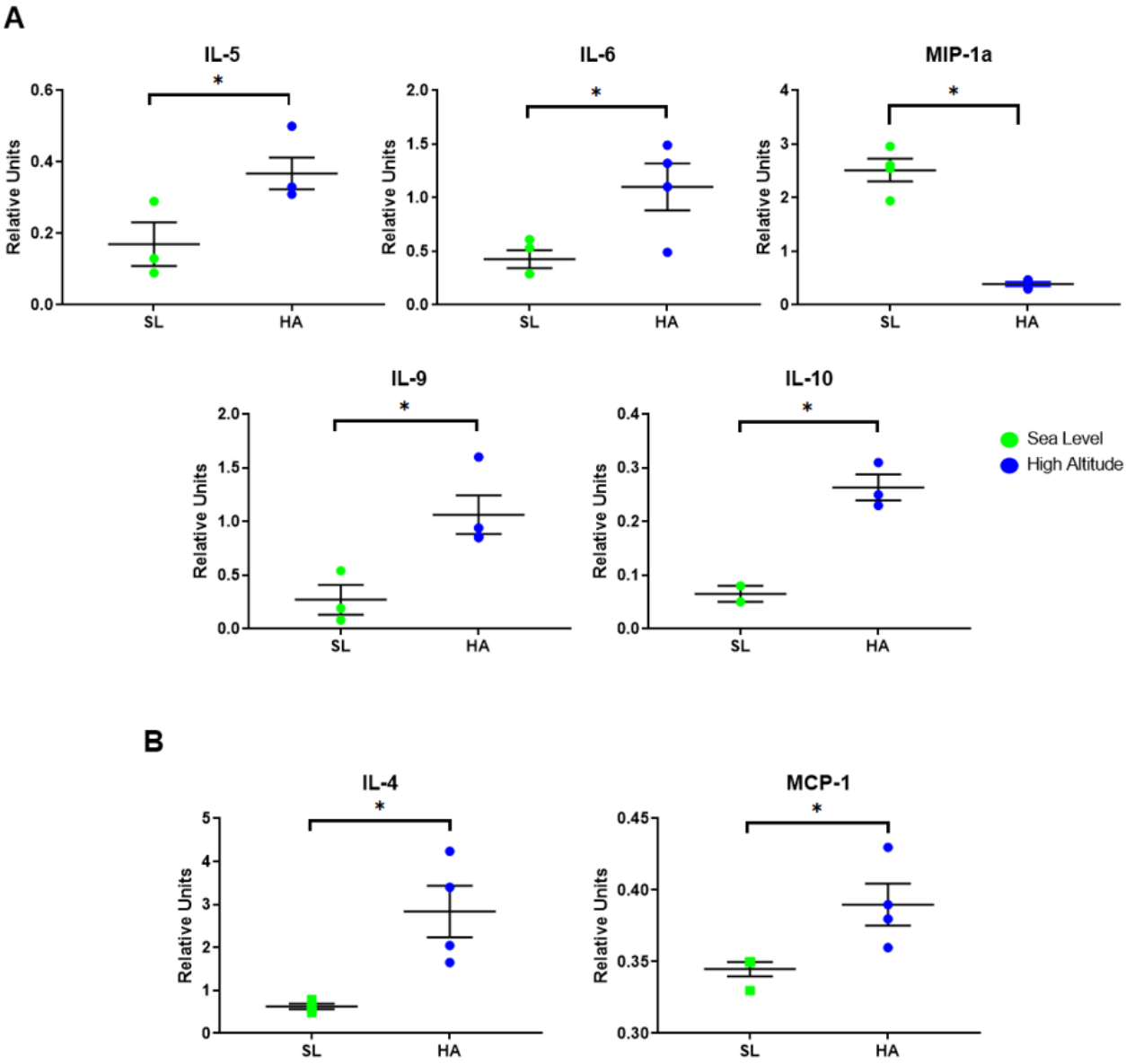

Figure 14. Region-specific changes in cytokine levels of cortex and hippocampus protein homogenate after 12 weeks high altitude.
Cortex (A) and hippocampus (B) significantly altered protein homogenate cytokine levels after 12 weeks high altitude exposure. Increased IL-5, IL-6, IL-9 and IL-10 and decreased MIP-1a is identified in the cortex, while increased IL-4 and MCP-1 is identified in the hippocampus. Unpaired t-tests, * $p < 0.05$, n = 3-4 mice per group, mean ± SEM.

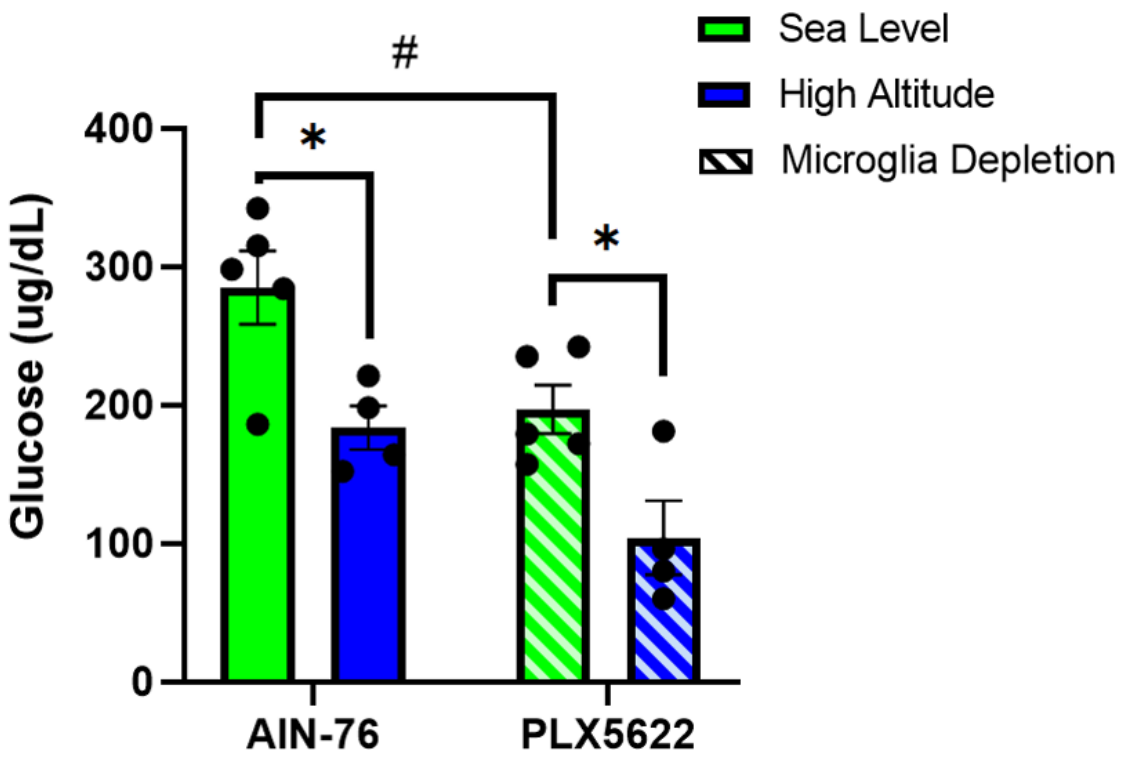

Figure 15. Peripheral blood glucose levels are decreased after high altitude exposure and after microglia depletion.
There is a significant main effect of high altitude exposure ($p = 0.0304$) as well as microglia depletion ($p = 0.0481$) on reducing blood glucose levels in female mice when measured with a traditional blood glucose meter. 2-way ANOVA with Tukey's multiple comparisons, n = 4-5 mice per group, mean ± SEM.

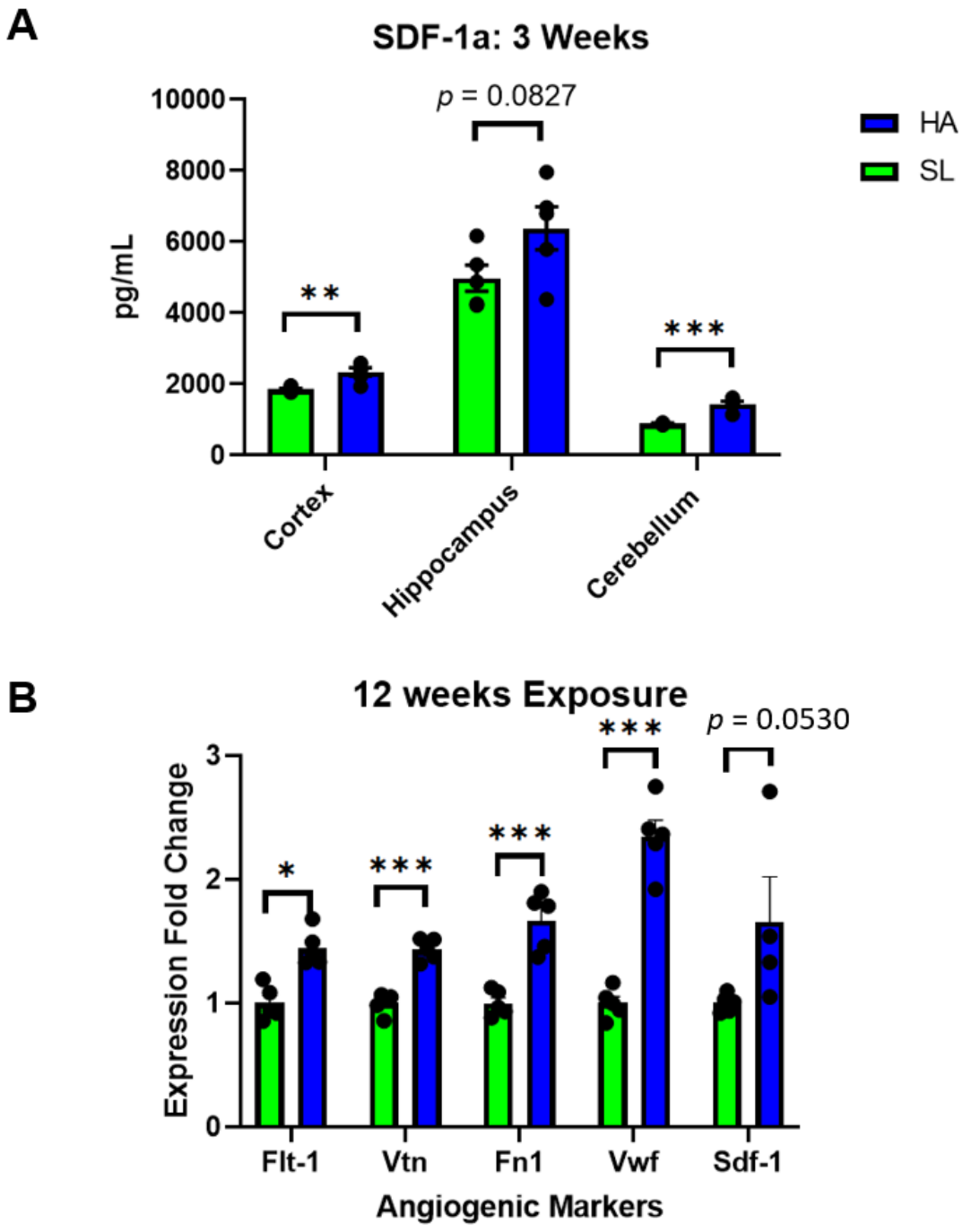

Figure 16. SDF-1a levels in protein homogenate are increased after 3 weeks high altitude.

SDF-1a protein levels are significantly increased in the cortex and cerebellum following 3 weeks high altitude exposure (A). This is consistent with previously identified increase in angiogenesis marker expression after 12 weeks high altitude exposure (B). Multiple unpaired t-test, n = 5 mice per group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, mean ± SEM..

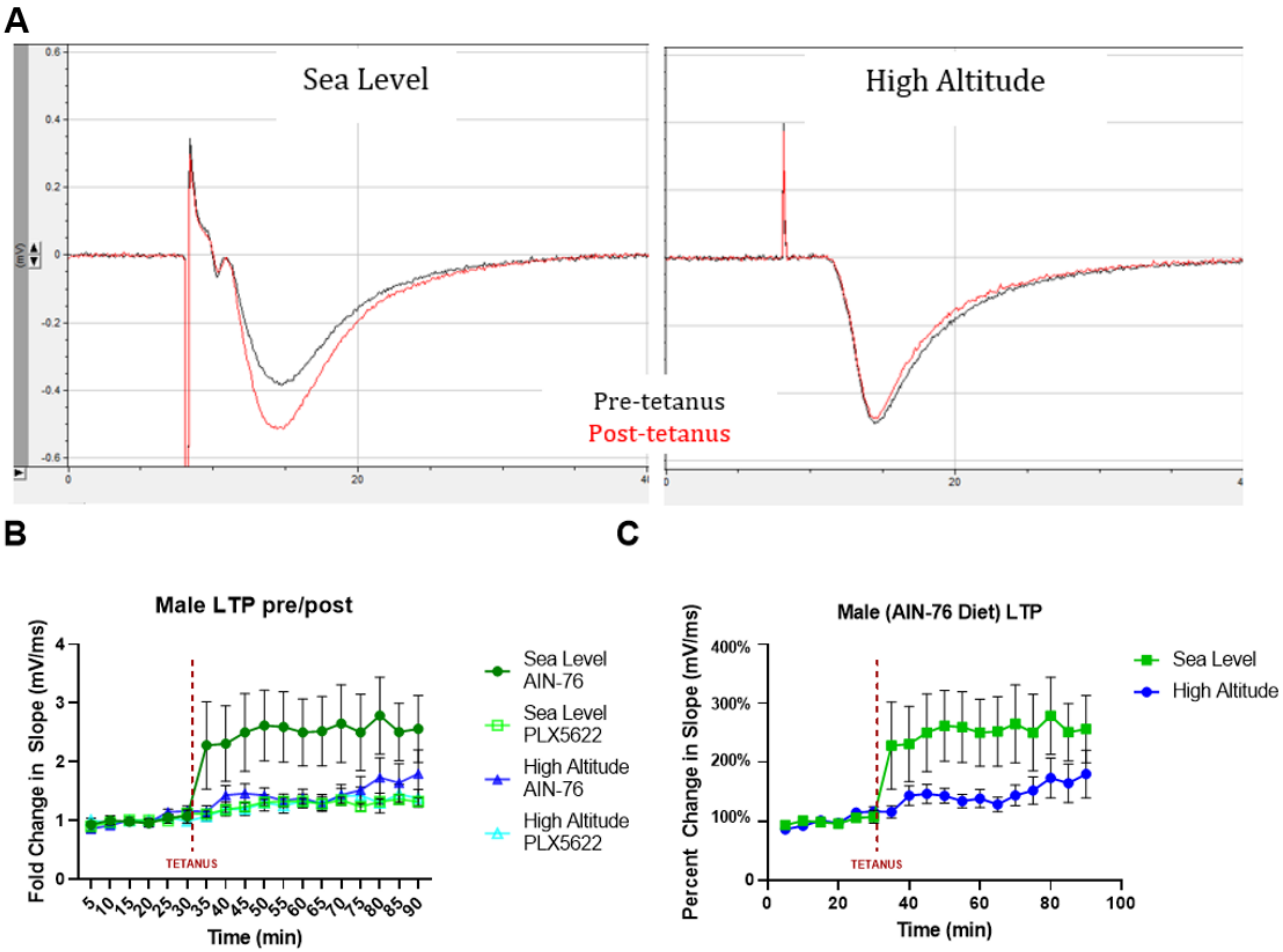

Figure 17. Reduction of CA1 hippocampal LTP after 3 weeks high altitude exposure.

Sample pre- (black) and post-tetanus (red) (tetanus: 100 Hz, 1 sec duration; Schaffer collaterals stimulated with electrode placed in CA3 area) traces for male sea level and high altitude control diet mice (A). Analysis using a 3-way ANOVA revealed a significant 3-way interaction effect of high altitude, sex and microglia depletion, so were performed an a priori analysis of male control diet mice to assess the sole effect of high altitude. While microglia depletion appeared to have similar effects on percent slope change as high altitude (B), we did not have the power to analyze these relationships. The a priori analysis revealed a significant reduction in percent slope change in LTP following 3 week high altitude exposure in male control diet mice compared to sea level (C). 2-way Repeat Measures ANOVA, $p < 0.05$, n = 6-14 mice per group, mean ± SEM.

CHAPTER 4: Discussion

THE INTERSECTION OF HIGH ALTITUDE, VASCULATURE AND INFLAMMATION

Oxidative stress and inflammatory microenvironment in the brain after high altitude and hypoxia exposure causes a shift in glial activity and metabolism that disrupts the regulatory balance of the neurovascular unit and leads to neurodegeneration (164). Cerebrovascular pathologies are significant factors in the development of dementia and neurodegenerative disorders (107; 294; 332). We have demonstrated alterations in vascular structure, integrity and function which contribute to the development of neuroinflammation after high altitude exposure. Accumulation of inflammatory mediators and toxins may be a primary contributor to the pathology of high altitude cognitive decline.

Hypoxia is a known driver of angiogenesis and vascularization, promoting transcriptional regulation of signaling cascades that contribute to increasing blood flow and brain oxygenation (156; 390). Hypoxia also induces oxidative stress that triggers extensive inflammation that can have negative structural impacts on blood-brain barrier integrity (21; 63; 120-122). In the present study, the role of vascular extension in high altitude adaptation is investigated. Evidence of blood-brain barrier disruption that may play a role in the observed changes to microglia process tip speed and proliferation in response to high altitude exposure is also identified.

Increased brain vascularization at high altitude

The brain is the primary source of oxygen and nutrient consumption in the brain; while it only represents 2% of total body weight, it consumes 20% of total body oxygen and 15% of the body's cardiac output (213; 278). Brain microvasculature plays a key role

in maintaining neurocircuitry/neuronal network homeostasis through oxygen and nutrient delivery and modulating inflammatory response, undergoing structural changes when necessary to regulate these processes (52; 145; 244). It is hypothesized that vasodilation is a transient adaptation mechanism during acute high altitude exposure that resolves and transitions to angiogenesis and vascular extension, primarily in the capillary beds. A significant increase in micro-vasculature in the brains of mice after high altitude exposure is expected. While increased vasculature after 12 weeks high altitude exposure has been established (63), quantification of the whole brain vasculature required the development of a cutting-edge scanning and analysis protocol. Preliminary quantification of 7 weeks high altitude exposure mice facilitated fine tuning of the quantification procedures while still maintaining high confidence in the presence of a high altitude effect that could be identified. After determining that 7 weeks high altitude increases whole brain vasculature volume (Fig. 5), it was important to establish how vascular remodeling occurs in the transitionary acclimatization period between acute and chronic exposure.

Analysis of mouse brains after 3 weeks exposure of high altitude revealed a more detailed picture of vascular changes, with increases in branching nodes and vessel endings indicative of angiogenesis as the driver (Fig. 7). The significant increased volume and length of vasculature with diameters in the 10-40 μm range is consistent with increases in arteriole/venule vessels and likely leading to increases of the capillary bed. Diving deeper into the region-specific analysis shows that brain regions are experiencing different levels of vascular remodeling, which may indicate different mechanisms participating in high altitude acclimatization in different regions, or that some regions are more susceptible to the effects of hypoxic stress due to differences in resting state

activities (Fig. 8). The hippocampus shows a greater degree of increased vasculature (fold change of volume and length), which could mean that it is particularly suited to vascular remodeling most likely because of hippocampal role in spatial memories formation. It is interesting to note that the role of microglia in this structural vascular adaptation is not initially a primary component of the angiogenesis mechanisms during high altitude exposure, but the microglia do seem to be important for maintaining the complexity of the vasculature at sea level (Fig. 9).

High altitude exposure induces chronic vascular leakage: implications for a role of putative inflammatory factors

Increased albumin staining after high altitude exposure suggests chronic leakage across the blood-brain barrier, and the extent of this leakage appears to be region specific (Fig. 10). Albumin maintains colloid osmotic pressure of plasma that facilitates the flow of interstitial fluid into the bloodstream. It has also been shown to play a neuroprotective role in some models of focal and global brain hypoxia (30; 31; 86; 263; 375). Astrocytes will take up albumin when the brain is exposed to serum albumin, mediated by TGF-β receptor activity and leading to neuronal hyperexcitability (144). Microglia have been shown to express albumin in the hippocampus following transient cerebral ischemia (256). Albumin increases microglia activation and proliferation, with microglia taking up extravasated albumin following blood-brain barrier disruption, but microglia are also capable of producing albumin (3; 8; 136). Others suggest albumin produced in microglia may actually promote neuronal death in neurodegenerative states (45). Studies of fetal growth restricted lambs involving chronic hypoxia showed reduced cerebral blood flow and increased oxidative stress, microglia activation and blood-brain barrier disruption (as assessed by albumin extravasation and increased albumin positive cells in the brain) (48;

215). Hypoxia in late gestation sheep has shown sequestration of serum albumin by cells in the thalamus and cerebellum, parenchyma albumin staining particularly around vessels and significant upregulation of albumin reactivity within hippocampal CA1, thalamus and cerebellar Purkinje cells (376). Neuronal hyperactivity in a mouse model of epilepsy is associated with blood-brain barrier disruption and increased leakage of serum albumin colocalized with neurovascular unit constituents including neurons, endothelial cells and microglia (22). It is possible that depletion of microglia reduces inhibitory action on neural circuitry, potentially promoting overexcitation that could damage blood-brain barrier integrity and contribute to the increased albumin expression seen in the cortex and hippocampus even in the absence of high altitude exposure (Fig. 10). The increased albumin in male mice after microglia depletion may support the theory that blood-brain barrier leakage after high altitude exposure is not solely a byproduct of angiogenic remodeling and may be affected by sex differences in microglial maintenance of blood-brain barrier integrity (122). Increased albumin staining after chronic high altitude exposure indicates that leakage across the blood-brain barrier results in the accumulation in the extracellular environment that can lead to inflammation, but this may be related to failed clearance of the albumin by glial cells or the glymphatic system. It demonstrates that integrity was compromised after high altitude exposure, but not whether the integrity is still compromised at a chronic timepoint. To assess this, future studies should utilize albumin or dextran dye injection prior to perfusion and immunohistochemical analysis to see the instantaneous measure of blood-brain barrier disruption. Assessment of endothelial tight junction proteins is also an alternative, as other studies have shown that 1 week hypoxia causes an increase in occludin and ZO-1 expression that is impaired

following microglia depletion (120; 121). Identifying changes in tight junction expression after 3 weeks high altitude exposure would further reveal the transitional acclimatization impact on blood-brain barrier integrity as exposure becomes more chronic.

Sex specific microglia chemotactic response and tip proliferation

Our experiments analyzing microglia motility have revealed a region and sex specific difference in microglia homeostatic and reactive activity. Females exhibit a greater degree of variability in tip speed during surveillance and have a greater number of process tips in the hippocampus compared to the cortex or males, possibly indicative of a greater degree of cell ramification during spontaneous activity (Fig. 11). In response to laser ablation, male microglia have a greater degree of tip proliferation in the cortex, while females show a particular sensitivity to vessel damage in the cortex. Estrogen receptor expression on microglia may contribute to differential motility profiles observed in our 2-photon experiments (341). Estrogen has been shown to promote anti-inflammatory microglia phenotypes, impacting microglia behavior and phagocytic activity and reducing neurodegeneration (195; 367). Female microglia also show a particular sensitivity to vessel ablation in the cortex, suggesting microglia respond to tissue damage using a nuanced signaling identification mechanism that goes beyond simple ATP sensing through purinergic signaling. Additionally, male microglia show a significant suppression of tip proliferation during ball formation response activity in the cortex after high altitude exposure, possibly relating to confusion of extracellular signaling gradients due to increased blood-brain barrier disruption. Most of the observed sex differences are only present at sea level, which suggests hypoxic stress may cause microglia to achieve a base level of activity dynamics which are intrinsically determined.

Preclinical evidence shows that males and females use different signaling mechanisms to overcome cellular stress of high altitude exposure, possibly involving cyclooxygenase-2 which is implicated in sex differences and inflammation after high altitude as well as microglia activation (53; 66; 372).

Metabolic and signaling pathways underlying mechanisms of high altitude adaptation

The differences in microglia activity profiles seen after high altitude support the idea that microenvironment in this newly vascularized extracellular space is contributing to creating an inflammatory microenvironment which could affect glial and neuronal interactions. Extended exposure to reduced oxygen levels activates gene transcription of VEGF and HIF that facilitate the initiation of angiogenesis (156; 390). VEGF concentrations work as a signal to guide vascular branch patterns and the extension and sprouting of endothelial tip cells (90; 102; 104; 110; 267). Wnt signaling, which is critical for maintenance of blood-brain barrier integrity, decreases in endothelial cells after angiogenesis, suggesting a period of leakiness associated with vascular remodeling (59; 90; 274; 353; 388). High altitude exposure is suspected to result in gross blood-brain barrier dysfunction, in part due to free radical destabilization of membranes mediated by lipid peroxidation, inflammation, and activation of local HIF-1α and VEGF signaling cascades (21; 63; 163). In addition to shifting the brain microenvironment to a proinflammatory profile, hypoxia also affects the transport of glucose across the blood-brain barrier, impacting cellular metabolism (126; 168; 251). Alterations in aquaporin-4 (water channel membrane protein) expression/distribution on astrocytic endfeet increasing water permeability facilitates the development of high altitude cerebral edema (387), which may have implications for waste clearance via the perivascular spaces.

Changes in the extracellular environment associated with the structural augmentation of the neurovasculature after high altitude may lead to regional inflammation with an effect on microglial and neuronal activity. This is supported by the increased expression of pro-inflammatory cytokines in brain homogenate after 12 weeks high altitude exposure (Fig. 12). Increased IL-10 is associated with hyperactivity of hippocampal neurons in patients with temporal lobe epilepsy, SDF-1a is involved in silencing tonic activity of neurons in the hippocampus, and IL-6 has a negative regulatory role in memory acquisition (14). Cytokine modulation relating to increased blood-brain barrier disruption and neuroinflammation after high altitude exposure may disrupt the delicate balance of neurotransmission required to maintain healthy levels of synaptic plasticity.

Hypoglycemia can also impair LTP induction (290). Mice exhibit decreased levels of peripheral glucose. During hypoxia, a shift towards anaerobic respiration in processes involving cellular metabolism may increase demand for glucose and cause hypoglycemia, potentially contributing to reduced levels of hippocampal synaptic plasticity. Metabolic efficiency is affected during adaptation to chronic hypoxia, as corroborated by clinical positron emission tomography (PET) imaging that shows increased glucose uptake in the heart after high altitude exposure (54; 313), as well as significant changes in regional cerebral glucose metabolic rates after chronic high altitude exposure, with glucose metabolism observed to be increased in the cerebellum, altered in the thalamus and decreased in the occipital and frontal lobes (134; 227). Short term hypoxia has been shown to increase cerebral lactate and glucose concentrations, as well as increasing cerebral metabolism (340). The impact of high altitude on blood glucose

levels is contradictory, with studies showing both increased and decreased glucose readings after altitude exposure (81; 127; 133; 167).

Hippocampal synaptic plasticity, hypobaric hypoxia and the role of microglia

The present study has revealed changes in synaptic plasticity expressed as impaired LTP in the hippocampus of male mice after 3 weeks high altitude exposure. Memory consolidation and learning rely on synaptic changes relating to the induction of LTP and LTD. Inability to elicit these lasting changing in neurocircuitry communication results in functional deficits. Cognitive impairment after high altitude exposure is clinically reported, and we have previously reported deficits in hippocampal mediated memory in our mouse model (63). Future studies are necessary to determine if microglia depletion may alter synaptic plasticity. Removal of microglia could prevent inhibitory activity at the synapse, causes hyperexcitability of neurons and preventing induction of LTP. This is supported by data showing that seizure hyperactivity of neurons in a mouse model of epilepsy causes excitotoxicity and also inhibits LTP (109). For male mice exposed to high altitude but with microglia intact, impairment of LTP could be associated with overactivation of microglia in a pro-inflammatory manner due to oxidative stress (76). Chronic hypoxia has been shown to increase endothelial fractalkine (CX3CL1) expression and the activation of its cleaving enzyme ADAM17, which could increase the distribution of soluble CX3CL1 and disrupt the normal CX3CL1/CX3CR1 axis activity between microglia and neurons, thereby contributing to elevations in IL-1β and subsequent reduction of LTP (288; 368; 373; 381). There are significance evidence showing important role of hypothalamus in regulation of systemic glucose

metabolism(273) and recent study show involvement of microglia in hypothalamic regulation of glucose homeostasis (364).

The role of microglia in synaptic plasticity changes after high altitude is further supported by behavioral data from fear conditioning and Y-maze assays (Fig. 19). In contrast to the experiments presented previously, mice were depleted of microglia prior to high altitude exposure (or given control chow), depletion treatment continued throughout the 3 weeks of high altitude exposure, and then mice were brought to sea level and given control chow for 2 weeks to allow microglia to repopulate the brain, essentially resetting their activity to the pre high altitude state. Hippocampal mediated deficits in learning and memory after high altitude exposure were rescued by microglia repopulation. There is further evidence that the role of microglia in synaptic activity is region specific, as shown by the enhancement of amygdala mediated learning and memory in sea level and high altitude exposed mice.

LIMITATIONS

Environmental enrichment

Several factors may influence some of the physiological changes observed in the studies presented here. One such consideration is the presence of environmental enrichment in the mouse cages. Throughout our studies we provide nestlets as a source material for nest building activities, but the mice are not offered any toys or additional structural elements to provide cognitive stimulation. Rodent studies have demonstrated that environmental enrichment can enhance synaptic plasticity in the hippocampus, affecting the capacity for induction of long-term potentiation and long term depression (44; 306). There is also evidence that environmental enrichment can prevent cognitive

impairment in a rat model of high altitude exposure, working via VEGF signaling (159). Future studies should explore if increasing the degree of environmental enrichment offered in our mouse model could have an impact on neuronal activity and behavioral outcomes.

Vasculature quantification and sex differences

Despite having the scanning resolution to image microvasculature (capillaries < 10 μm diameter), the algorithm used for tracing did not capture many vessel segments in that range, possibly due to poorer contrast between signal and background (Fig. 20). It is likely that improving quantitative assessment of microvasculature will improve understanding of vessel-specific mechanisms involved in high altitude acclimatization. Additionally, greater sample sizes are needed to confirm that there are no sex differences in high altitude induced vascular remodeling and angiogenesis. Females have greater pools of endothelial progenitor cells, which may contribute to the ability to compensate for hypoxic stress with angiogenesis (94). Sex differences in microvascular morphology and function suggests that males and females may maintain vascular homeostasis through different mechanisms, possibly involving sex specific hormones (40; 68; 139; 140; 252). One study found that testosterone actually helps to alleviate hypertension induced by hypoxia at high altitude (147).

Albumin positive cells

During assessment of immunohistochemical staining for albumin after high altitude exposure as a measure of chronic blood-brain barrier leakage, a substantial number of cells that appear to be albumin positive were observed. These cells seem to be most abundant in the thalamus, although albumin positive cells were observed in the

cortex and hippocampus as well. These cells were not quantified, but appeared less prevalent in microglia depleted samples, suggesting the possibility that the albumin positive cells are either microglia or that microglia facilitate the albumin accumulation on these cells. A 2-photon image was taken to provide an example of this albumin positive cell population located in the thalamus of a high altitude exposed male mouse, demonstrating that the albumin is present in the cytoplasm of the cells and not just on the membrane surface. (Fig. 18)

FUTURE DIRECTIONS AND TRANSLATIONAL IMPLICATIONS

Glymphatic Clearance and Sleep

Due to the observed increases in neurovasculature and altered microglia activity and inflammation after high altitude exposure, assessment of the glymphatic system is a natural progression of this research. The glymphatic system is a perivascular waste clearance system which eliminates soluble proteins and metabolites from the central nervous system and facilitates distribution of critical compounds necessary for brain functioning (146; 196; 229; 246). This system is primarily active during sleep (particularly slow wave sleep) and utilizes pulsation of the vasculature paired with glial cell cooperation to aid in efficient clearance (115; 146; 246; 270; 333). Aquaporin-4 is the main water channel component of the glymphatic system, and increased aquaporin-4 expression by astrocytes is associated with the development of high altitude cerebral edema (302; 333). Hypoxia exposure simulating an altitude of 4500 m has been shown to reduce total sleep time and efficiency, as well as decreasing time spent in slow-wave sleep and rapid eye movement (REM) sleep stages (71). Cognitive function and mood also declined after hypoxia in association with altered sleep patterns (71). While

normobaric and hypobaric hypoxia both result in altered sleep architecture, increased heart rate and reduced oxygen saturation, the effects of hypobaric hypoxia were more severe, suggesting an important role of hypobaria in high altitude acclimatization and possibly implicating a more robust impact on glymphatic function (130). Clinical investigations have used cranial magnetic resonance imaging to discover a potential link between enlarged Virchow-robin spaces in high altitude associated headache (7).

Indeed, small vessel disease related dementia is associated with oxidative stress, chronic hypoxia, neuroinflammation, neurovascular and microvascular dysfunction, mitochondrial/metabolic dysfunction, and white matter damage/neurodegeneration (234). Impaired elimination of interstitial fluid and hypoxia contribute to white matter hyperintensities in dementia (209). Impairment of the glymphatic system is associated with sleep disturbance, neuroinflammation and tau pathology after brain injury (41; 79; 141; 268). Increased vascularization to accommodate the need for improved oxygen delivery after high altitude exposure may exacerbate malfunctioning of the glymphatic system and related sleep disruptions. Cerebral blood flow is increased during REM sleep in most brain regions indicating a higher rate of energy consumption through cerebral metabolic rate, but cerebral synaptic activity during REM sleep is comparable to wakefulness and blood-brain barrier permeability to glucose is not altered to accommodate changes in region specific glucose metabolism (119; 205; 211; 301). The incidence of sleep disruption at high altitude and the increased utilization of glucose to compensate for oxidative stress may be indicative of additional strain on neurological function leading to cognitive impairment. This would be consistent with findings that sleep deprivation prevents rhythmic modification of perineuronal nets that are necessary

for memory consolidation during sleep (255). Additionally, brain injury and cognitive dysfunction related to diabetes and hyper- and hypo- glycemia are associated with vascular pathologies relating to oxidative stress and neuroinflammation, mitochondrial dysfunction, and impaired glymphatic clearance (123).

Potential molecular targets

Stromal cell derived factor 1, SDF-1, (also known as C-X-C motif chemokine ligand 12, CXCL12) is a ligand for the chemokine (C-X-C motif) receptors 4 and 7 (comprising the CXCL12 axis), and transcriptionally regulated by hypoxia inducible factor 1 (HIF-1) (50). SDF-1 is transcriptionally upregulated in the hippocampus and amygdala following 3 and 12 weeks high altitude exposure (63). It is strongly evolutionarily conserved, with ubiquitous expression among humans, mice and lower vertebrates (299). It plays a critical role in development, regulating cell migration and vascular guidance (39; 186; 312; 330; 343). Interneuron leading process branching behavior and migration speed is heavily dependent on this axis, as well as regional interneuron distribution and the development of inhibitory tone (177; 207; 208; 331). The axis is also essential for cerebellar development, regulating the chemotaxis and proliferation of granule cells and mediating axonal growth cone guidance (158; 199; 342; 370).

During adulthood, CXCL12 axis modulates synaptic transmission in the cerebellum, altering communication between parallel fibers and Purkinje neurons (187; 279). It is necessary for the successful migration of endothelial progenitor cells from bone marrow stroma, increasing their proliferation and adhesion, and promotes angiogenesis (58; 175; 180; 249; 304; 349; 350). Evidence suggests that the CXCL12

axis is involved in brain injury recovery and repair, enhancing remyelination and neuroblast migration, reducing apoptosis, and stimulating neovascularization (12; 61; 114; 143; 181; 216-218; 315; 317). It is also implicated in regulating CNS immune privilege and leukocyte infiltration at the blood-brain barrier, which may influence interactions with inflammatory mechanisms following high altitude exposure and injury, as hypoxia (a critical factor in both conditions) induces substantial upregulation of CXCL12 (91; 113; 196; 223; 295; 296; 318). The CXCL12 axis is also involved in mitochondrial cell function and metabolism, another important factor in hypoxia (70; 226; 228; 235; 383).

CXCR4 and CXCR7 (also known as atypical chemokine receptor 3) are members of the G-protein coupled receptor (GPCRs) family. The receptors can exist in monomer, homodimer or heterodimer forms, and are involved in numerous diverse downstream signaling pathways (259). In areas of focal brain ischemia, CXCR4 promotes monocyte infiltration across the blood-brain barrier and mediates microglia response in the infarct area (357). SDF-1α stimulation of microglia via CXCR4 signaling after stroke upregulates pro-inflammatory IL-6 production (198). Interestingly, CXCR4 expression has been shown to increase following dexamethasone treatment, which is one of the current medications prescribed to reduce symptoms of acute mountain sickness (49).

Adenosine is a metabolite that works to maintain balance between energy supply and demand during periods of oxygen deprivation or increased energy requirements (97). Hypoxia modulates the expression of adenosine receptors, promoting an angiogenic phenotype and contributing to vasodilation (97). Adenosine activation exerts anti-inflammatory modulation on HIF-1 expression, reducing accumulation in astrocytes after

hypoxia (103). Adenosine A1 receptor signaling has also been shown to promote enhanced memory and long-term potentiation in a model of chronic intermittent hypoxia, playing a neuroprotective role to prevent cognitive dysfunction associated with hypoxia (382). However, hypoxia induced adenosine activation can also cause persistent synaptic depression through internalization of GluA1 and GluA2 leading to hippocampal neurodegeneration (55). Interestingly, the effect of adenosine activity is highly region dependent, with enhanced adenosine A1 receptor expression in the cortex shown to be antidepressant but overexpression in the hippocampus causing impaired long-term potentiation (297). We previously identified an increase in hippocampal A1R transcription in the hippocampus after 3 weeks high altitude exposure, which may be a contributing factor in our observation of reduced hippocampal long-term potentiation (63).

Erythropoietin (EPO) is a hormone primarily produced by the kidneys to promote formation of red blood cells by the bone marrow; it can cross the blood-brain barrier, but is also endogenously expressed under hypoxic conditions by neurons and astrocytes in the brain, and EPO receptors are expressed by neurons, glia, endothelial cells and neural progenitor cells (243; 344). It has been used to stimulate neurogenesis and improve cognition, promoting anti-inflammatory signaling and regulated by HIF-1 transcription and exhibiting neuroprotective effects (193; 243; 291; 344; 345; 347). EPO promotes a balance between microglia activation, apoptosis and neurodifferentiation (42; 98). Microglia express EPO receptors which are involved in attenuating the production of inflammatory cytokines and reducing morphological changes related to microglia activation (325). EPO facilitates cell migration via interactions with the SDF-1 axis (179;

316), further implicating a role in high altitude adaptation. Erythropoietin contributes to sex-specific glucose sensitivity (384). EPO activity regulates metabolic and glucose homeostasis and contributes to promoting angiogenesis and vascular response to hypoxia (319). Increased vasculature in the kidneys and spleen following hypoxia induced angiogenesis during high altitude exposure may contribute to increased EPO production and recruitment of hematopoietic stem cells to induce the generation of erythrocytes for restoration of tissue oxygenation and thus contribute to alleviating hypoxic stress on cognitive function (Fig. 21). Additionally, the spleen, as a reservoir for endothelial progenitor cells via the SDF-1 axis, may play a crucial role in promoting the vascular remodeling observed after high altitude exposure (385).

Clinical directions

In recent years the significance of the gut-brain axis has gained increasing attention, with gastrointestinal environment and gut microbiota recognized as having a significant impact on nervous system function. Evidence suggests the gut microbiome may contribute to variability in metabolic and physiological response to high altitude exposure, including severity of acute mountain sickness (150). Hypoxic and oxidative stress at high altitude can cause injury to intestinal barrier leading to inflammatory response and potentially compromising the gut microbiota (154; 225). Furthermore, several circulating metabolites have been identified that are released by gut bacteria and exert effects on components of the vascular system including endothelial cell function (9). This could have serious implications for angiogenesis and vasodilation function of vasculature in chronic high altitude adaptation response.

Ischemic preconditioning has been explored as a possible tool to reduce the maladaptive effects of high altitude exposure, with studies focusing on improving mechanisms of acute physiological adaptation like hypoxic ventilatory response and arterial oxygen saturation (32; 286). Remarkably, preconditioning paradigms range from short-term high altitude simulations to repeated bouts of peripheral limb ischemia (286). Intermittent normobaric hypoxia has been shown to facilitate acclimatization to high altitude by reducing inflammation (101). Evidence suggests the protective mechanisms of hypoxic preconditioning work through adenosine receptor 1 signaling, promoting recovery of hippocampal synaptic plasticity following hypoxia exposure (275).

Future directions may investigate how microglia depletion can be utilized as a treatment following high altitude exposure, to reset microglia activity and hopefully restore normative synaptic plasticity by interrupting the feed forward cycle of neuroinflammation. Perturbation of vascular adaptation mechanisms would be risky, but we should assess whether changes to cerebrovasculature are reversible following return to sea level. Given the differential impact of high altitude on males and females, further investigation into its effect on the reproductive cycle may be warranted.

SUMMARY

The experiments performed here demonstrate that chronic high altitude exposure induces structural neurovascular adaptation that is not initially dependent on microglia (Fig. 22). Robust adaptation is seen in the cortex and hippocampus, and may rely on different mechanisms. High altitude exposure compromises blood-brain barrier integrity, with different brain regions showing greater vulnerability to disruption; however the leakage in blood-brain barrier is not a byproduct of angiogenesis. Increased extracellular

inflammation affects microglia chemotaxis in a sex and region dependent manner during homeostatic surveillance activity and during directed reactivity. Microglia response dynamics are impacted primarily by changes in degree of process tip proliferation after high altitude and these changes are region and sex specific. Peripheral glucose levels are reduced under hypoxic conditions as well as following microglia depletion, suggesting a role of microglia in metabolic regulation. Inflammatory cytokine fluctuations including increased SDF-1 expression are region specific. High altitude exposure causes impairment of synaptic plasticity in the CA1 area of the hippocampus in males, which is consistent with previously reported functional deficits and may be influenced by the new microenvironment and microglial interactions. The signaling pathways relating to hypoxia induced factors are complex and interconnected, with molecular targets often exhibiting protective and maladaptive activity profiles. This makes it quite challenging to identify promising simple therapeutic targets to improve cognitive outcomes. Future research may focus on improving systemic function of integrated processes like sleep and glymphatic clearance over antagonism of individual receptor/ligand interactions.

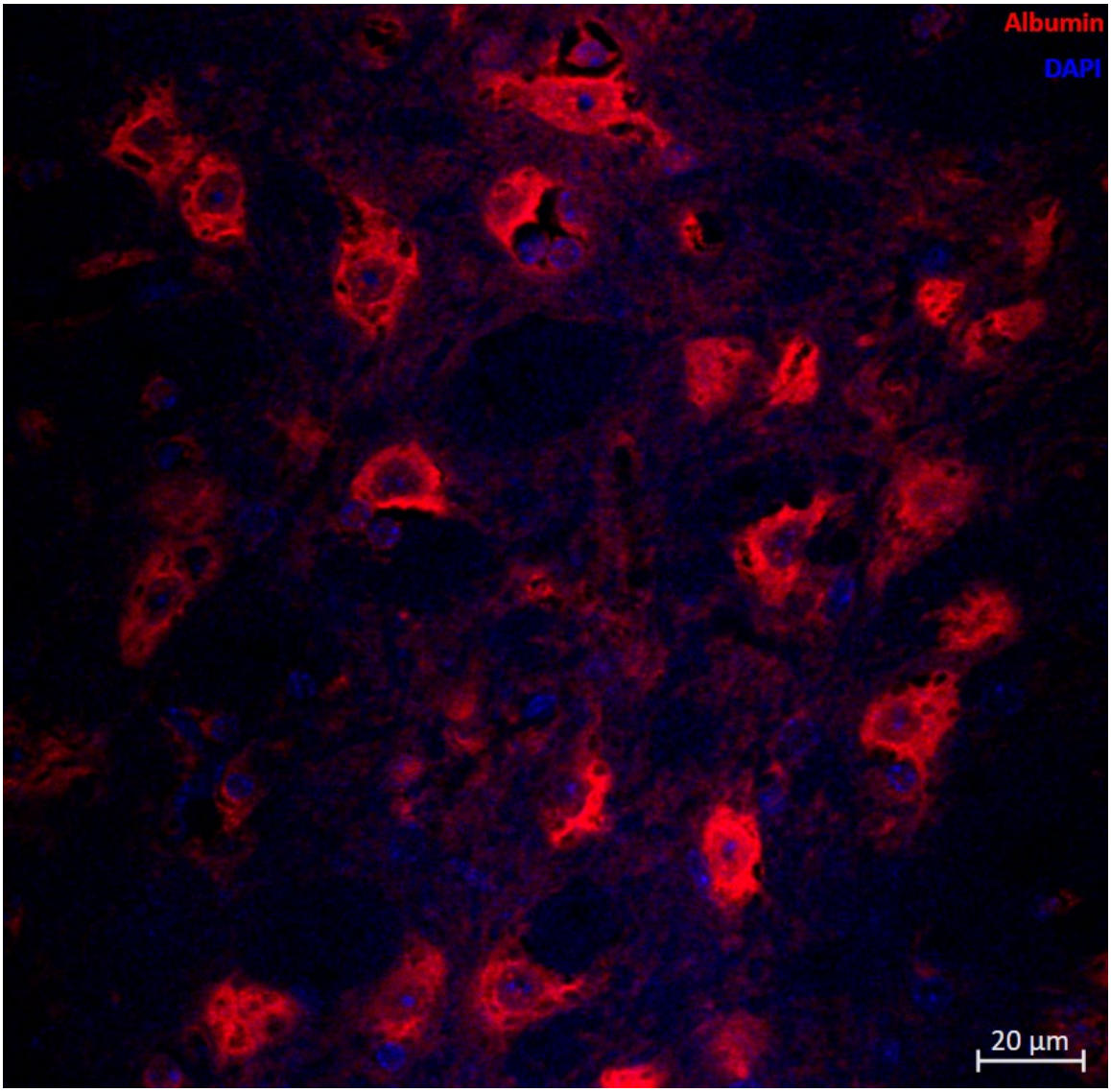

Figure 18. Albumin positive cells identified through immunohistochemistry.
Sample 2-photon image from the thalamus in control diet male mouse with microglia intact after 3 weeks high altitude showing albumin positive cells with expression in the cytoplasm, not surface membrane staining.

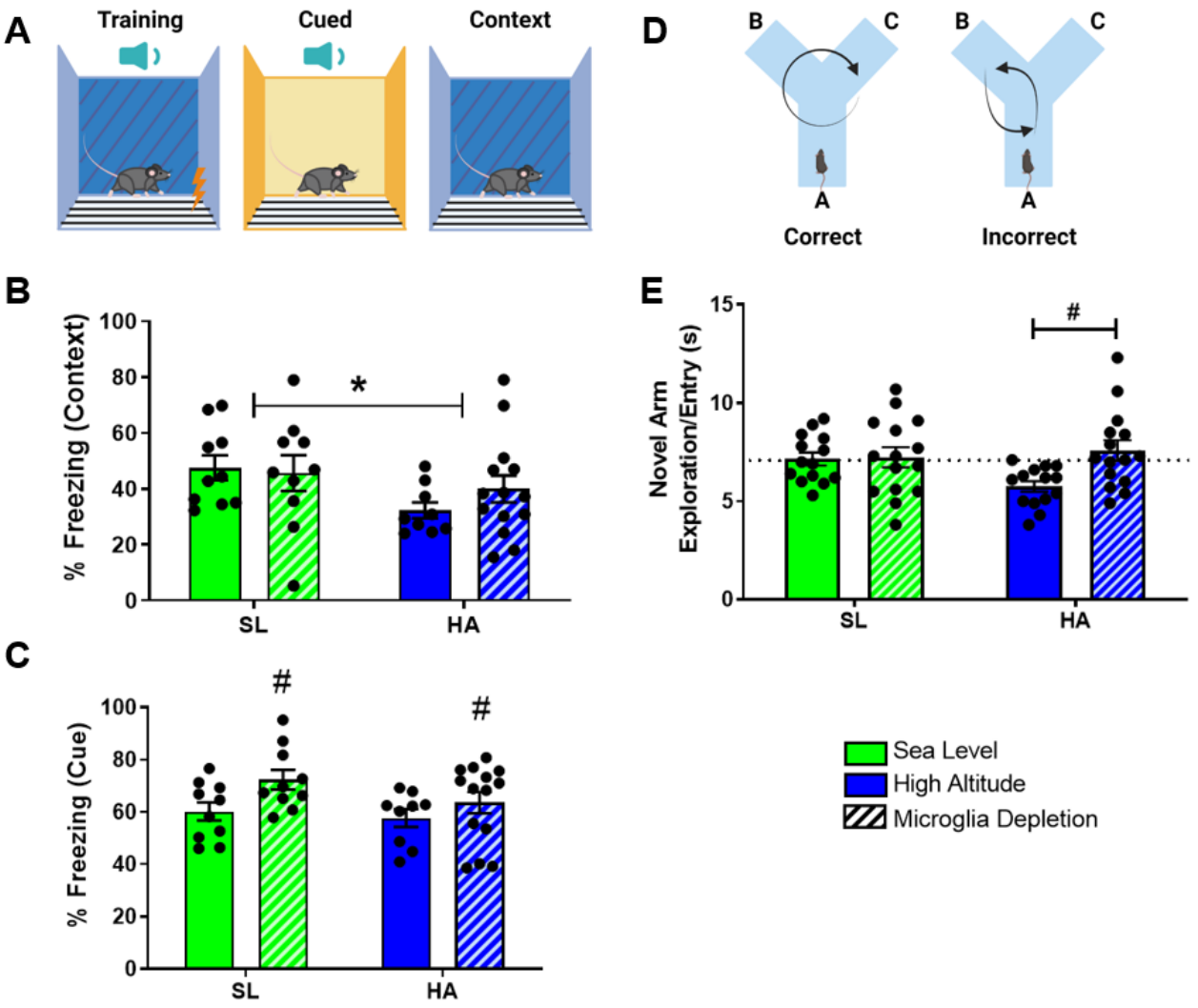

Figure 19. Behavioral deficits after 3 weeks high altitude exposure can be rescued by microglia repopulation.

For the fear conditioning assay (A-C), mice are trained in an associative learning task to pair environmental context and auditory cue with a foot shock, and subsequently tested on the percent time freezing when presented with the same context or cue as a test of fear memory (A). The spontaneous Y-maze assay (D-E) tests short term spatial memory by assessing how often the mice explore a novel arm instead of doubling back to a previously explored are (D). In this figure, mice experienced microglia depletion or control diet prior to and during high altitude or sea level exposure, followed by 2 weeks at sea level for microglia repopulation. 3 weeks high altitude exposure causes deficits in hippocampal mediated learning and memory as seen by decreased freezing in the context fear condition (B). Hippocampal memory is rescued by resetting microglia through repopulation (E). Microglia depletion enhances amygdala mediated memory as shown by increased freezing in the cued condition (C). 2-way ANOVA, Bonferroni posttests, n = 15 mice per group, df = 54, $p < 0.05$

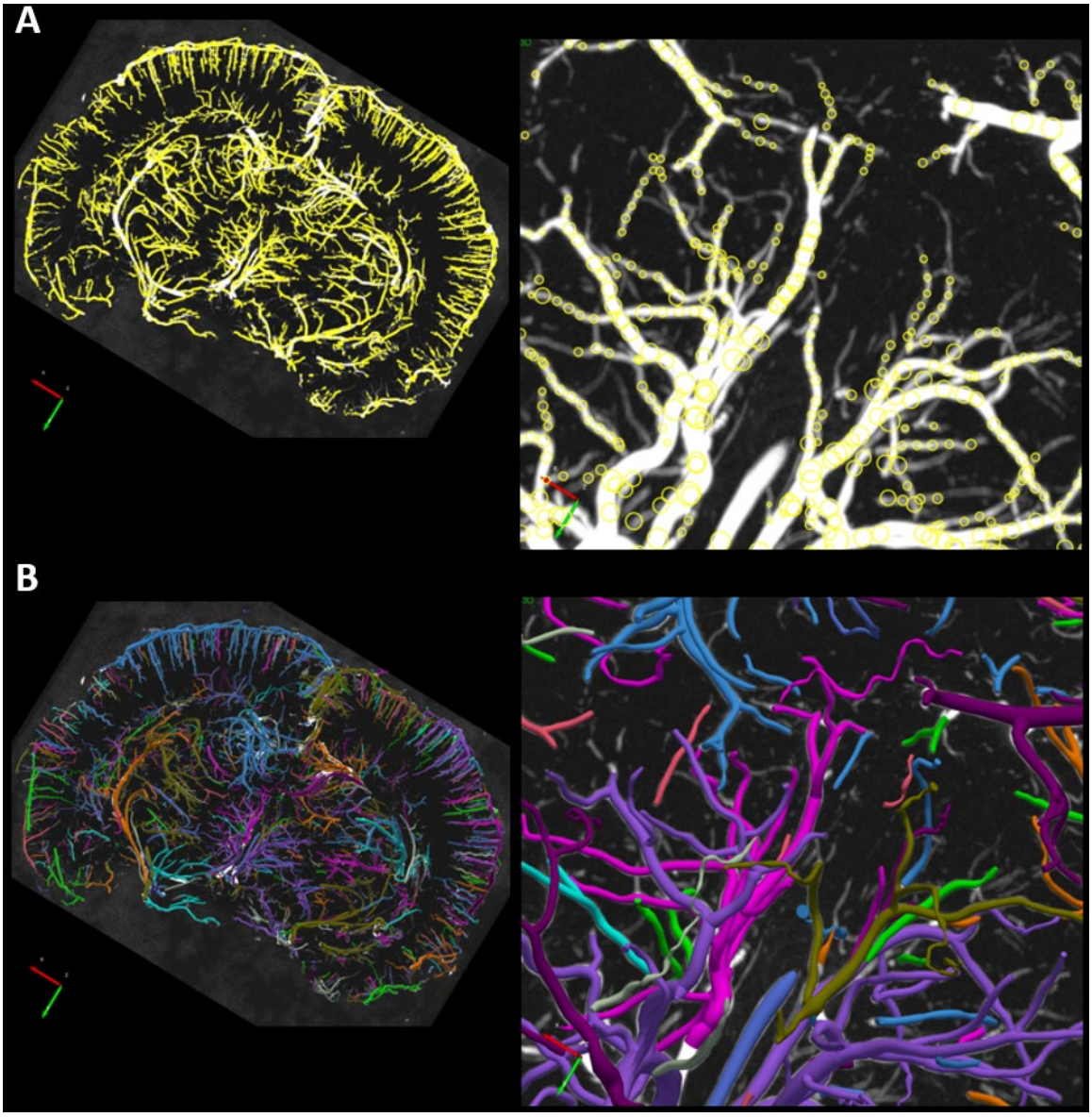

Figure 20. Limitations of Vesselucida software for tracing capillaries in whole brain datasets.

Vesselucida seed detection/validation (A) algorithms in dense vasculature data sets can get overwhelmed by the abundance of vessels and omit small diameter capillaries and microvessels in final tracings (B).

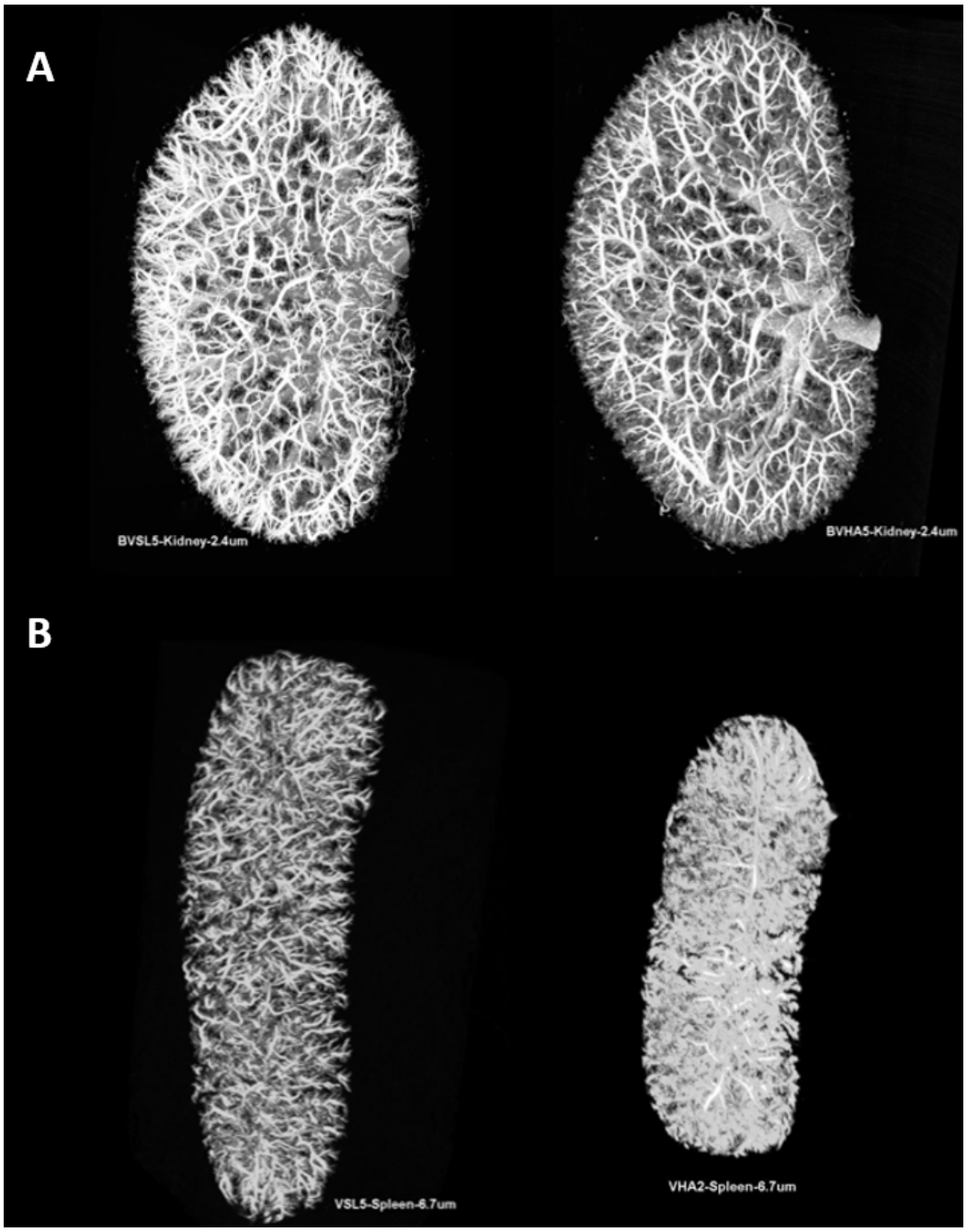

Figure 21. Micro-CT imaging of kidney and spleen after high altitude.
High resolution micro-CT images of kidney (A) and spleen (B) after sea level (left) or 7 weeks high altitude exposure (right) and subsequent perfusion with high contrast agent BriteVu. Kidneys were scanned at 2.4 μm resolution and spleens were scanned at 6.7 μm resolution.

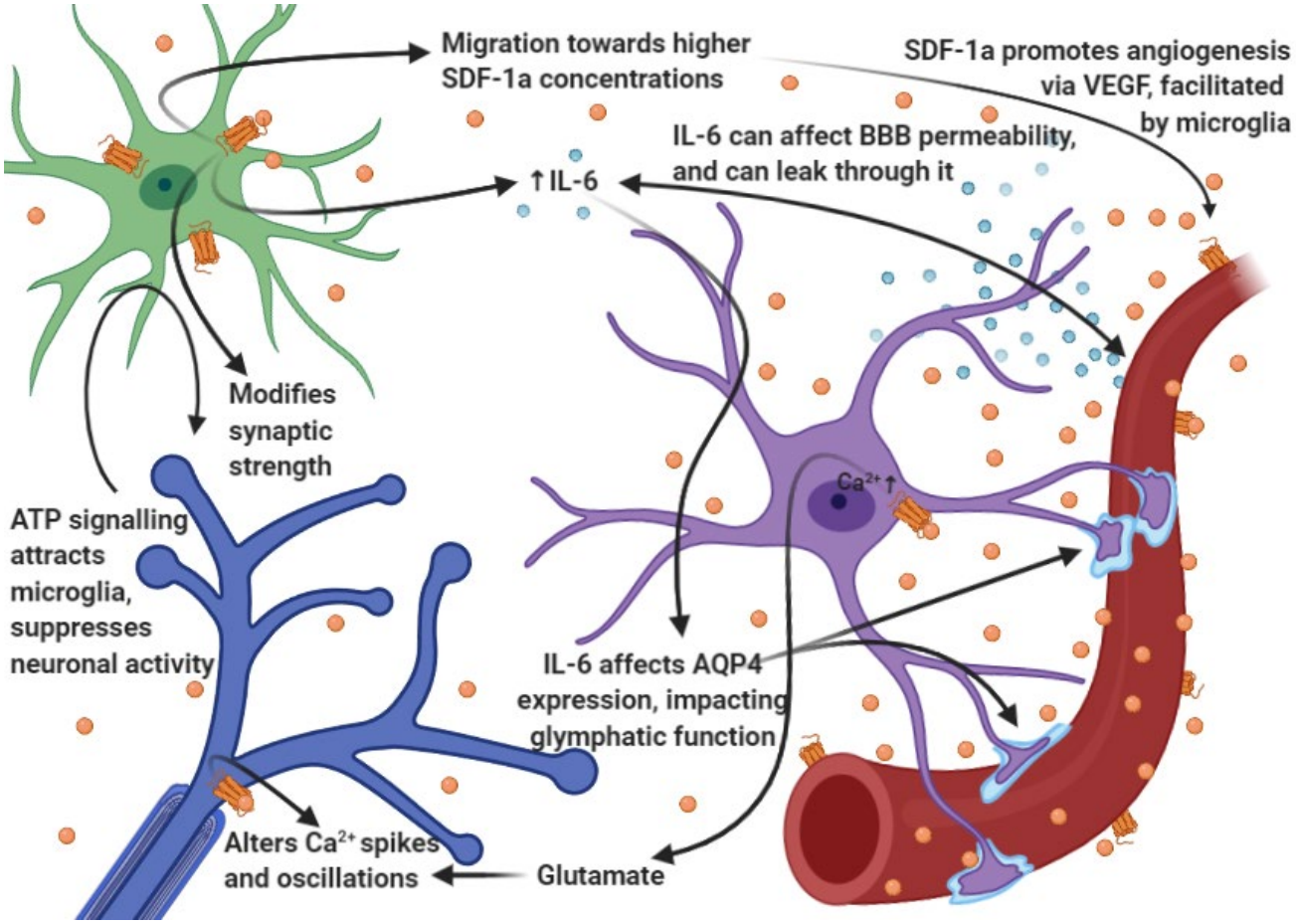

Figure 22. Summary of mechanisms behind 3 week high altitude acclimatization. High altitude causes angiogenesis and vascular remodeling which is not initially dependent on microglia activity. Blood-brain barrier integrity is disrupted at high altitude, with microglia (green) playing a protective role in stabilization. The microenvironment resulting from leakage and oxidative stress causes inflammatory crosstalk with mechanisms of high altitude acclimatization that involve metabolic regulation. Changes in the extracellular signaling environment and microglia activity contribute to dysfunctional neuronal (blue) and synaptic plasticity, leading to reduced long-term potentiation after high altitude that is consistent with observed cognitive deficits. The role of microglia in this process is further confirmed by restoration of function after microglia repopulation. Components of the perivascular spaces (including astrocytes, purple), glymphatic system and neurovascular unit are likely involved in chronic high altitude acclimatization. (Created with BioRender.com)

SUPPLEMENTAL

MatLab Coding Script for Micro-CT Regional Analysis by Dr. Andrew Knutsen

```
%%
%%% Step 0: Update paths
RD = '/Volumes/My Passport/BVHAFP-2_Rec/'; % folder with raw jpg
files

nm = 'BVHAFP-2'; % subject ID
MD = ['/Users/tif/Research/microCT/' nm]; % folder to write
processed data
if ~exist(MD,'dir')
    mkdir(MD)
end

AR =
'/Users/tif/Research/microCT/ProcessingPipeline/ANTs_AffineRegist
ration.sh'; % affine registration shell script
TRi =
'/Users/tif/Research/microCT/ProcessingPipeline/ANTs_ApplyTransfo
rmInverse.sh'; % inverse transformation

template =
'/Users/tif/Research/microCT/ProcessingPipeline/mMaMouseT2Mod.nii
'; % template
seg =
'/Users/tif/Research/microCT/ProcessingPipeline/MaMouseSegMod.nii
'; % atlas labels

%%
%%% Step 1: Create subsampled micro-CT image
cd(RD)
lst = dir('BV*jpg'); % find JPGs in raw data folder

res = 2.98; % microns per pixel
delta = ceil(100 / res); % number of pixels in ~100 microns

im0 = rgb2gray(imread(lst(1).name)); % load in the first image
[s1,s2] = size(im0); % image dimensions

% arrays dividing image into 100-micron sections
arr1 = 1:delta:s1;
arr2 = 1:delta:s2;

% create a subsampled image in dimensions 1 and 2 based on
maximum intensity within 100 micron volumes
IM = [];
H = waitbar(0/length(lst),'Create subsampled image...');
for ix = 1:length(lst)
    im = rgb2gray(imread(lst(ix).name)); % read image #ix and
convert to grayscale
```

```
    imSS = zeros(length(arr1),length(arr2)); % subsample image
    ii = 0;
    for iy = 1:(length(arr1)-1)
        for iz = 1:(length(arr2)-1)
            ii = ii + 1;
            tmp = im(arr1(iy):(arr1(iy+1)-
1),arr2(iz):(arr2(iz+1)-1));
            imSS(iy,iz) = max(tmp(:)); % assign maximum value
        end
    end
    IM = cat(3,IM,imSS); % concatenate in 3rd dimension

    waitbar(ix/length(lst),H);
end
close(H);

% subsample image in dimension 3
arr3 = 1:delta:length(lst);
IM_SS = zeros(size(IM,1),size(IM,2),length(arr3));
for ix = 1:(length(arr3)-1)
    IM_SS(:,:,ix) = max(IM(:,:,arr3(ix):(arr3(ix+1)-1)),[],3);
end

res2 = [res res res]/1000*delta; % subsampled image resolution
~100 microns isotropic
cd(MD)

% write NIFTIs of subsampled image before and after smoothing
WriteNIFTI([nm '_microCT_SSmax.nii'],res2,IM_SS)
WriteNIFTI([nm
'_microCT_SSmax_smooth.nii'],res2,smooth3(IM_SS,'gaussian',[5 5
5],2));

%%
%%% Step 2: Register subsampled micro CT to MRI template and
transform
%%% labels to micro-CT space

% load subsampled micro-CT image
N = nifti([nm '_microCT_SSmax_smooth.nii']);
mat = N.mat;
CT = N.dat(:,:,:);
clear N

% create brain mask
m = zeros(size(CT));
thresh = max(CT(:))*graythresh(CT(:)/max(CT(:)))*0.9; % adjust
this is need to modify mask => change 0.9 to smaller value for
more included, larger value for less included
```

```
m(CT > thresh) = 1; % anything with signal > thresh*0.9 is
assigned 1 in the mask

mD = imdilate(m,strel('sphere',3)); % dilate the mask
mF = zeros(size(m));
for ix = 1:size(m,3)
    mF(:,:,ix) = imfill(mD(:,:,ix)); % fill any holes
end
mE = imerode(mF,strel('sphere',3)); % erode the mask

CTsmooth = mE.*CT; % apply mask to smoothed micro-CT data
WriteNIFTI([nm '_mCT.nii'],mat,CTsmooth) % write NIFTI

% make template look like micro-CT data
ct = double(niftiread([nm '_mCT.nii']));
im = double(niftiread(template));
im_seg = double(niftiread(seg));

viewer3d(ct)
viewer3d(im) % use viewer3d to check orientation of images

im_RO = flip(flip(im,2),3);% flip(permute(im,[2 1 3]),3); % will
need to adjust based on the data => flip and permute are commands
to use - check help on these if needed
im_seg_RO = flip(flip(im_seg,2),3); % flip(permute(im_seg,[2 1
3]),3); % make this match above

viewer3d(im_RO) % check RO image and adjust as needed

template_RO =
'/Users/tif/Research/microCT/ProcessingPipeline/mMaMouseT2Mod_RO.
nii'; % template
seg_RO =
'/Users/tif/Research/microCT/ProcessingPipeline/MaMouseSegMod_RO.
nii'; % atlas labels

WriteNIFTI(template_RO,[0.1 0.1 0.1],im_RO)
WriteNIFTI(seg_RO,[0.1 0.1 0.1],im_seg_RO)

% run registration
eval(['!bash ' AR ' ' template_RO ' ' nm '_mCT.nii ' nm
'_MicroCTtoMRI']) % register subject to template

eval(['!bash ' TRi ' ' nm '_mCT.nii ' template_RO ' ' nm
'_MicroCTtoMRI0GenericAffine.mat BSpline']) % transform template
to subject
eval(['!bash ' TRi ' ' nm '_mCT.nii ' seg_RO ' ' nm
'_MicroCTtoMRI0GenericAffine.mat MultiLabel']) % transform atlas
labels to subject

% visually inspect registration results using ITK-Snap or similar
```

```
%%
%%% Step 3: Write JPGs in each of the ROIs

seg = double(niftiread('sMaMouseSegMod_RO.nii')); % load atlas
labels in subject space

segArray = [1 7 8 14]; % atlas labels associated with ROIs
segLabels{1} = 'Hippocampus';segLabels{2} =
'Thalamus';segLabels{3} = 'Cerebellum';segLabels{4} = 'Cortex';

[X1,X2] = ndgrid(arr1,arr2); % indices associated with subsampled
image
[x1,x2] = ndgrid(1:size(im0,1),1:size(im0,2)); % indices
associated with raw data

amin = 0;amax = (2^8-1); % intensity range

for ix = 1:length(segArray)
    ND = [MD filesep segLabels{ix}];
    if ~exist(ND,'dir')
        mkdir(ND)
    end
end

numImages = length(lst); % total # of images
H = waitbar(0,'Write microCT data for each ROI...');
tic
ii = 0;
for iy = 1:size(seg,3)
    % interpolate atlas labels to raw data size
    F =
griddedInterpolant(X1,X2,seg(:,:,iy),'nearest','nearest');
    seg_hr = F(x1,x2);

    sl = arr3(iy); % slice #
    for iz = 0:(delta-1)
        ii = ii + 1;
        % sl_nm = lst(sl+iz).name;

        if (sl+iz) <= length(lst)
            cd(RD) % chage to directory with raw JPGs
            im = rgb2gray(imread(lst(sl+iz).name)); % load raw
JPG file

            % slice number for writing files
            if sl+iz < 10
                SL = ['SL000' mat2str(sl+iz)];
            elseif sl+iz >= 10 && sl+iz < 100
                SL = ['SL00' mat2str(sl+iz)];
            elseif sl+iz >= 100 && sl+iz < 1000
```

```
                SL = ['SL0' mat2str(sl+iz)];
            else
                SL = ['SL' mat2str(sl+iz)];
            end

            for ix = 1:length(segArray) % loop through all ROIs
                ND = [MD filesep segLabels{ix}];

                % create mask for slice "sl" based on ROI(ix)
                m_hr = zeros(size(seg_hr));
                m_hr(seg_hr == segArray(ix)) = 1;
                m_hr = uint8(m_hr);

                im_mask = m_hr.*im; % image * mask

                cd(ND) % change to processed data folder for
ROI(ix)
                ind = strfind(nm,'_');
                new = [nm '_' SL '_' segLabels{ix} '.jpg']; % new
name => output as jp2000 file type
                imwrite(im_mask,new) % write image
            end
            waitbar(ii/numImages,H); % update progress
        end
    end
end
close(H);
toc
```

REFERENCES

1. Abiega O, Beccari S, Diaz-Aparicio I, Nadjar A, Layé S, et al. 2016. Neuronal Hyperactivity Disturbs ATP Microgradients, Impairs Microglial Motility, and Reduces Phagocytic Receptor Expression Triggering Apoptosis/Microglial Phagocytosis Uncoupling. *PLoS Biol* 14:e1002466
2. Aebi MR, Bourdillon N, Kunz A, Bron D, Millet GP. 2020. Specific effect of hypobaria on cerebrovascular hypercapnic responses in hypoxia. *Physiol Rep* 8:e14372
3. Ahn SM, Byun K, Cho K, Kim JY, Yoo JS, et al. 2008. Human microglial cells synthesize albumin in brain. *PLoS One* 3:e2829
4. Akiyoshi R, Wake H, Kato D, Horiuchi H, Ono R, et al. 2018. Microglia Enhance Synapse Activity to Promote Local Network Synchronization. *eNeuro* 5
5. Akrami H, Aminzadeh S, Fallahi H. 2015. Inhibitory effect of ibuprofen on tumor survival and angiogenesis in gastric cancer cell. *Tumour Biol* 36:3237-43
6. Akunov A, Sydykov A, Toktash T, Doolotova A, Sarybaev A. 2018. Hemoglobin Changes After Long-Term Intermittent Work at High Altitude. *Frontiers in Physiology* 9
7. Akyol A, Özsunar Y, Amasyalı SY, Arıkan Z, Özkul A. 2021. Giant Virchow-Robin spaces may play a role at headache attributed to hypoxia and/or hypercapnia. *Agri* 33:190-3
8. Alonso A, Reinz E, Fatar M, Hennerici MG, Meairs S. 2011. Clearance of albumin following ultrasound-induced blood-brain barrier opening is mediated by glial but not neuronal cells. *Brain Res* 1411:9-16
9. Amedei A, Morbidelli L. 2019. Circulating Metabolites Originating from Gut Microbiota Control Endothelial Cell Function. *Molecules* 24
10. Amin N, Chen S, Ren Q, Tan X, Botchway BOA, et al. 2021. Hypoxia Inducible Factor-1α Attenuates Ischemic Brain Damage by Modulating Inflammatory Response and Glial Activity. *Cells* 10
11. Anderson WW, Collingridge GL. 2001. The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. *Journal of Neuroscience Methods* 108:71-83
12. Ardelt AA, Bhattacharyya BJ, Belmadani A, Ren D, Miller RJ. 2013. Stromal derived growth factor-1 (CXCL12) modulates synaptic transmission to immature neurons during post-ischemic cerebral repair. *Experimental neurology* 248:246-53
13. Arias-Cavieres A, Fonteh A, Castro-Rivera CI, Garcia AJ, 3rd. 2021. Intermittent Hypoxia causes targeted disruption to NMDA receptor dependent synaptic plasticity in area CA1 of the hippocampus. *Exp Neurol* 344:113808
14. Arisi GM. 2014. Nervous and immune systems signals and connections: cytokines in hippocampus physiology and pathology. *Epilepsy Behav* 38:43-7
15. Askew EW. 2002. Work at high altitude and oxidative stress: antioxidant nutrients. *Toxicology* 180:107-19
16. Au - Djurisic M. 2020. Minimizing Hypoxia in Hippocampal Slices from Adult and Aging Mice. *JoVE*:e61377

17. Augusto-Oliveira M, Arrifano GP, Delage CI, Tremblay M, Crespo-Lopez ME, Verkhratsky A. 2022. Plasticity of microglia. *Biol Rev Camb Philos Soc* 97:217-50
18. Avignone E, Lepleux M, Angibaud J, Nägerl UV. 2015. Altered morphological dynamics of activated microglia after induction of status epilepticus. *J Neuroinflammation* 12:202
19. Badimon A, Strasburger HJ, Ayata P, Chen X, Nair A, et al. 2020. Negative feedback control of neuronal activity by microglia. *Nature* 586:417-23
20. Ballabh P, Braun A, Nedergaard M. 2004. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 16:1-13
21. Baneke A. 2010. What role does the blood brain barrier play in acute mountain sickness? *Travel Med Infect Dis* 8:257-62
22. Bankstahl M, Breuer H, Leiter I, Märkel M, Bascuñana P, et al. 2018. Blood-Brain Barrier Leakage during Early Epileptogenesis Is Associated with Rapid Remodeling of the Neurovascular Unit. *eNeuro* 5
23. Bärtsch P, Gibbs JS. 2007. Effect of altitude on the heart and the lungs. *Circulation* 116:2191-202
24. Bärtsch P, Swenson ER. 2013. Acute high-altitude illnesses. *N Engl J Med* 369:1666-7
25. Basaran KE, Villongco M, Ho B, Ellis E, Zarndt R, et al. 2016. Ibuprofen Blunts Ventilatory Acclimatization to Sustained Hypoxia in Humans. *PLoS One* 11:e0146087
26. Basnyat B, Lemaster J, Litch JA. 1999. Everest or bust: a cross sectional, epidemiological study of acute mountain sickness at 4243 meters in the Himalayas. *Aviat Space Environ Med* 70:867-73
27. Basnyat B, Wu T, Gertsch JH. 2004. Neurological conditions at altitude that fall outside the usual definition of altitude sickness. *High Alt Med Biol* 5:171-9
28. Beer JMA, Shender BS, Chauvin D, Dart TS, Fischer J. 2017. Cognitive Deterioration in Moderate and Severe Hypobaric Hypoxia Conditions. *Aerosp Med Hum Perform* 88:617-26
29. Beidleman BA, Muza SR, Fulco CS, Cymerman A, Ditzler D, et al. 2004. Intermittent altitude exposures reduce acute mountain sickness at 4300 m. *Clin Sci (Lond)* 106:321-8
30. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD. 2001. Human albumin therapy of acute ischemic stroke: marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. *Stroke* 32:553-60
31. Belayev L, Saul I, Huh PW, Finotti N, Zhao W, et al. 1999. Neuroprotective effect of high-dose albumin therapy against global ischemic brain injury in rats. *Brain Res* 845:107-11
32. Berger MM, Macholz F, Mairbäurl H, Bärtsch P. 2015. Remote ischemic preconditioning for prevention of high-altitude diseases: fact or fiction? *J Appl Physiol (1985)* 119:1143-51
33. Bernier L-P, York EM, Kamyabi A, Choi HB, Weilinger NL, MacVicar BA. 2020. Microglial metabolic flexibility supports immune surveillance of the brain parenchyma. *Nature Communications* 11:1559

34. Bliss TV, Collingridge GL. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31-9
35. Bliss TV, Cooke SF. 2011. Long-term potentiation and long-term depression: a clinical perspective. *Clinics (Sao Paulo)* 66 Suppl 1:3-17
36. Bliss TV, Lomo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232:331-56
37. Bloch KE, Buenzli JC, Latshang TD, Ulrich S. 2015. Sleep at high altitude: guesses and facts. *J Appl Physiol (1985)* 119:1466-80
38. Bloch KE, Turk AJ, Maggiorini M, Hess T, Merz T, et al. 2009. Effect of ascent protocol on acute mountain sickness and success at Muztagh Ata, 7546 m. *High Alt Med Biol* 10:25-32
39. Bodea GO, Spille JH, Abe P, Andersson AS, Acker-Palmer A, et al. 2014. Reelin and CXCL12 regulate distinct migratory behaviors during the development of the dopaminergic system. *Development* 141:661-73
40. Boese AC, Kim SC, Yin KJ, Lee JP, Hamblin MH. 2017. Sex differences in vascular physiology and pathophysiology: estrogen and androgen signaling in health and disease. *Am J Physiol Heart Circ Physiol* 313:H524-h45
41. Bolte AC, Dutta AB, Hurt ME, Smirnov I, Kovacs MA, et al. 2020. Meningeal lymphatic dysfunction exacerbates traumatic brain injury pathogenesis. *Nat Commun* 11:4524
42. Bond WS, Rex TS. 2014. Evidence That Erythropoietin Modulates Neuroinflammation through Differential Action on Neurons, Astrocytes, and Microglia. *Front Immunol* 5:523
43. Boos CJ, Woods DR, Varias A, Biscocho S, Heseltine P, Mellor AJ. 2016. High Altitude and Acute Mountain Sickness and Changes in Circulating Endothelin-1, Interleukin-6, and Interleukin-17a. *High Alt Med Biol* 17:25-31
44. Buschler A, Manahan-Vaughan D. 2012. Brief environmental enrichment elicits metaplasticity of hippocampal synaptic potentiation in vivo. *Front Behav Neurosci* 6:85
45. Byun K, Bayarsaikhan E, Kim D, Kim CY, Mook-Jung I, et al. 2012. Induction of neuronal death by microglial AGE-albumin: implications for Alzheimer's disease. *PLoS One* 7:e37917
46. Caretti A, Bianciardi P, Ronchi R, Fantacci M, Guazzi M, Samaja M. 2008. Phosphodiesterase-5 inhibition abolishes neuron apoptosis induced by chronic hypoxia independently of hypoxia-inducible factor-1alpha signaling. *Exp Biol Med (Maywood)* 233:1222-30
47. Carod-Artal FJ. 2014. High-altitude headache and acute mountain sickness. *Neurologia* 29:533-40
48. Castillo-Melendez M, Yawno T, Allison BJ, Jenkin G, Wallace EM, Miller SL. 2015. Cerebrovascular adaptations to chronic hypoxia in the growth restricted lamb. *Int J Dev Neurosci* 45:55-65
49. Caulfield J, Fernandez M, Snetkov V, Lee T, Hawrylowicz C. 2002. CXCR4 expression on monocytes is up-regulated by dexamethasone and is modulated by autologous CD3+ T cells. *Immunology* 105:155-62

50. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, et al. 2004. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 10:858-64
51. Chai M, Gu C, Shen Q, Liu J, Zhou Y, et al. 2020. Hypoxia alleviates dexamethasone-induced inhibition of angiogenesis in cocultures of HUVECs and rBMSCs via HIF-1α. *Stem Cell Res Ther* 11:343
52. Chambers R, Zweifach BW. 1947. Intercellular cement and capillary permeability. *Physiol Rev* 27:436-63
53. Chauhan G, Roy K, Kumar G, Kumari P, Alam S, et al. 2019. Distinct influence of COX-1 and COX-2 on neuroinflammatory response and associated cognitive deficits during high altitude hypoxia. *Neuropharmacology* 146:138-48
54. Chen CH, Liu YF, Lee SD, Huang CY, Lee WC, et al. 2009. Altitude hypoxia increases glucose uptake in human heart. *High Alt Med Biol* 10:83-6
55. Chen PZ, He WJ, Zhu ZR, E GJ, Xu G, et al. 2018. Adenosine A(2A) receptor involves in neuroinflammation-mediated cognitive decline through activating microglia under acute hypobaric hypoxia. *Behav Brain Res* 347:99-107
56. Chen Y, Chen S, Kawazoe N, Chen G. 2018. Promoted Angiogenesis and Osteogenesis by Dexamethasone-loaded Calcium Phosphate Nanoparticles/Collagen Composite Scaffolds with Microgroove Networks. *Sci Rep* 8:14143
57. Chen Z, Jalabi W, Hu W, Park H-J, Gale JT, et al. 2014. Microglial displacement of inhibitory synapses provides neuroprotection in the adult brain. *Nature Communications* 5:4486
58. Cheng M, Huang K, Zhou J, Yan D, Tang YL, et al. 2015. A critical role of Src family kinase in SDF-1/CXCR4-mediated bone-marrow progenitor cell recruitment to the ischemic heart. *Journal of molecular and cellular cardiology* 81:49-53
59. Cho C, Smallwood PM, Nathans J. 2017. Reck and Gpr124 Are Essential Receptor Cofactors for Wnt7a/Wnt7b-Specific Signaling in Mammalian CNS Angiogenesis and Blood-Brain Barrier Regulation. *Neuron* 95:1056-73.e5
60. Choudhury ME, Miyanishi K, Takeda H, Islam A, Matsuoka N, et al. 2020. Phagocytic elimination of synapses by microglia during sleep. *Glia* 68:44-59
61. Chu T, Shields LBE, Zhang YP, Feng S, Shields CB, Cai J. 2017. CXCL12/CXCR4/CXCR7 chemokine axis in the central nervous system: Therapeutic targets for remyelination in demyelinating diseases. *Neuroscientist* 23:627-48
62. Corfield DR, McKay LC. 2016. Regional Cerebrovascular Responses to Hypercapnia and Hypoxia. *Adv Exp Med Biol* 903:157-67
63. Cramer NP, Korotcov A, Bosomtwi A, Xu X, Holman DR, et al. 2019. Neuronal and vascular deficits following chronic adaptation to high altitude. *Exp Neurol* 311:293-304
64. Cramer NP, Xu X, Christensen C, Bierman A, Tankersley CG, Galdzicki Z. 2015. Strain variation in the adaptation of C57Bl6 and BALBc mice to chronic hypobaric hypoxia. *Physiology & Behavior* 143:158-65

65. Cui M, Bai X, Li T, Chen F, Dong Q, et al. 2013. Decreased Extracellular Adenosine Levels Lead to Loss of Hypoxia-Induced Neuroprotection after Repeated Episodes of Exposure to Hypoxia. *PLOS ONE* 8:e57065
66. Członkowska A, Kurkowska-Jastrzebska I, Członkowski A, Peter D, Stefano GB. 2002. Immune processes in the pathogenesis of Parkinson's disease - a potential role for microglia and nitric oxide. *Med Sci Monit* 8:Ra165-77
67. Dagher NN, Najafi AR, Kayala KMN, Elmore MRP, White TE, et al. 2015. Colony-stimulating factor 1 receptor inhibition prevents microglial plaque association and improves cognition in 3xTg-AD mice. *Journal of Neuroinflammation* 12:139
68. Dama A, Baggio C, Boscaro C, Albiero M, Cignarella A. 2021. Estrogen Receptor Functions and Pathways at the Vascular Immune Interface. *Int J Mol Sci* 22
69. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, et al. 2005. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* 8:752-8
70. Davids MS, Deng J, Wiestner A, Lannutti BJ, Wang L, et al. 2012. Decreased mitochondrial apoptotic priming underlies stroma-mediated treatment resistance in chronic lymphocytic leukemia. *Blood* 120:3501-9
71. de Aquino Lemos V, Antunes HK, dos Santos RV, Lira FS, Tufik S, de Mello MT. 2012. High altitude exposure impairs sleep patterns, mood, and cognitive functions. *Psychophysiology* 49:1298-306
72. De Biase LM, Schuebel KE, Fusfeld ZH, Jair K, Hawes IA, et al. 2017. Local Cues Establish and Maintain Region-Specific Phenotypes of Basal Ganglia Microglia. *Neuron* 95:341-56.e6
73. De La Zerda DJ, Stokes JA, Do J, Go A, Fu Z, Powell FL. 2018. Ibuprofen does not reverse ventilatory acclimatization to chronic hypoxia. *Respir Physiol Neurobiol* 256:29-35
74. Dehnert C, Böhm A, Grigoriev I, Menold E, Bärtsch P. 2014. Sleeping in moderate hypoxia at home for prevention of acute mountain sickness (AMS): a placebo-controlled, randomized double-blind study. *Wilderness Environ Med* 25:263-71
75. Dettori I, Fusco I, Bulli I, Gaviano L, Coppi E, et al. 2021. Protective effects of carbonic anhydrase inhibition in brain ischaemia in vitro and in vivo models. *J Enzyme Inhib Med Chem* 36:964-76
76. Di Filippo M, Chiasserini D, Gardoni F, Viviani B, Tozzi A, et al. 2013. Effects of central and peripheral inflammation on hippocampal synaptic plasticity. *Neurobiol Dis* 52:229-36
77. Dorward DA, Thompson AA, Baillie JK, MacDougall M, Hirani N. 2007. Change in plasma vascular endothelial growth factor during onset and recovery from acute mountain sickness. *Respir Med* 101:587-94
78. Dos Santos Quaresma M, Souza W, Lemos VA, Caris AV, Thomatieli-Santos RV. 2020. The Possible Importance of Glutamine Supplementation to Mood and Cognition in Hypoxia from High Altitude. *Nutrients* 12
79. Doustar J, Danan IJ. 2022. Glymphatic System Dysfunction in Mild Traumatic Brain Injury. *Neurology* 98:S24-s5

80. Du KX, Dong Y, Zhang Y, Hou LW, Fan DX, et al. 2015. Effects of dexamethasone on aquaporin-4 expression in brain tissue of rat with bacterial meningitis. *Int J Clin Exp Pathol* 8:3090-6
81. Dugan CW, Maloney SK, Abramoff KJ, Panag SS, Davis EA, et al. 2021. Effects of simulated high altitude on blood glucose levels during exercise in individuals with Type 1 Diabetes. *J Clin Endocrinol Metab*
82. Dumont L, Mardirosoff C, Tramèr MR. 2000. Efficacy and harm of pharmacological prevention of acute mountain sickness: quantitative systematic review. *Bmj* 321:267-72
83. Dunham-Snary KJ, Wu D, Sykes EA, Thakrar A, Parlow LRG, et al. 2017. Hypoxic Pulmonary Vasoconstriction: From Molecular Mechanisms to Medicine. *Chest* 151:181-92
84. Dunn JF, Isaacs AM. 2021. The impact of hypoxia on blood-brain, blood-CSF, and CSF-brain barriers. *J Appl Physiol (1985)* 131:977-85
85. Dzau VJ, Gibbons GH. 1993. Vascular remodeling: mechanisms and implications. *J Cardiovasc Pharmacol* 21 Suppl 1:S1-5
86. Eady TN, Khoutorova L, Obenaus A, Mohd-Yusof A, Bazan NG, Belayev L. 2014. Docosahexaenoic acid complexed to albumin provides neuroprotection after experimental stroke in aged rats. *Neurobiol Dis* 62:1-7
87. Ellsworth AJ, Larson EB, Strickland D. 1987. A randomized trial of dexamethasone and acetazolamide for acute mountain sickness prophylaxis. *Am J Med* 83:1024-30
88. Elsaid N, Saied A, Kandil H, Soliman A, Taher F, et al. 2021. Impact of stress and hypertension on the cerebrovasculature. *Front Biosci (Landmark Ed)* 26:1643-52
89. Eltzschig HK, Carmeliet P. 2011. Hypoxia and inflammation. *N Engl J Med* 364:656-65
90. Engelhardt B, Liebner S. 2014. Novel insights into the development and maintenance of the blood-brain barrier. *Cell Tissue Res* 355:687-99
91. Engelhardt B, Ransohoff RM. 2012. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends in immunology* 33:579-89
92. Eyo UB, Haruwaka K, Mo M, Campos-Salazar AB, Wang L, et al. 2021. Microglia provide structural resolution to injured dendrites after severe seizures. *Cell Reports* 35:109080
93. Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ. 2014. Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. *J Neurosci* 34:10528-40
94. Fadini GP, de Kreutzenberg S, Albiero M, Coracina A, Pagnin E, et al. 2008. Gender differences in endothelial progenitor cells and cardiovascular risk profile: the role of female estrogens. *Arterioscler Thromb Vasc Biol* 28:997-1004
95. Fan N, Liu C, Ren M. 2020. Effect of different high altitudes on vascular endothelial function in healthy people. *Medicine* 99:e19292
96. Fan Z, Sehm T, Rauh M, Buchfelder M, Eyupoglu IY, Savaskan NE. 2014. Dexamethasone alleviates tumor-associated brain damage and angiogenesis. *PLoS One* 9:e93264

97. Feoktistov I, Ryzhov S, Zhong H, Goldstein AE, Matafonov A, et al. 2004. Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A2B angiogenic phenotype. *Hypertension* 44:649-54
98. Fernandez Garcia-Agudo L, Steixner-Kumar AA, Curto Y, Barnkothe N, Hassouna I, et al. 2021. Brain erythropoietin fine-tunes a counterbalance between neurodifferentiation and microglia in the adult hippocampus. *Cell Rep* 36:109548
99. Filipowska J, Tomaszewski KA, Niedźwiedzki Ł, Walocha JA, Niedźwiedzki T. 2017. The role of vasculature in bone development, regeneration and proper systemic functioning. *Angiogenesis* 20:291-302
100. Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J. 1998. Multipotent progenitor cells in the adult dentate gyrus. *J Neurobiol* 36:249-66
101. Gangwar A, Pooja, Sharma M, Singh K, Patyal A, et al. 2019. Intermittent normobaric hypoxia facilitates high altitude acclimatization by curtailing hypoxia-induced inflammation and dyslipidemia. *Pflugers Arch* 471:949-59
102. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, et al. 2003. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* 161:1163-77
103. Gessi S, Merighi S, Stefanelli A, Fazzi D, Varani K, Borea PA. 2013. A(1) and A(3) adenosine receptors inhibit LPS-induced hypoxia-inducible factor-1 accumulation in murine astrocytes. *Pharmacol Res* 76:157-70
104. Geudens I, Gerhardt H. 2011. Coordinating cell behaviour during blood vessel formation. *Development* 138:4569-83
105. Glober NK, Sprague S, Ahmad S, Mayfield KG, Fletcher LM, et al. 2019. Acetazolamide Treatment Prevents Redistribution of Astrocyte Aquaporin 4 after Murine Traumatic Brain Injury. *Neurosci J* 2019:2831501
106. Golia MT, Poggini S, Alboni S, Garofalo S, Ciano Albanese N, et al. 2019. Interplay between inflammation and neural plasticity: Both immune activation and suppression impair LTP and BDNF expression. *Brain Behav Immun* 81:484-94
107. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, et al. 2011. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 42:2672-713
108. Green KN, Crapser JD, Hohsfield LA. 2020. To Kill a Microglia: A Case for CSF1R Inhibitors. *Trends in Immunology* 41:771-84
109. Grosser S, Buck N, Braunewell K-H, Gilling KE, Wozny C, et al. 2020. Loss of Long-Term Potentiation at Hippocampal Output Synapses in Experimental Temporal Lobe Epilepsy. *Frontiers in Molecular Neuroscience* 13
110. Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, et al. 2003. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. *Dev Cell* 5:45-57
111. Gu YT, Zhang H, Xue YX. 2007. Dexamethasone treatment modulates aquaporin-4 expression after intracerebral hemorrhage in rats. *Neurosci Lett* 413:126-31
112. Guo F, Hua Y, Wang J, Keep RF, Xi G. 2012. Inhibition of carbonic anhydrase reduces brain injury after intracerebral hemorrhage. *Transl Stroke Res* 3:130-7

113. Guyon A. 2014. CXCL12 chemokine and its receptors as major players in the interactions between immune and nervous systems. *Frontiers in cellular neuroscience* 8:65
114. Gyoneva S, Ransohoff RM. 2015. Inflammatory reaction after traumatic brain injury: Therapeutic potential of targeting cell-cell communication by chemokines. *Trends in pharmacological sciences* 36:471-80
115. Hablitz LM, Nedergaard M. 2021. The Glymphatic System: A Novel Component of Fundamental Neurobiology. *J Neurosci* 41:7698-711
116. Hackett PH, Roach RC. 2001. High-altitude illness. *N Engl J Med* 345:107-14
117. Hackett PH, Roach RC. 2004. High altitude cerebral edema. *High Alt Med Biol* 5:136-46
118. Hackett PH, Roach RC, Wood RA, Foutch RG, Meehan RT, et al. 1988. Dexamethasone for prevention and treatment of acute mountain sickness. *Aviat Space Environ Med* 59:950-4
119. Hajak G, Klingelhöfer J, Schulz-Varszegi M, Rüther E. 1993. [Blood circulation and energy metabolism of the brain in healthy sleep]. *Nervenarzt* 64:456-67
120. Halder SK, Milner R. 2019. A critical role for microglia in maintaining vascular integrity in the hypoxic spinal cord. *Proc Natl Acad Sci U S A* 116:26029-37
121. Halder SK, Milner R. 2020. Mild hypoxia triggers transient blood–brain barrier disruption: a fundamental protective role for microglia. *Acta Neuropathologica Communications* 8:175
122. Halder SK, Milner R. 2021. The impact of chronic mild hypoxia on cerebrovascular remodelling; uncoupling of angiogenesis and vascular breakdown. *Fluids Barriers CNS* 18:50
123. Hamed SA. 2017. Brain injury with diabetes mellitus: evidence, mechanisms and treatment implications. *Expert Rev Clin Pharmacol* 10:409-28
124. Hamm S, Dehouck B, Kraus J, Wolburg-Buchholz K, Wolburg H, et al. 2004. Astrocyte mediated modulation of blood-brain barrier permeability does not correlate with a loss of tight junction proteins from the cellular contacts. *Cell Tissue Res* 315:157-66
125. Hao JQ, He XY, Yang X, Xiao YC, Duan SQ, et al. 2022. Acetazolamide Alleviate Cerebral Edema Induced by Ischemic Stroke Through Inhibiting the Expression of AQP4 mRNA. *Neurocrit Care* 36:97-105
126. Harik SI, Behmand RA, LaManna JC. 1994. Hypoxia increases glucose transport at blood-brain barrier in rats. *J Appl Physiol (1985)* 77:896-901
127. Hassan B. 2020. Interpretation of maternal blood glucose during pregnancy at high altitude area, Abha-Saudi Arabia. *J Family Med Prim Care* 9:4633-6
128. Hawkins BT, Davis TP. 2005. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 57:173-85
129. Hedley-Whyte ET, Hsu DW. 1986. Effect of dexamethasone on blood-brain barrier in the normal mouse. *Ann Neurol* 19:373-7
130. Heinzer R, Saugy JJ, Rupp T, Tobback N, Faiss R, et al. 2016. Comparison of Sleep Disorders between Real and Simulated 3,450-m Altitude. *Sleep* 39:1517-23
131. Henstridge CM, Tzioras M, Paolicelli RC. 2019. Glial Contribution to Excitatory and Inhibitory Synapse Loss in Neurodegeneration. *Frontiers in Cellular Neuroscience* 13

132. Heynen A, Bear M, Abraham W. Low-frequency stimulation of the Schaffer collaterals produces homosynaptic LTD in area CA1 of the adult rat hippocampus in vivo. *Proc. Soc Neurosci Abstr*, *1995*, 21:2005:
133. Hill NE, Deighton K, Matu J, Misra S, Oliver NS, et al. 2018. Continuous Glucose Monitoring at High Altitude-Effects on Glucose Homeostasis. *Med Sci Sports Exerc* 50:1679-86
134. Hochachka PW, Clark CM, Matheson GO, Brown WD, Stone CK, et al. 1999. Effects on regional brain metabolism of high-altitude hypoxia: a study of six US marines. *Am J Physiol* 277:R314-9
135. Holden JE, Stone CK, Clark CM, Brown WD, Nickles RJ, et al. 1995. Enhanced cardiac metabolism of plasma glucose in high-altitude natives: adaptation against chronic hypoxia. *J Appl Physiol (1985)* 79:222-8
136. Hooper C, Taylor DL, Pocock JM. 2005. Pure albumin is a potent trigger of calcium signalling and proliferation in microglia but not macrophages or astrocytes. *J Neurochem* 92:1363-76
137. Hristovska I, Pascual O. 2015. Deciphering Resting Microglial Morphology and Process Motility from a Synaptic Prospect. *Front Integr Neurosci* 9:73
138. Huang YJ, Nan GX. 2019. Oxidative stress-induced angiogenesis. *J Clin Neurosci* 63:13-6
139. Huxley VH. 2007. Sex and the cardiovascular system: the intriguing tale of how women and men regulate cardiovascular function differently. *Adv Physiol Educ* 31:17-22
140. Huxley VH, Kemp SS. 2018. Sex-Specific Characteristics of the Microcirculation. *Adv Exp Med Biol* 1065:307-28
141. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, et al. 2014. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *J Neurosci* 34:16180-93
142. Irarrázaval S, Allard C, Campodónico J, Pérez D, Strobel P, et al. 2017. Oxidative Stress in Acute Hypobaric Hypoxia. *High Alt Med Biol* 18:128-34
143. Itoh T, Satou T, Ishida H, Nishida S, Tsubaki M, et al. 2009. The relationship between SDF-1alpha/CXCR4 and neural stem cells appearing in damaged area after traumatic brain injury in rats. *Neurological research* 31:90-102
144. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, et al. 2006. TGF-β receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain* 130:535-47
145. Jacob M, Chappell D, Becker BF. 2016. Regulation of blood flow and volume exchange across the microcirculation. *Crit Care* 20:319
146. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. 2015. The Glymphatic System: A Beginner's Guide. *Neurochem Res* 40:2583-99
147. Jiang S, Chen G, Yang Z, Wang D, Lu Y, et al. 2021. Testosterone attenuates hypoxia-induced hypertension by affecting NRF1-mediated transcriptional regulation of ET-1 and ACE. *Hypertens Res* 44:1395-405
148. Johnson PL, Edwards N, Burgess KR, Sullivan CE. 2010. Sleep architecture changes during a trek from 1400 to 5000 m in the Nepal Himalaya. *J Sleep Res* 19:148-56

149. Kaplan L, Chow BW, Gu C. 2020. Neuronal regulation of the blood-brain barrier and neurovascular coupling. *Nat Rev Neurosci* 21:416-32
150. Karl JP, Berryman CE, Young AJ, Radcliffe PN, Branck TA, et al. 2018. Associations between the gut microbiota and host responses to high altitude. *Am J Physiol Gastrointest Liver Physiol* 315:G1003-g15
151. Kato G, Inada H, Wake H, Akiyoshi R, Miyamoto A, et al. 2016. Microglial Contact Prevents Excess Depolarization and Rescues Neurons from Excitotoxicity. *eNeuro* 3
152. Kayser B, Hulsebosch R, Bosch F. 2008. Low-dose acetylsalicylic acid analog and acetazolamide for prevention of acute mountain sickness. *High Alt Med Biol* 9:15-23
153. Kemp A, Manahan-Vaughan D. 2004. Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proc Natl Acad Sci U S A* 101:8192-7
154. Khanna K, Mishra KP, Ganju L, Kumar B, Singh SB. 2018. High-Altitude-Induced alterations in Gut-Immune Axis: A review. *Int Rev Immunol* 37:119-26
155. Khuu MA, Pagan CM, Nallamothu T, Hevner RF, Hodge RD, et al. 2019. Intermittent Hypoxia Disrupts Adult Neurogenesis and Synaptic Plasticity in the Dentate Gyrus. *J Neurosci* 39:1320-31
156. Kim YW, Byzova TV. 2014. Oxidative stress in angiogenesis and vascular disease. *Blood* 123:625-31
157. Kisler K, Nelson AR, Montagne A, Zlokovic BV. 2017. Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. *Nat Rev Neurosci* 18:419-34
158. Klein RS, Rubin JB, Gibson HD, DeHaan EN, Alvarez-Hernandez X, et al. 2001. SDF-1 alpha induces chemotaxis and enhances Sonic hedgehog-induced proliferation of cerebellar granule cells. *Development* 128:1971-81
159. Koester-Hegmann C, Bengoetxea H, Kosenkov D, Thiersch M, Haider T, et al. 2018. High-Altitude Cognitive Impairment Is Prevented by Enriched Environment Including Exercise via VEGF Signaling. *Front Cell Neurosci* 12:532
160. Kotilinek LA, Westerman MA, Wang Q, Panizzon K, Lim GP, et al. 2008. Cyclooxygenase-2 inhibition improves amyloid-beta-mediated suppression of memory and synaptic plasticity. *Brain* 131:651-64
161. Kramer AF, Coyne JT, Strayer DL. 1993. Cognitive function at high altitude. *Hum Factors* 35:329-44
162. Kretschmer M, Rüdiger D, Zahler S. 2021. Mechanical Aspects of Angiogenesis. *Cancers (Basel)* 13
163. Lafuente JV, Bermudez G, Camargo-Arce L, Bulnes S. 2016. Blood-Brain Barrier Changes in High Altitude. *CNS Neurol Disord Drug Targets* 15:1188-97
164. Lana D, Ugolini F, Giovannini MG. 2020. An Overview on the Differential Interplay Among Neurons-Astrocytes-Microglia in CA1 and CA3 Hippocampus in Hypoxia/Ischemia. *Front Cell Neurosci* 14:585833
165. Langendorf EK, Rommens PM, Drees P, Ritz U. 2021. Dexamethasone Inhibits the Pro-Angiogenic Potential of Primary Human Myoblasts. *Int J Mol Sci* 22
166. Laprell L, Schulze C, Brehme M-L, Oertner TG. 2021. The role of microglia membrane potential in chemotaxis. *Journal of Neuroinflammation* 18:21

167. Larsen JJ, Hansen JM, Olsen NV, Galbo H, Dela F. 1997. The effect of altitude hypoxia on glucose homeostasis in men. *J Physiol* 504 (Pt 1):241-9
168. Lataste X. 1992. The blood-brain barrier in hypoxia. *Int J Sports Med* 13 Suppl 1:S45-7
169. Latshang TD, Lo Cascio CM, Stöwhas AC, Grimm M, Stadelmann K, et al. 2013. Are nocturnal breathing, sleep, and cognitive performance impaired at moderate altitude (1,630-2,590 m)? *Sleep* 36:1969-76
170. Lawley JS, Levine BD, Williams MA, Malm J, Eklund A, et al. 2016. Cerebral spinal fluid dynamics: effect of hypoxia and implications for high-altitude illness. *J Appl Physiol (1985)* 120:251-62
171. Leaf DE, Goldfarb DS. 2007. Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness. *J Appl Physiol (1985)* 102:1313-22
172. Lemos Vde A, dos Santos RV, Lira FS, Rodrigues B, Tufik S, de Mello MT. 2013. Can high altitude influence cytokines and sleep? *Mediators Inflamm* 2013:279365
173. Lenzlinger PM, Morganti-Kossmann MC, Laurer HL, McIntosh TK. 2001. The duality of the inflammatory response to traumatic brain injury. *Mol Neurobiol* 24:169-81
174. Levett DZ, Radford EJ, Menassa DA, Graber EF, Morash AJ, et al. 2012. Acclimatization of skeletal muscle mitochondria to high-altitude hypoxia during an ascent of Everest. *Faseb j* 26:1431-41
175. Li B, Bai W, Sun P, Zhou B, Hu B, Ying J. 2015. The effect of CXCL12 on endothelial progenitor cells: potential target for angiogenesis in intracerebral hemorrhage. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* 35:23-31
176. Li D, Zhang L, Huang X, Liu L, He Y, et al. 2017. WIP1 Phosphatase Plays a Critical Neuroprotective Role in Brain Injury Induced by High-Altitude Hypoxic Inflammation. *Neurosci Bull* 33:292-8
177. Li G, Adesnik H, Li J, Long J, Nicoll RA, et al. 2008. Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. *J Neurosci* 28:1085-98
178. Li J, Chen K, Zhu L, Pollard JW. 2006. Conditional deletion of the colony stimulating factor-1 receptor (c-fms proto-oncogene) in mice. *Genesis* 44:328-35
179. Li J, Guo W, Xiong M, Han H, Chen J, et al. 2015. Effect of SDF-1/CXCR4 axis on the migration of transplanted bone mesenchymal stem cells mobilized by erythropoietin toward lesion sites following spinal cord injury. *Int J Mol Med* 36:1205-14
180. Li L, Chu L, Fang Y, Yang Y, Qu T, et al. 2017. Preconditioning of bone marrow-derived mesenchymal stromal cells by tetramethylpyrazine enhances cell migration and improves functional recovery after focal cerebral ischemia in rats. *Stem cell research & therapy* 8:112
181. Li S, Ming W, Zhou Z, Wang B, Zhao X, Zhang J. 2012. SDF-1α induces angiogenesis after traumatic brain injury. *J Brain Res* 1444:76-86

182. Li XC, Hong FF, Tu YJ, Li YA, Ma CY, et al. 2022. Blockade of adenosine A(2A) receptor alleviates cognitive dysfunction after chronic exposure to intermittent hypoxia in mice. *Exp Neurol* 350:113929
183. Li Y, Du X-f, Liu C-s, Wen Z-l, Du J-l. 2012. Reciprocal Regulation between Resting Microglial Dynamics and Neuronal Activity In Vivo. *Developmental Cell* 23:1189-202
184. Li Y, Wang Y, Wang B, Sui L. 2015. Nose tip detection on three-dimensional faces using pose-invariant differential surface features. *IET Computer Vision* 9:75-84
185. Li Y, Zhang Y, Zhang Y. 2018. Research advances in pathogenesis and prophylactic measures of acute high altitude illness. *Respir Med* 145:145-52
186. Liapi A, Pritchett J, Jones O, Fujii N, Parnavelas JG, Nadarajah B. 2008. Stromal-derived factor 1 signalling regulates radial and tangential migration in the developing cerebral cortex. *Dev Neurosci* 30:117-31
187. Limatola C, Giovannelli A, Maggi L, Ragozzino D, Castellani L, et al. 2000. SDF-1alpha-mediated modulation of synaptic transmission in rat cerebellum. *Eur J Neurosci* 12:2497-504
188. Lin H, Lee E, Hestir K, Leo C, Huang M, et al. 2008. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* 320:807-11
189. Lipman GS, Kanaan NC, Holck PS, Constance BB, Gertsch JH. 2012. Ibuprofen prevents altitude illness: a randomized controlled trial for prevention of altitude illness with nonsteroidal anti-inflammatories. *Ann Emerg Med* 59:484-90
190. Liu C, Croft QP, Kalidhar S, Brooks JT, Herigstad M, et al. 2013. Dexamethasone mimics aspects of physiological acclimatization to 8 hours of hypoxia but suppresses plasma erythropoietin. *J Appl Physiol (1985)* 114:948-56
191. Liu M-C, Liu X-Q, Wang W, Shen X-F, Che H-L, et al. 2012. Involvement of Microglia Activation in the Lead Induced Long-Term Potentiation Impairment. *PLOS ONE* 7:e43924
192. Liu M, Gong R, Chen W, Peng H. 2018. 3D neuron tip detection in volumetric microscopy images using an adaptive ray-shooting model. *Pattern Recognition* 75:263-71
193. Liu M, Wang AJ, Chen Y, Zhao G, Jiang Z, et al. 2020. Efficacy and safety of erythropoietin for traumatic brain injury. *BMC Neurology* 20:399
194. Logue OC, Cramer NP, Xu X, Perl DP, Galdzicki Z. 2016. Alterations of functional properties of hippocampal networks following repetitive closed-head injury. *Exp Neurol* 277:227-43
195. Loiola RA, Wickstead ES, Solito E, McArthur S. 2019. Estrogen Promotes Pro-resolving Microglial Behavior and Phagocytic Cell Clearance Through the Actions of Annexin A1. *Frontiers in Endocrinology* 10
196. Louveau A, Da Mesquita S, Kipnis J. 2016. Lymphatics in Neurological Disorders: A Neuro-Lympho-Vascular Component of Multiple Sclerosis and Alzheimer’s Disease? *Neuron* 91:957-73
197. Low EV, Avery AJ, Gupta V, Schedlbauer A, Grocott MP. 2012. Identifying the lowest effective dose of acetazolamide for the prophylaxis of acute mountain sickness: systematic review and meta-analysis. *Bmj* 345:e6779

198. Lu DY, Tang CH, Yeh WL, Wong KL, Lin CP, et al. 2009. SDF-1alpha up-regulates interleukin-6 through CXCR4, PI3K/Akt, ERK, and NF-kappaB-dependent pathway in microglia. *Eur J Pharmacol* 613:146-54
199. Lu Q, Sun EE, Klein RS, Flanagan JG. 2001. Ephrin-B reverse signaling is mediated by a novel PDZ-RGS protein and selectively inhibits G protein-coupled chemoattraction. *Cell* 105:69-79
200. Luedi MM, Singh SK, Mosley JC, Hassan ISA, Hatami M, et al. 2018. Dexamethasone-mediated oncogenicity in vitro and in an animal model of glioblastoma. *J Neurosurg* 129:1446-55
201. Luks AM. 2015. Physiology in Medicine: A physiologic approach to prevention and treatment of acute high-altitude illnesses. *J Appl Physiol (1985)* 118:509-19
202. Luks AM, Auerbach PS, Freer L, Grissom CK, Keyes LE, et al. 2019. Wilderness Medical Society Clinical Practice Guidelines for the Prevention and Treatment of Acute Altitude Illness: 2019 Update. *Wilderness Environ Med* 30:S3-s18
203. Luks AM, Swenson ER, Bärtsch P. 2017. Acute high-altitude sickness. *Eur Respir Rev* 26
204. Lundeberg J, Feiner JR, Schober A, Sall JW, Eilers H, Bickler PE. 2018. Increased Cytokines at High Altitude: Lack of Effect of Ibuprofen on Acute Mountain Sickness, Physiological Variables, or Cytokine Levels. *High Alt Med Biol* 19:249-58
205. Lydic R, Baghdoyan HA, Hibbard L, Bonyak EV, DeJoseph MR, Hawkins RA. 1991. Regional brain glucose metabolism is altered during rapid eye movement sleep in the cat: a preliminary study. *J Comp Neurol* 304:517-29
206. Lyons SA, Kettenmann H. 1998. Oligodendrocytes and microglia are selectively vulnerable to combined hypoxia and hypoglycemia injury in vitro. *J Cereb Blood Flow Metab* 18:521-30
207. Lysko DE, Putt M, Golden JA. 2011. SDF1 regulates leading process branching and speed of migrating interneurons. *J Neurosci* 31:1739-45
208. Lysko DE, Putt M, Golden JA. 2014. SDF1 reduces interneuron leading process branching through dual regulation of actin and microtubules. *J Neurosci* 34:4941-62
209. MacGregor Sharp M, Saito S, Keable A, Gatherer M, Aldea R, et al. 2020. Demonstrating a reduced capacity for removal of fluid from cerebral white matter and hypoxia in areas of white matter hyperintensity associated with age and dementia. *Acta Neuropathol Commun* 8:131
210. Madry C, Kyrargyri V, Arancibia-Cárcamo IL, Jolivet R, Kohsaka S, et al. 2018. Microglial Ramification, Surveillance, and Interleukin-1β Release Are Regulated by the Two-Pore Domain K(+) Channel THIK-1. *Neuron* 97:299-312.e6
211. Madsen PL, Vorstrup S. 1991. Cerebral blood flow and metabolism during sleep. *Cerebrovasc Brain Metab Rev* 3:281-96
212. Maggiorini M. 2006. High altitude-induced pulmonary oedema. *Cardiovasc Res* 72:41-50
213. Magistretti PJ, Pellerin L. 1996. Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a prominent role of astrocytes. *Cereb Cortex* 6:50-61

214. Mahalingam S, McClelland GB, Scott GR. 2017. Evolved changes in the intracellular distribution and physiology of muscle mitochondria in high-altitude native deer mice. *J Physiol* 595:4785-801
215. Malhotra A, Castillo-Melendez M, Allison BJ, Sutherland AE, Nitsos I, et al. 2018. Neuropathology as a consequence of neonatal ventilation in premature growth-restricted lambs. *Am J Physiol Regul Integr Comp Physiol* 315:R1183-r94
216. Mao L, Huang M, Chen SC, Li YN, Xia YP, et al. 2014. Endogenous endothelial progenitor cells participate in neovascularization via CXCR4/SDF-1 axis and improve outcome after stroke. *CNS neuroscience & therapeutics* 20:460-8
217. Mao W, Yi X, Qin J, Tian M, Jin G. 2013. *CXCL12 inhibits cortical neuron apoptosis by increasing the ratio of Bcl-2/Bax after traumatic brain injury*.
218. Mao W, Yi X, Qin J, Tian M, Jin G. 2016. *CXCL12/CXCR4 Axis Improves Migration of Neuroblasts Along Corpus Callosum by Stimulating MMP-2 Secretion After Traumatic Brain Injury in Rats*.
219. March-Diaz R, Lara-Ureña N, Romero-Molina C, Heras-Garvin A, Ortega-de San Luis C, et al. 2021. Hypoxia compromises the mitochondrial metabolism of Alzheimer's disease microglia via HIF1. *Nature Aging* 1:385-99
220. Martin SJ. 1998. Time-dependent reversal of dentate LTP by 5 Hz stimulation. *Neuroreport* 9:3775-81
221. Martin SJ, Grimwood PD, Morris RG. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* 23:649-711
222. Martin SJ, Shires KL, Spooner PA. 2013. The relationship between tetanus intensity and the magnitude of hippocampal long-term potentiation in vivo. *Neuroscience* 231:363-72
223. McCandless EE, Piccio L, Woerner BM, Schmidt RE, Rubin JB, et al. 2008. Pathological Expression of CXCL12 at the Blood-Brain Barrier Correlates with Severity of Multiple Sclerosis. *The American Journal of Pathology* 172:799-808
224. McGrath JC, Deighan C, Briones AM, Shafaroudi MM, McBride M, et al. 2005. New aspects of vascular remodelling: the involvement of all vascular cell types. *Exp Physiol* 90:469-75
225. McKenna ZJ, Gorini Pereira F, Gillum TL, Amorim FT, Deyhle MR, Mermier CM. 2022. High-altitude exposures and intestinal barrier dysfunction. *Am J Physiol Regul Integr Comp Physiol* 322:R192-r203
226. Merino JJ, Garcimartin A, Lopez-Oliva ME, Benedi J, Gonzalez MP. 2016. The Impact of CXCR4 Blockade on the Survival of Rat Brain Cortical Neurons. *International journal of molecular sciences* 17
227. Merz TM, Treyer V, Hefti U, Spengler CM, Schwarz U, et al. 2006. Changes in cerebral glucose metabolism after an expedition to high altitudes. *High Alt Med Biol* 7:28-38
228. Messina-Graham S, Broxmeyer H. 2016. SDF-1/CXCL12 modulates mitochondrial respiration of immature blood cells in a bi-phasic manner. *Blood cells, molecules & diseases* 58:13-8
229. Mestre H, Mori Y, Nedergaard M. 2020. The Brain's Glymphatic System: Current Controversies. *Trends Neurosci* 43:458-66

230. Mirrakhimov AE, Strohl KP. 2016. High-altitude Pulmonary Hypertension: an Update on Disease Pathogenesis and Management. *Open Cardiovasc Med J* 10:19-27
231. Miyamoto A, Wake H, Ishikawa AW, Eto K, Shibata K, et al. 2016. Microglia contact induces synapse formation in developing somatosensory cortex. *Nat Commun* 7:12540
232. Mondo E, Becker SC, Kautzman AG, Schifferer M, Baer CE, et al. 2020. A Developmental Analysis of Juxtavascular Microglia Dynamics and Interactions with the Vasculature. *The Journal of Neuroscience* 40:6503-21
233. Moore LG. 2017. Measuring high-altitude adaptation. *J Appl Physiol (1985)* 123:1371-85
234. Moretti R, Caruso P. 2020. Small Vessel Disease-Related Dementia: An Invalid Neurovascular Coupling? *Int J Mol Sci* 21
235. Morlino G, Barreiro O, Baixauli F, Robles-Valero J, Gonzalez-Granado JM, et al. 2014. Miro-1 links mitochondria and microtubule Dynein motors to control lymphocyte migration and polarity. *Molecular and cellular biology* 34:1412-26
236. Moya EA, Go A, Kim CB, Fu Z, Simonson TS, Powell FL. 2020. Neuronal HIF-1α in the nucleus tractus solitarius contributes to ventilatory acclimatization to hypoxia. *J Physiol* 598:2021-34
237. Murray AJ. 2016. Energy metabolism and the high-altitude environment. *Exp Physiol* 101:23-7
238. Murray AJ, Horscroft JA. 2016. Mitochondrial function at extreme high altitude. *J Physiol* 594:1137-49
239. Murray AJ, Montgomery HE, Feelisch M, Grocott MPW, Martin DS. 2018. Metabolic adjustment to high-altitude hypoxia: from genetic signals to physiological implications. *Biochem Soc Trans* 46:599-607
240. Neves G, Cooke SF, Bliss TV. 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. *Nat Rev Neurosci* 9:65-75
241. Nguyen KT, Gates CA, Hassell JE, Jr., Foxx CL, Salazar SN, et al. 2021. Evaluation of the effects of altitude on biological signatures of inflammation and anxiety- and depressive-like behavioral responses. *Prog Neuropsychopharmacol Biol Psychiatry* 111:110331
242. Nimmerjahn A, Kirchhoff F, Helmchen F. 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308:1314-8
243. Noguchi CT, Asavaritikrai P, Teng R, Jia Y. 2007. Role of erythropoietin in the brain. *Crit Rev Oncol Hematol* 64:159-71
244. Nourshargh S, Alon R. 2014. Leukocyte migration into inflamed tissues. *Immunity* 41:694-707
245. Nussbaumer-Ochsner Y, Ursprung J, Siebenmann C, Maggiorini M, Bloch KE. 2012. Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing. *Sleep* 35:419-23
246. Nycz B, Mandera M. 2021. The features of the glymphatic system. *Auton Neurosci* 232:102774
247. O'Hara R, Serres J, Dodson W, Wright B, Ordway J, et al. 2014. The use of dexamethasone in support of high-altitude ground operations and physical performance: review of the literature. *J Spec Oper Med* 14:53-8

248. Oberman K, Hovens I, de Haan J, Falcao-Salles J, van Leeuwen B, Schoemaker R. 2021. Acute pre-operative ibuprofen improves cognition in a rat model for postoperative cognitive dysfunction. *J Neuroinflammation* 18:156
249. Ohab JJ, Fleming S, Blesch A, Carmichael ST. 2006. A neurovascular niche for neurogenesis after stroke. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26:13007-16
250. Oliveira AC, Sharma RK, Aquino V, Lobaton G, Bryant AJ, et al. 2018. Involvement of Microglial Cells in Hypoxia-induced Pulmonary Hypertension. *Am J Respir Cell Mol Biol* 59:271-3
251. Ozgür B, Helms HCC, Tornabene E, Brodin B. 2022. Hypoxia increases expression of selected blood-brain barrier transporters GLUT-1, P-gp, SLC7A5 and TFRC, while maintaining barrier integrity, in brain capillary endothelial monolayers. *Fluids Barriers CNS* 19:1
252. Pabbidi MR, Kuppusamy M, Didion SP, Sanapureddy P, Reed JT, Sontakke SP. 2018. Sex differences in the vascular function and related mechanisms: role of 17β-estradiol. *Am J Physiol Heart Circ Physiol* 315:H1499-h518
253. Paddenberg R, Stieger P, von Lilien AL, Faulhammer P, Goldenberg A, et al. 2007. Rapamycin attenuates hypoxia-induced pulmonary vascular remodeling and right ventricular hypertrophy in mice. *Respir Res* 8:15
254. Pandit A, Karmacharya P, Pathak R, Giri S, Aryal MR. 2014. Efficacy of NSAIDs for the prevention of acute mountain sickness: a systematic review and meta-analysis. *J Community Hosp Intern Med Perspect* 4
255. Pantazopoulos H, Gisabella B, Rexrode L, Benefield D, Yildiz E, et al. 2020. Circadian Rhythms of Perineuronal Net Composition. *eNeuro* 7
256. Park JH, Park JA, Ahn JH, Kim YH, Kang IJ, et al. 2017. Transient cerebral ischemia induces albumin expression in microglia only in the CA1 region of the gerbil hippocampus. *Mol Med Rep* 16:661-5
257. Park MJ, Park HS, You MJ, Yoo J, Kim SH, Kwon MS. 2019. Dexamethasone Induces a Specific Form of Ramified Dysfunctional Microglia. *Mol Neurobiol* 56:1421-36
258. Parkes I, Chintawar S, Cader MZ. 2018. Neurovascular dysfunction in dementia - human cellular models and molecular mechanisms. *Clin Sci (Lond)* 132:399-418
259. Pawig L, Klasen C, Weber C, Bernhagen J, Noels H. 2015. Diversity and Inter-Connections in the CXCR4 Chemokine Receptor/Ligand Family: Molecular Perspectives. *Front Immunol* 6:429
260. Peers C, Dallas ML, Boycott HE, Scragg JL, Pearson HA, Boyle JP. 2009. Hypoxia and neurodegeneration. *Ann N Y Acad Sci* 1177:169-77
261. Pena E, Brito J, El Alam S, Siques P. 2020. Oxidative Stress, Kinase Activity and Inflammatory Implications in Right Ventricular Hypertrophy and Heart Failure under Hypobaric Hypoxia. *Int J Mol Sci* 21
262. Pena E, El Alam S, Siques P, Brito J. 2022. Oxidative Stress and Diseases Associated with High-Altitude Exposure. *Antioxidants (Basel)* 11
263. Petricoin EF, Belluco C, Araujo RP, Liotta LA. 2006. The blood peptidome: a higher dimension of information content for cancer biomarker discovery. *Nat Rev Cancer* 6:961-7

264. Pfeiffer T, Avignone E, Nägerl UV. 2016. Induction of hippocampal long-term potentiation increases the morphological dynamics of microglial processes and prolongs their contacts with dendritic spines. *Scientific Reports* 6:32422
265. Pham K, Parikh K, Heinrich EC. 2021. Hypoxia and Inflammation: Insights From High-Altitude Physiology. *Front Physiol* 12:676782
266. Phillips L, Basnyat B, Chang Y, Swenson ER, Harris NS. 2017. Findings of Cognitive Impairment at High Altitude: Relationships to Acetazolamide Use and Acute Mountain Sickness. *High Alt Med Biol* 18:121-7
267. Phng LK, Gerhardt H. 2009. Angiogenesis: a team effort coordinated by notch. *Dev Cell* 16:196-208
268. Piantino JA, Iliff JJ, Lim MM. 2022. The Bidirectional Link Between Sleep Disturbances and Traumatic Brain Injury Symptoms: A Role for Glymphatic Dysfunction? *Biol Psychiatry* 91:478-87
269. Pinto B, Morelli G, Rastogi M, Savardi A, Fumagalli A, et al. 2020. Rescuing Over-activated Microglia Restores Cognitive Performance in Juvenile Animals of the Dp(16) Mouse Model of Down Syndrome. *Neuron* 108:887-904.e12
270. Plog BA, Nedergaard M. 2018. The Glymphatic System in Central Nervous System Health and Disease: Past, Present, and Future. *Annu Rev Pathol* 13:379-94
271. Powell FL. 2007. The influence of chronic hypoxia upon chemoreception. *Respir Physiol Neurobiol* 157:154-61
272. Powell FL, Fu Z. 2008. HIF-1 and ventilatory acclimatization to chronic hypoxia. *Respir Physiol Neurobiol* 164:282-7
273. Pozo M, Claret M. 2018. Hypothalamic Control of Systemic Glucose Homeostasis: The Pancreas Connection. *Trends Endocrinol Metab* 29:581-94
274. Profaci CP, Munji RN, Pulido RS, Daneman R. 2020. The blood-brain barrier in health and disease: Important unanswered questions. *J Exp Med* 217
275. Pugliese AM, Latini S, Corradetti R, Pedata F. 2003. Brief, repeated, oxygen-glucose deprivation episodes protect neurotransmission from a longer ischemic episode in the in vitro hippocampus: role of adenosine receptors. *Br J Pharmacol* 140:305-14
276. Pugsley MK, Tabrizchi R. 2000. The vascular system. An overview of structure and function. *J Pharmacol Toxicol Methods* 44:333-40
277. Pun M, Hartmann SE, Furian M, Dyck AM, Muralt L, et al. 2018. Effect of Acute, Subacute, and Repeated Exposure to High Altitude (5050 m) on Psychomotor Vigilance. *Frontiers in Physiology* 9
278. Quastel JH, Wheatley AH. 1932. Oxidations by the brain. *Biochem J* 26:725-44
279. Ragozzino D, Renzi M, Giovannelli A, Eusebi F. 2002. Stimulation of chemokine CXC receptor 4 induces synaptic depression of evoked parallel fibers inputs onto Purkinje neurons in mouse cerebellum. *J Neuroimmunol* 127:30-6
280. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, et al. 2011. Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol* 70:374-83
281. Ran X, Wang H, Chen Y, Zeng Z, Zhou Q, et al. 2010. Aquaporin-1 expression and angiogenesis in rabbit chronic myocardial ischemia is decreased by acetazolamide. *Heart Vessels* 25:237-47

282. Reddy OC, van der Werf YD. 2020. The Sleeping Brain: Harnessing the Power of the Glymphatic System through Lifestyle Choices. *Brain Sci* 10
283. Rice RA, Spangenberg EE, Yamate-Morgan H, Lee RJ, Arora RP, et al. 2015. Elimination of Microglia Improves Functional Outcomes Following Extensive Neuronal Loss in the Hippocampus. *J Neurosci* 35:9977-89
284. Richalet JP, Larmignat P, Poitrine E, Letournel M, Canouï-Poitrine F. 2012. Physiological risk factors for severe high-altitude illness: a prospective cohort study. *Am J Respir Crit Care Med* 185:192-8
285. Richalet JP, Rivera M, Bouchet P, Chirinos E, Onnen I, et al. 2005. Acetazolamide: a treatment for chronic mountain sickness. *Am J Respir Crit Care Med* 172:1427-33
286. Rieger MG, Hoiland RL, Tremblay JC, Stembridge M, Bain AR, et al. 2017. One session of remote ischemic preconditioning does not improve vascular function in acute normobaric and chronic hypobaric hypoxia. *Exp Physiol* 102:1143-57
287. Roach EB, Bleiberg J, Lathan CE, Wolpert L, Tsao JW, Roach RC. 2014. AltitudeOmics: Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment. *Neuroreport* 25:814-8
288. Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, et al. 2011. CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J Neurosci* 31:16241-50
289. Sa de Almeida J, Vargas M, Fonseca-Gomes J, Tanqueiro SR, Belo RF, et al. 2020. Microglial Sirtuin 2 Shapes Long-Term Potentiation in Hippocampal Slices. *Front Neurosci* 14:614
290. Sadgrove MP, Beaver CJ, Turner DA. 2007. Effects of relative hypoglycemia on LTP and NADH imaging in rat hippocampal slices. *Brain Res* 1165:30-9
291. Said MF, Islam AA, Massi MN, Prihantono, Hatta M, et al. 2021. Effect of erythropoietin administration on expression of mRNA brain-derived Neutrophic factor, levels of stromal cell-derived Factor-1, and neuron specific enolase in brain injury model Sprague Dawley. *Annals of Medicine and Surgery* 70:102877
292. Sanes JR, Lichtman JW. 1999. Can molecules explain long-term potentiation? *Nat Neurosci* 2:597-604
293. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, et al. 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74:691-705
294. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. 2007. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 69:2197-204
295. Selvaraj UM, Ortega SB, Hu R, Gilchrist R, Kong X, et al. 2017. Preconditioning-induced CXCL12 upregulation minimizes leukocyte infiltration after stroke in ischemia-tolerant mice. *J Cereb Blood Flow Metab* 37:801-13
296. Semaan A, Dietrich D, Bergheim D, Dietrich J, C. Kalff J, et al. 2016. *CXCL12 expression and PD-L1 expression serve as prognostic biomarkers in HCC and are induced by hypoxia*. 1-12 pp.
297. Serchov T, Schwarz I, Theiss A, Sun L, Holz A, et al. 2020. Enhanced adenosine A1 receptor and Homer1a expression in hippocampus modulates the resilience to stress-induced depression-like behavior. *Neuropharmacology* 162:107834

298. Sharma Kandel R, Mishra R, Gautam J, Alaref A, Hassan A, Jahan N. 2020. Patchy Vasoconstriction Versus Inflammation: A Debate in the Pathogenesis of High Altitude Pulmonary Edema. *Cureus* 12:e10371
299. Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, et al. 1995. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics* 28:495-500
300. Siarey RJ, Kline-Burgess A, Cho M, Balbo A, Best TK, et al. 2006. Altered signaling pathways underlying abnormal hippocampal synaptic plasticity in the Ts65Dn mouse model of Down syndrome. *J Neurochem* 98:1266-77
301. Silvani A, Asti V, Berteotti C, Bojic T, Cianci T, et al. 2005. Sleep-related brain activation does not increase the permeability of the blood-brain barrier to glucose. *J Cereb Blood Flow Metab* 25:990-7
302. Simka M, Latacz P, Czaja J. 2018. Possible Role of Glymphatic System of the Brain in the Pathogenesis of High-Altitude Cerebral Edema. *High Alt Med Biol* 19:394-7
303. Simonson TS. 2015. Altitude Adaptation: A Glimpse Through Various Lenses. *High Alt Med Biol* 16:125-37
304. Song ZY, Wang F, Cui SX, Qu XJ. 2018. Knockdown of CXCR4 Inhibits CXCL12-Induced Angiogenesis in HUVECs through Downregulation of the MAPK/ERK and PI3K/AKT and the Wnt/beta-Catenin Pathways. *Cancer investigation* 36:10-8
305. Spangenberg E, Severson PL, Hohsfield LA, Crapser J, Zhang J, et al. 2019. Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. *Nature Communications* 10:3758
306. Stein LR, O'Dell KA, Funatsu M, Zorumski CF, Izumi Y. 2016. Short-term environmental enrichment enhances synaptic plasticity in hippocampal slices from aged rats. *Neuroscience* 329:294-305
307. Steinback CD, Poulin MJ. 2016. Influence of Hypoxia on Cerebral Blood Flow Regulation in Humans. *Adv Exp Med Biol* 903:131-44
308. Stolp HB, Dziegielewska KM. 2009. Review: Role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. *Neuropathol Appl Neurobiol* 35:132-46
309. Storz JF, Moriyama H. 2008. Mechanisms of hemoglobin adaptation to high altitude hypoxia. *High Alt Med Biol* 9:148-57
310. Strapazzon G, Brugger H, Dal Cappello T, Procter E, Hofer G, Lochner P. 2014. Factors associated with optic nerve sheath diameter during exposure to hypobaric hypoxia. *Neurology* 82:1914-8
311. Strapazzon G, Vezzaro R, Hofer G, Dal Cappello T, Procter E, et al. 2015. Factors associated with B-lines after exposure to hypobaric hypoxia. *Eur Heart J Cardiovasc Imaging* 16:1241-6
312. Stumm R, Kolodziej A, Schulz S, Kohtz JD, Höllt V. 2007. Patterns of SDF-1alpha and SDF-1gamma mRNAs, migration pathways, and phenotypes of CXCR4-expressing neurons in the developing rat telencephalon. *J Comp Neurol* 502:382-99
313. Su Z, Liu Y, Zhang H. 2021. Adaptive Cardiac Metabolism Under Chronic Hypoxia: Mechanism and Clinical Implications. *Front Cell Dev Biol* 9:625524

314. Südhof TC. 2021. The cell biology of synapse formation. *J Cell Biol* 220
315. Sun BW, Shen HM, Liu BC, Fang HL. 2017. Research on the effect and mechanism of the CXCR-4-overexpressing BMSCs combined with SDF-1alpha for the cure of acute SCI in rats. *European review for medical and pharmacological sciences* 21:167-74
316. Sun C, Zhang S, Wang J, Jiang W, Xin Q, et al. 2019. EPO enhances the protective effects of MSCs in experimental hyperoxia-induced neonatal mice by promoting angiogenesis. *Aging (Albany NY)* 11:2477-87
317. Sun W, Liu J, Huan Y, Zhang C. 2014. Intracranial injection of recombinant stromal-derived factor-1 alpha (SDF-1alpha) attenuates traumatic brain injury in rats. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* 63:287-97
318. Sun Y, Mao X, Fan C, Liu C, Guo A, et al. 2014. CXCL12-CXCR4 axis promotes the natural selection of breast cancer cell metastasis. *Tumour Biology* 35:7765-73
319. Suresh S, Rajvanshi PK, Noguchi CT. 2019. The Many Facets of Erythropoietin Physiologic and Metabolic Response. *Front Physiol* 10:1534
320. Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. 2019. Blood-Brain Barrier: From Physiology to Disease and Back. *Physiol Rev* 99:21-78
321. Swenson ER. 2014. Carbonic anhydrase inhibitors and high altitude illnesses. *Subcell Biochem* 75:361-86
322. Swenson ER. 2016. Pharmacology of acute mountain sickness: old drugs and newer thinking. *J Appl Physiol (1985)* 120:204-15
323. Swiatkowski P, Murugan M, Eyo UB, Wang Y, Rangaraju S, et al. 2016. Activation of microglial P2Y12 receptor is required for outward potassium currents in response to neuronal injury. *Neuroscience* 318:22-33
324. Szepesi Z, Manouchehrian O, Bachiller S, Deierborg T. 2018. Bidirectional Microglia–Neuron Communication in Health and Disease. *Frontiers in Cellular Neuroscience* 12
325. Tamura T, Aoyama M, Ukai S, Kakita H, Sobue K, Asai K. 2017. Neuroprotective erythropoietin attenuates microglial activation, including morphological changes, phagocytosis, and cytokine production. *Brain Res* 1662:65-74
326. Teema AM, Zaitone SA, Moustafa YM. 2016. Ibuprofen or piroxicam protects nigral neurons and delays the development of l-dopa induced dyskinesia in rats with experimental Parkinsonism: Influence on angiogenesis. *Neuropharmacology* 107:432-50
327. Teyler TJ. 2001. In Vitro Neurophysiology. In *International Encyclopedia of the Social & Behavioral Sciences*, ed. NJ Smelser, PB Baltes:7251-4. Oxford: Pergamon. Number of 7251-4 pp.
328. Thal SC, Schaible EV, Neuhaus W, Scheffer D, Brandstetter M, et al. 2013. Inhibition of proteasomal glucocorticoid receptor degradation restores dexamethasone-mediated stabilization of the blood-brain barrier after traumatic brain injury. *Crit Care Med* 41:1305-15
329. Ting JT, Lee BR, Chong P, Soler-Llavina G, Cobbs C, et al. 2018. Preparation of Acute Brain Slices Using an Optimized N-Methyl-D-glucamine Protective Recovery Method. *J Vis Exp*

330. Tiveron MC, Rossel M, Moepps B, Zhang YL, Seidenfaden R, et al. 2006. Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. *J Neurosci* 26:13273-8
331. Toba Y, Tiong JD, Ma Q, Wray S. 2008. CXCR4/SDF-1 system modulates development of GnRH-1 neurons and the olfactory system. *Dev Neurobiol* 68:487-503
332. Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, et al. 2013. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain* 136:2697-706
333. Toriello M, González-Quintanilla V, Pascual J. 2021. The glymphatic system and its involvement in disorders of the nervous system. *Med Clin (Barc)* 156:339-43
334. Tremblay M-È, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. 2011. The Role of Microglia in the Healthy Brain. *The Journal of Neuroscience* 31:16064-9
335. Uldall M, Botfield H, Jansen-Olesen I, Sinclair A, Jensen R. 2017. Acetazolamide lowers intracranial pressure and modulates the cerebrospinal fluid secretion pathway in healthy rats. *Neurosci Lett* 645:33-9
336. Ulrich S, Nussbaumer-Ochsner Y, Vasic I, Hasler E, Latshang TD, et al. 2014. Cerebral oxygenation in patients with OSA: effects of hypoxia at altitude and impact of acetazolamide. *Chest* 146:299-308
337. Um JW. 2017. Roles of Glial Cells in Sculpting Inhibitory Synapses and Neural Circuits. *Front Mol Neurosci* 10:381
338. Umpierre AD, Bystrom LL, Ying Y, Liu YU, Worrell G, Wu LJ. 2020. Microglial calcium signaling is attuned to neuronal activity in awake mice. *Elife* 9
339. Vandebroek A, Yasui M. 2020. Regulation of AQP4 in the Central Nervous System. *Int J Mol Sci* 21
340. Vestergaard MB, Lindberg U, Aachmann-Andersen NJ, Lisbjerg K, Christensen SJ, et al. 2016. Acute hypoxia increases the cerebral metabolic rate - a magnetic resonance imaging study. *J Cereb Blood Flow Metab* 36:1046-58
341. Villa A, Vegeto E, Poletti A, Maggi A. 2016. Estrogens, Neuroinflammation, and Neurodegeneration. *Endocr Rev* 37:372-402
342. Vilz TO, Moepps B, Engele J, Molly S, Littman DR, Schilling K. 2005. The SDF-1/CXCR4 pathway and the development of the cerebellar system. *Eur J Neurosci* 22:1831-9
343. Virgintino D, Errede M, Rizzi M, Girolamo F, Strippoli M, et al. 2013. The CXCL12/CXCR4/CXCR7 ligand-receptor system regulates neuro-glio-vascular interactions and vessel growth during human brain development. *J Inherit Metab Dis* 36:455-66
344. Vittori DC, Chamorro ME, Hernández YV, Maltaneri RE, Nesse AB. 2021. Erythropoietin and derivatives: Potential beneficial effects on the brain. *J Neurochem* 158:1032-57
345. Voit RA, Sankaran VG. 2020. Stabilizing HIF to Ameliorate Anemia. *Cell* 180:6
346. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. 2009. Resting Microglia Directly Monitor the Functional State of Synapses In Vivo

and Determine the Fate of Ischemic Terminals. *The Journal of Neuroscience* 29:3974-80
347. Wakhloo D, Scharkowski F, Curto Y, Javed Butt U, Bansal V, et al. 2020. Functional hypoxia drives neuroplasticity and neurogenesis via brain erythropoietin. *Nat Commun* 11:1313
348. Wang C, Yan M, Jiang H, Wang Q, He S, et al. 2018. Mechanism of aquaporin 4 (AQP 4) up-regulation in rat cerebral edema under hypobaric hypoxia and the preventative effect of puerarin. *Life Sci* 193:270-81
349. Wang J, Knaut H. 2014. Chemokine signaling in development and disease. *Development (Cambridge, England)* 141:4199-205
350. Wang LL, Chen D, Lee J, Gu X, Alaaeddine G, et al. 2014. Mobilization of endogenous bone marrow derived endothelial progenitor cells and therapeutic potential of parathyroid hormone after ischemic stroke in mice. *PLoS One* 9:e87284
351. Wang M, Whiting K, Lischka F, Galdzicki Z, Yu G. 2022. An unsupervised approach to detect microglia tip in volumetric fluorescence imaging data. In *IEEE International Symposium on Biomedical Imaging*
352. Wang Q, Tang XN, Yenari MA. 2007. The inflammatory response in stroke. *J Neuroimmunol* 184:53-68
353. Wang Y, Rattner A, Zhou Y, Williams J, Smallwood PM, Nathans J. 2012. Norrin/Frizzled4 signaling in retinal vascular development and blood brain barrier plasticity. *Cell* 151:1332-44
354. Wei F, Song J, Zhang C, Lin J, Xue R, et al. 2019. Chronic stress impairs the aquaporin-4-mediated glymphatic transport through glucocorticoid signaling. *Psychopharmacology (Berl)* 236:1367-84
355. Wei M, Bo X, Xiaopeng Z, Hongbin Z. Decomposition of branching volume data by tip detection. *Proc. 2008 15th IEEE International Conference on Image Processing*, *2008*:1948-51:
356. Werneburg S, Feinberg PA, Johnson KM, Schafer DP. 2017. A microglia-cytokine axis to modulate synaptic connectivity and function. *Curr Opin Neurobiol* 47:138-45
357. Werner Y, Mass E, Ashok Kumar P, Ulas T, Händler K, et al. 2020. Cxcr4 distinguishes HSC-derived monocytes from microglia and reveals monocyte immune responses to experimental stroke. *Nature Neuroscience* 23:351-62
358. West JB. 1996. Prediction of barometric pressures at high altitude with the use of model atmospheres. *J Appl Physiol (1985)* 81:1850-4
359. West JB. 2008. A new approach to very-high-altitude land travel: the train to Lhasa, Tibet. *Ann Intern Med* 149:898-900
360. Whayne TF, Jr. 2014. Cardiovascular medicine at high altitude. *Angiology* 65:459-72
361. Wiktorowska-Owczarek A, Namiecińska M, Owczarek J. 2015. THE EFFECT OF IBUPROFEN ON bFGF, VEGF SECRETION AND CELL PROLIFERATION IN THE PRESENCE OF LPS IN HMEC-1 CELLS. *Acta Pol Pharm* 72:889-94
362. Wilson MH, Newman S, Imray CH. 2009. The cerebral effects of ascent to high altitudes. *Lancet Neurol* 8:175-91

363. Windsor JS, Rodway GW. 2007. Heights and haematology: the story of haemoglobin at altitude. *Postgrad Med J* 83:148-51
364. Winkler Z, Kuti D, Polyák Á, Juhász B, Gulyás K, et al. 2019. Hypoglycemia-activated Hypothalamic Microglia Impairs Glucose Counterregulatory Responses. *Sci Rep* 9:6224
365. Wolburg H, Noell S, Mack A, Wolburg-Buchholz K, Fallier-Becker P. 2009. Brain endothelial cells and the glio-vascular complex. *Cell Tissue Res* 335:75-96
366. Wong BW, Marsch E, Treps L, Baes M, Carmeliet P. 2017. Endothelial cell metabolism in health and disease: impact of hypoxia. *Embo j* 36:2187-203
367. Wu WF, Tan XJ, Dai YB, Krishnan V, Warner M, Gustafsson J. 2013. Targeting estrogen receptor β in microglia and T cells to treat experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 110:3543-8
368. Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B. 2015. Microglia: Dynamic Mediators of Synapse Development and Plasticity. *Trends Immunol* 36:605-13
369. Wyss-Coray T, Lin C, Yan F, Yu GQ, Rohde M, et al. 2001. TGF-beta1 promotes microglial amyloid-beta clearance and reduces plaque burden in transgenic mice. *Nat Med* 7:612-8
370. Xiang Y, Li Y, Zhang Z, Cui K, Wang S, et al. 2002. Nerve growth cone guidance mediated by G protein-coupled receptors. *Nat Neurosci* 5:843-8
371. Xiang Y, Ma B, Li T, Gao JW, Yu HM, Li XJ. 2004. Acetazolamide inhibits aquaporin-1 protein expression and angiogenesis. *Acta Pharmacol Sin* 25:812-6
372. Xu K, Sun X, Benderro GF, Tsipis CP, LaManna JC. 2017. Gender differences in hypoxic acclimatization in cyclooxygenase-2-deficient mice. *Physiol Rep* 5
373. Yamashita K, Imaizumi T, Hatakeyama M, Tamo W, Kimura D, et al. 2003. Effect of hypoxia on the expression of fractalkine in human endothelial cells. *Tohoku J Exp Med* 200:187-94
374. Yang MT, Chien WL, Lu DH, Liou HC, Fu WM. 2013. Acetazolamide impairs fear memory consolidation in rodents. *Neuropharmacology* 67:412-8
375. Yao X, Miao W, Li M, Wang M, Ma J, et al. 2010. Protective effect of albumin on VEGF and brain edema in acute ischemia in rats. *Neurosci Lett* 472:179-83
376. Yawno T, Castillo-Melendez M, Jenkin G, Wallace EM, Walker DW, Miller SL. 2012. Mechanisms of Melatonin-Induced Protection in the Brain of Late Gestation Fetal Sheep in Response to Hypoxia. *Developmental Neuroscience* 34:543-51
377. Yi H, Yu Q, Zeng D, Shen Z, Li J, et al. 2021. Serum Inflammatory Factor Profiles in the Pathogenesis of High-Altitude Polycythemia and Mechanisms of Acclimation to High Altitudes. *Mediators Inflamm* 2021:8844438
378. York EM, Zhang J, Choi HB, MacVicar BA. 2021. Neuroinflammatory inhibition of synaptic long-term potentiation requires immunometabolic reprogramming of microglia. *Glia* 69:567-78
379. Yu X, Ji C, Shao A. 2020. Neurovascular Unit Dysfunction and Neurodegenerative Disorders. *Front Neurosci* 14:334
380. Zhang G, Zhou SM, Yuan C, Tian HJ, Li P, Gao YQ. 2013. The effects of short-term and long-term exposure to a high altitude hypoxic environment on neurobehavioral function. *High Alt Med Biol* 14:338-41

381. Zhang J, Hu H, Palma NL, Harrison JK, Mubarak KK, et al. 2012. Hypoxia-induced endothelial CX3CL1 triggers lung smooth muscle cell phenotypic switching and proliferative expansion. *Am J Physiol Lung Cell Mol Physiol* 303:L912-22
382. Zhang Y, Cao H, Qiu X, Xu D, Chen Y, et al. 2020. Neuroprotective Effects of Adenosine A1 Receptor Signaling on Cognitive Impairment Induced by Chronic Intermittent Hypoxia in Mice. *Frontiers in Cellular Neuroscience* 14
383. Zhang Y, Dépond M, He L, Foudi A, Kwarteng EO, et al. 2016. CXCR4/CXCL12 axis counteracts hematopoietic stem cell exhaustion through selective protection against oxidative stress. *Scientific reports* 6:37827
384. Zhang Y, Rogers HM, Zhang X, Noguchi CT. 2017. Sex difference in mouse metabolic response to erythropoietin. *Faseb j* 31:2661-73
385. Zhao X, Qian D, Wu N, Yin Y, Song M, et al. 2011. Spleen recruits endothelial progenitor cells (EPCs) via SDF-1/CXCR4 axis(*). *Heart* 97:A92-A3
386. Zhou H, Saidel GM, LaManna JC. 2008. Cerebral blood flow adaptation to chronic hypoxia. *Adv Exp Med Biol* 614:371-7
387. Zhou Y, Huang X, Zhao T, Qiao M, Zhao X, et al. 2017. Hypoxia augments LPS-induced inflammation and triggers high altitude cerebral edema in mice. *Brain Behav Immun* 64:266-75
388. Zhou Y, Wang Y, Tischfield M, Williams J, Smallwood PM, et al. 2014. Canonical WNT signaling components in vascular development and barrier formation. *J Clin Invest* 124:3825-46
389. Zhuravin IA, Dubrovskaya NM, Vasilev DS, Postnikova TY, Zaitsev AV. 2019. Prenatal hypoxia produces memory deficits associated with impairment of long-term synaptic plasticity in young rats. *Neurobiol Learn Mem* 164:107066
390. Zimna A, Kurpisz M. 2015. Hypoxia-Inducible Factor-1 in Physiological and Pathophysiological Angiogenesis: Applications and Therapies. *Biomed Res Int* 2015:549412
391. Zlokovic BV. 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57:178-201
392. Zorumski CF, Izumi Y. 1998. Modulation of LTP induction by NMDA receptor activation and nitric oxide release. *Prog Brain Res* 118:173-82