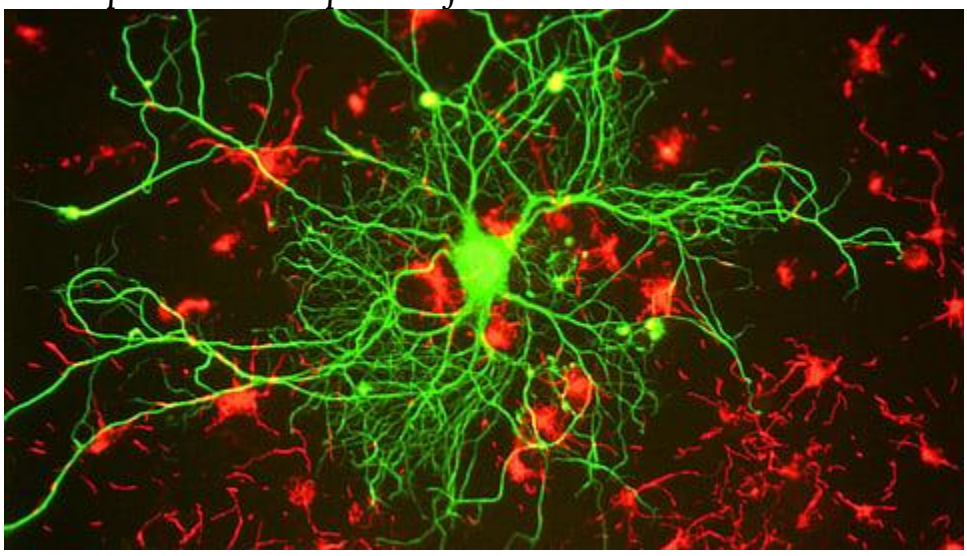
BRAIN INTRACRANIAL VOL 11

Calcium-mediated dendritic action potentials (dCaAPs)

Recent research has revealed a **unique type of electrical signal** in the human brain, known as **calcium-mediated dendritic action potentials (dCaAPs)**. This discovery focuses on dendrites, the branch-like extensions of neurons, and indicates that these signals exhibit a **graded response**, meaning their strength varies with input intensity. Unlike typical all-or-none responses, dCaAPs allow individual neurons to process complex information independently, challenging previous beliefs about how neural networks function. This finding opens new avenues for understanding brain computation and cognition, highlighting the sophistication of the human brain compared to other animals.

Groundbreaking Discovery of Unique Electrical Signals in the Human Brain

New research reveals distinct brain signals in dendrites, redefining how neurons process complex information



Newly discovered brain signals reveal the human brain's unmatched computational abilities. (Representational image: Wikimedia Commons)

For the first time, researchers have identified a unique type of electrical signal in the human brain, providing new insights into how information is processed. This breakthrough focuses on dendrites, the branch-like extensions of neurons responsible for receiving and transmitting signals. While previous studies on dendritic electrical activity were primarily conducted on rodents, this research examined human brain tissue, specifically from layer 2/3 (L2/3) pyramidal neurons in the cerebral cortex. This region is known for its vital role in advanced thinking and problem-solving.

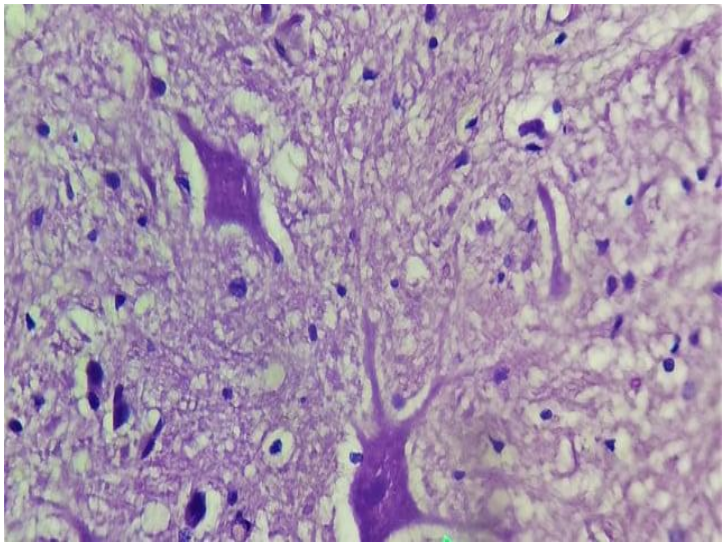
Dendrites are crucial components of neurons, acting as the main receivers of signals from other nerve cells. These signals are transmitted to the neuron's soma, or main body, where they are further processed. Understanding dendritic activity has been a central focus in neuroscience, but the findings from this study have revealed entirely new dynamics in human neurons.

Discovery of a New Signal

A previously unknown type of electrical signal, referred to as calcium-mediated dendritic action potentials (dCaAPs), was discovered during the study. Unlike the typical "all-or-none" responses that are standard in neuronal signaling, these dCaAPs exhibited a "graded" response. The strength of these signals varied based on the level of input, reaching maximum efficiency with moderate stimulation but diminishing when stronger inputs were applied.

This discovery challenges traditional views of neural communication. While it was previously believed that complex problems required the involvement of entire networks of neurons, the study demonstrated that individual neurons, through these unique dendritic signals, can process intricate information independently. Essentially, a single

neuron is capable of classifying and interpreting inputs that were once thought to be beyond the capacity of a single cell.



Unique dendritic signals challenge traditional views on neuron networks and brain function. (Representational image: Wikimedia Commons)

Implications for Brain Function

The findings highlight the advanced computational abilities of human neurons, distinguishing them from those of other species. Researchers suggest that these distinctive dendritic properties may contribute to the human brain's remarkable cognitive abilities, including problem-solving and abstract thought. This study challenges long-standing assumptions about the function of neural networks, emphasizing the sophistication of human brain activity.

Applications in Neurological Research

Beyond deepening the understanding of brain computation, the discovery opens new doors for exploring neurological disorders. Many conditions, such as Alzheimer's disease, epilepsy, and autism, involve disruptions in how the brain processes information. By understanding the role of these unique signals in healthy brain function, researchers can better explore how their dysfunction contributes to such disorders.

This groundbreaking research also offers potential for developing targeted therapies. Insights into how neurons independently process

complex information could inspire new treatments for neurological conditions where brain computation fails.

The identification of calcium-mediated dendritic action potentials in the human brain marks a significant advancement in neuroscience. This discovery not only enhances our understanding of human brain function but also underscores the complexity and uniqueness of human cognition. By further studying these signals, scientists may uncover new ways to address neurological disorders, advancing both our knowledge of the brain and our ability to treat its ailments.

References:

1. Tingley, Anna. “A First-of-Its-Kind Signal Has Been Detected in Human Brains.” *ScienceAlert*. Last modified December 10, 2024. https://www.sciencealert.com/a-first-of-its-kind-signal-has-been-detected-in-human-brains.
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Brain Cells Use Muscle-Like Signals to Strengthen Learning and Memory

·February 7, 2025

Summary: New research reveals that brain cells use a muscle-like signaling mechanism to relay information over long distances. Scientists discovered that dendrites, the branch-like extensions of neurons, contain a structured network of contact sites that amplify

calcium signals—similar to how muscles contract. These contact sites regulate calcium release, activating key proteins involved in learning and memory.

This mechanism explains how neurons process information received at specific points and relay it to the cell body. Understanding this process sheds light on synaptic plasticity, which underlies learning and memory formation. The findings could provide new insights into neurodegenerative diseases like Alzheimer's.

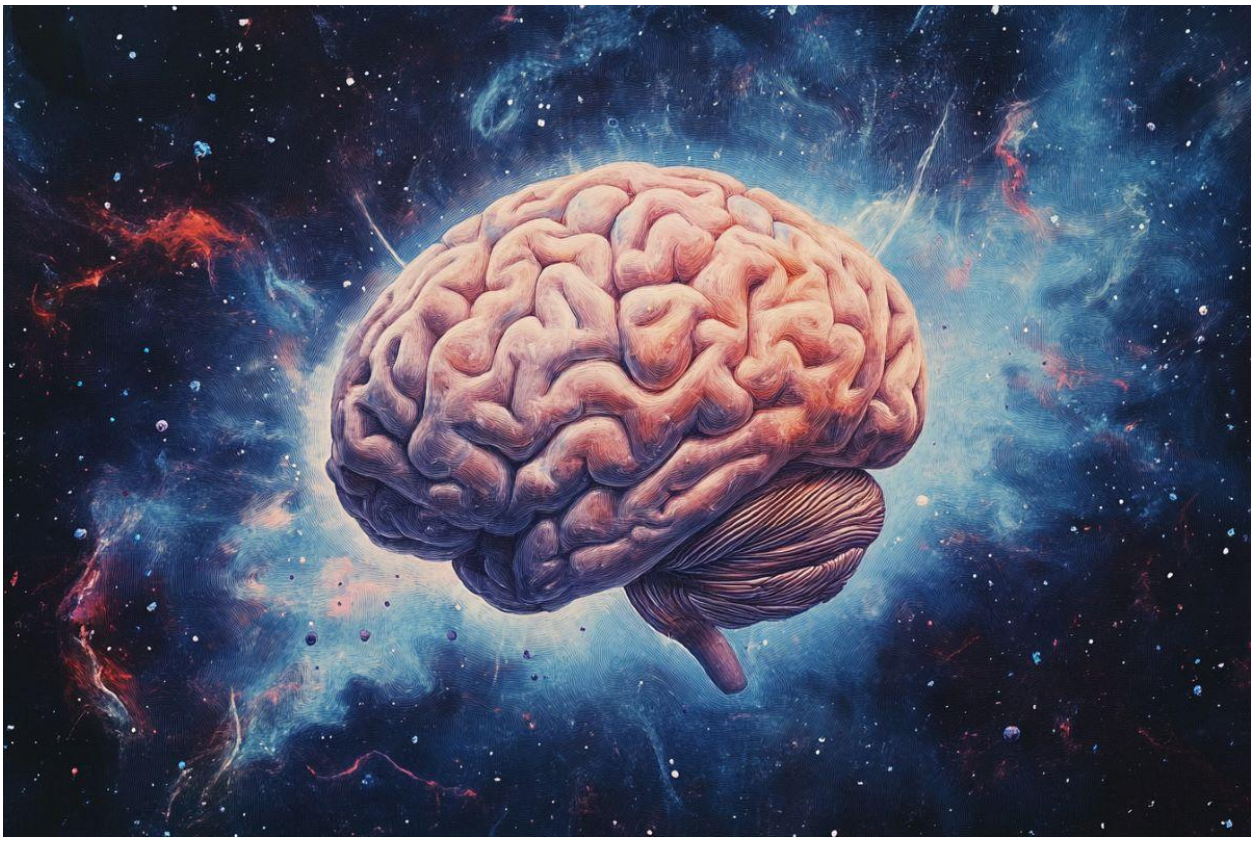
Key Facts:

- **Neural Calcium Amplification:** Brain cells use structured ER contact sites to amplify calcium signals, similar to how muscle cells trigger contractions.
- **Memory and Learning Connection:** These calcium signals activate CaMKII, a protein critical for strengthening neuronal connections and memory formation.
- **Potential Disease Insights:** Understanding this mechanism could help explain cognitive dysfunction in conditions like Alzheimer's disease.

Source: HHMI

Our biceps and our brain cells may have more in common than previously thought.

New research led by the Lippincott-Schwartz Lab shows that a network of subcellular structures similar to those responsible for propagating molecular signals that make muscles contract are also responsible for transmitting signals in the brain that may facilitate learning and memory.



This process continues from contact site to contact site all along the dendrite to the cell body, where the neuron decides how it will communicate with other neurons. Credit: Neuroscience News

“Einstein said that when he uses his brain, it is like he is using a muscle, and in that respect, there is some parallel here,” says Janelia Senior Group Leader Jennifer Lippincott-Schwartz.

“The same machinery is operating in both cases but with different readouts.”

The first clue about the possible connection between brain and muscle cells came when Janelia scientists noticed something strange about the endoplasmic reticulum, or ER – the membranous sheets and folds inside cells that are crucial for many cellular functions.

Lorena Benedetti, a research scientist in the Lippincott-Schwartz Lab, was tracking molecules at high resolution along the surface of the ER in mammalian neurons when she saw that the molecules were tracing a

repeating, ladder-like pattern along the entire length of the dendrites — the branch-like extensions on brain cells that receive incoming signals.

Around the same time, Senior Group Leader Stephan Saalfeld alerted Lippincott-Schwartz to high-resolution 3D electron microscopy images of neurons in the fly brain where the ER was also forming regularly spaced, transversal structures.

The ER normally appears like a huge, dynamic net, so as soon as Lippincott-Schwartz saw the structures, she knew her lab needed to figure out what they were for.

“In science, structure is function,” says Lippincott-Schwartz, who also heads Janelia’s 4D Cellular Physiology research area.

“This is an unusual, beautiful structure that we are seeing throughout the whole dendrite, so we just had this feeling that it must have some important function.”

The researchers, led by Benedetti, started by looking at the only other area of the body known to have similar, ladder-like ER structures: muscle tissue.

In muscle cells, the ER and the plasma membrane – the outer membrane of the cell – meet at periodic contact sites, an arrangement controlled by a molecule called junctophilin.

Using high-resolution imaging, the researchers discovered that dendrites also contain a form of junctophilin that controls contact sites between their ER and plasma membrane.

Further, the team found that the same molecular machinery controlling calcium release at muscle cells’ contact sites — where calcium drives muscle contraction — was also present at dendrite contact sites — where calcium regulates neuronal signaling.

Because of these clues, the researchers had a hunch that the molecular machinery at the dendritic contact sites must also be important for transmitting calcium signals, which cells use to communicate.

They suspected that the contact sites along the dendrites might act like a repeater on a telegraph machine: receiving, amplifying, and propagating signals over long distances. In neurons, this could explain how signals received at specific sites on dendrites are relayed to the cell body hundreds of micrometers away.

“How that information travels over long distances and how the calcium signal gets specifically amplified was not known,” says Benedetti.

“We thought that ER could play that role, and that these regularly distributed contact sites are spatially and temporally localized amplifiers: they can receive this calcium signal, locally amplify this calcium signal, and relay this calcium signal over a distance.”

The researchers found that this process is triggered when a neuronal signal causes calcium to enter the dendrite through voltage-gated ion channel proteins, which are positioned at the contact sites. Although this initial calcium signal dissipates quickly, it triggers the release of additional calcium from the ER at the contact site.

This influx of calcium at the contact site attracts and activates a kinase called CaMKII, a protein known to be important in memory. CaMKII alters the plasma membrane’s biochemical properties, changing the strength of the signal that is passed down the plasma membrane.

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It also sheds light on the molecular mechanisms underlying synaptic plasticity – the strengthening or weakening of neuronal connections that enables learning and memory.

Figuring out this process at the molecular level could increase understanding of how the brain works normally and in diseases where these processes go awry, like Alzheimer's.

"We are showing that a structure – a beautiful structure – operating at a level of subcellular organization is having a huge effect on the way the entire neuronal system is operating vis-à-vis calcium signaling," Lippincott-Schwartz says.

"This is a great example of how, in doing science, if you see a beautiful structure, it can take you into a whole new world."

About this learning and memory research news

"Periodic ER-plasma membrane junctions support long-range Ca2+ signal integration in dendrites" by Jennifer Lippincott-Schwartz et al. *Cell*

Abstract

Periodic ER-plasma membrane junctions support long-range Ca2+ signal integration in dendrites

Neuronal dendrites must relay synaptic inputs over long distances, but the mechanisms by which activity-evoked intracellular signals propagate over macroscopic distances remain unclear.

Here, we discovered a system of periodically arranged endoplasmic reticulum-plasma membrane (ER-PM) junctions tiling the plasma membrane of dendrites at ~1 µm intervals, interlinked by a meshwork of ER tubules patterned in a ladder-like array.

Populated with Junctophilin-linked plasma membrane voltage-gated Ca^{2+} channels and ER Ca^{2+}-release channels (ryanodine receptors), ER-PM junctions are hubs for ER-PM crosstalk, fine-tuning of Ca^{2+} homeostasis, and local activation of the Ca^{2+}/calmodulin-dependent protein kinase II.

Local spine stimulation activates the Ca^{2+} modulatory machinery, facilitating signal transmission and ryanodine-receptor-dependent Ca^{2+} release at ER-PM junctions over 20 µm away.

Thus, interconnected ER-PM junctions support signal propagation and Ca^{2+} release from the spine-adjacent ER.

The capacity of this subcellular architecture to modify both local and distant membrane-proximal biochemistry potentially contributes to dendritic computations.

New discovery links brain and muscle cells in signal transmission

*Howard Hughes Medical Institute*Feb 8 2025

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Using high-resolution imaging, the researchers discovered that dendrites also contain a form of junctophilin that controls contact sites between their ER and plasma membrane. Further, the team found that the same molecular machinery controlling calcium release at muscle cells' contact sites - where calcium drives muscle contraction - was also present at dendrite contact sites - where calcium regulates neuronal signaling.

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The researchers found that this process is triggered when a neuronal signal causes calcium to enter the dendrite through voltage-gated ion channel proteins, which are positioned at the contact sites. Although this initial calcium signal dissipates quickly, it triggers the release of additional calcium from the ER at the contact site.

This influx of calcium at the contact site attracts and activates a kinase called CaMKII, a protein known to be important in memory. CaMKII alters the plasma membrane's biochemical properties, changing the strength of the signal that is passed down the plasma membrane.

This process continues from contact site to contact site all along the dendrite to the cell body, where the neuron decides how it will communicate with other neurons.

The new research reveals a novel mechanism for signal transmission in brain cells and helps answer an open question in neuroscience about how intracellular signals travel over long distances in neurons, enabling information received at specific sites on dendrites to be processed in the brain.

It also sheds light on the molecular mechanisms underlying synaptic plasticity – the strengthening or weakening of neuronal connections that enables learning and memory. Figuring out this process at the molecular level could increase understanding of how the brain works normally and in diseases where these processes go awry, like Alzheimer's.

"We are showing that a structure – a beautiful structure – operating at a level of subcellular organization is having a huge effect on the way the entire neuronal system is operating vis-à-vis calcium signaling," Lippincott-Schwartz says. "This is a great example of how, in doing science, if you see a beautiful structure, it can take you into a whole new world."

02/07/25 | From muscle to memory: new research uses clues from the body to understand signaling in the brain

New research shows that how a network of subcellular structures is responsible for transmitting signals in neurons. This movie shows 3D renderings of these structures in high-resolution 3D electron microscopy images of fruit fly neurons. The endoplasmic reticulum (green), plasma membrane (blue), mitochondria (pink), microtubules (tan), and ER-plasma membrane contacts (magenta) are segmented from FIB-SEM datasets of a Drosophila melanogaster MBON1 neuron. *Credit: Benedetti et al.*

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This movie shows time-lapse high-resolution imaging in neurons, revealing the dynamic behavior of ER tubules contrasted with the persistence of ER-PM junctional sites over time. Time-lapse acquired using 2D lattice-SIM in burst mode of HaloTag-Sec61β (labeled with JF585 HaloTag-ligand) expressing neurons. Scale bars: 0.5 µm. *Credit: Benedetti et al.*

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This movie shows high-resolution imaging of neurons, showing lysosomes swiftly moving through ladder-like ER arrays. Time-lapse acquired using Lattice-SIM of mEmerald-Sec61β expressing neurons. Scale bars: 0.5 µm. *Credit: Benedetti et al.*

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Lorena Benedetti, Ruolin Fan, Aubrey V Weigel, Andrew S Moore, Patrick R Houlihan, Mark Kittisopikul, Grace Park, Alyson Petruncio, Philip M Hubbard, Song Pang, C Shan Xu, Harald F Hess, Stephan Saalfeld, Vidhya Rangaraju, David E Clapham, Pietro De Camilli, Timothy A Ryan, Jennifer Lippincott-Schwartz. "Periodic ER-plasma membrane junctions support long-range Ca2+ signal integration in dendrites." PMID: 39708809

A First-of-Its-Kind Signal Has Been Detected in Human Brains

Scientists have identified a unique form of cell messaging occurring in the human brain, revealing just how much we still have to learn about its mysterious inner workings.

Excitingly, the discovery hints that our brains might be even more powerful units of computation than we realized.

In 2020, researchers from institutes in Germany and Greece reported a mechanism in the brain's outer cortical cells that produces a novel 'graded' signal all on its own, one that could provide individual neurons with another way to carry out their logical functions.

By measuring the electrical activity in sections of tissue removed during surgery on epileptic patients and analyzing their structure using fluorescent microscopy, the neurologists found individual cells in the cortex used not just the usual sodium ions to 'fire', but calcium as well.

This combination of positively charged ions kicked off waves of voltage that had never been seen before, referred to as a calcium-mediated dendritic action potentials, or dCaAPs.

Brains – especially those of the human variety – are often compared to computers. The analogy has its limits, but on some levels they perform tasks in similar ways.

Both use the power of an electrical voltage to carry out various operations. In computers it's in the form of a rather simple flow of electrons through intersections called transistors.

In neurons, the signal is in the form of a wave of opening and closing channels that exchange charged particles such as sodium, chloride, and potassium. This pulse of flowing ions is called an action potential.

Instead of transistors, neurons manage these messages chemically at the end of branches called dendrites.

"The dendrites are central to understanding the brain because they are at the core of what determines the computational power of single neurons," Humboldt University neuroscientist Matthew Larkum told Walter Beckwith at the American Association for the Advancement of Science.

Dendrites are the traffic lights of our nervous system. If an action potential is significant enough, it can be passed on to other nerves, which can block or pass on the message.

This is the logical underpinnings of our brain – ripples of voltage that can be communicated collectively in two forms: either an *AND* message (if x *and* y are triggered, the message is passed on); or an *OR* message (if x *or* y is triggered, the message is passed on).

Arguably, nowhere is this more complex than in the dense, wrinkled outer section of the human central nervous system; the cerebral cortex. The deeper second and third layers are especially thick, packed with branches that carry out high order functions we associate with sensation, thought, and motor control.

It was tissues from these layers that the researchers took a close look at, hooking up cells to a device called a somatodendritic patch clamp to send active potentials up and down each neuron, recording their signals.

"There was a 'eureka' moment when we saw the dendritic action potentials for the first time," said Larkum.

To ensure any discoveries weren't unique to people with epilepsy, they double checked their results in a handful of samples taken from brain tumors.

While the team had carried out similar experiments on rats, the kinds of signals they observed buzzing through the human cells were very different.

More importantly, when they dosed the cells with a sodium channel blocker called tetrodotoxin, they still found a signal. Only by blocking calcium did all fall quiet.

Finding an action-potential mediated by calcium is interesting enough. But modelling the way this sensitive new kind of signal worked in the cortex revealed a surprise.

In addition to the logical *AND* and *OR*-type functions, these individual neurons could act as 'exclusive' *OR* (*XOR*) intersections, which only permit a signal when another signal is graded in a particular fashion.

"Traditionally, the *XOR* operation has been thought to require a network solution," the researchers wrote.

More work needs to be done to see how dCaAPs behave across entire neurons, and in a living system. Not to mention whether it's a human-thing, or if similar mechanisms have evolved elsewhere in the animal kingdom.

Technology is also looking to our own nervous system for inspiration on how to develop better hardware; knowing our own individual cells have a few more tricks up their sleeves could lead to new ways to network transistors.

Exactly how this new logic tool squeezed into a single nerve cell translates into higher functions is a question for future researchers to answer.

This research was published in *Science*.

Scientists Uncover a New Signal in Human Brain Cells: A Potential Leap in Understanding Neural Power

The human brain, often described as the most complex organ in the universe, continues to reveal its secrets. In a groundbreaking discovery, scientists have identified a new type of signal within human brain cells that could reshape our understanding of how the brain functions as a computational unit. This new signal, found in cortical cells, suggests that the brain's capabilities might far exceed previous estimations.

The Discovery: A New Wave of Voltage

Researchers have uncovered a unique graded signal within neurons, called a **calcium-mediated dendritic action potential (dCaAP)**. Unlike the traditional action potentials generated by sodium ions, this new signal involves the flow of calcium ions, creating a distinct wave of voltage. This finding challenges the established notion that neuron communication is exclusively governed by sodium-based action potentials.

Key Features of the Discovery:

- **Calcium Ions in Action**: While sodium ions are the main drivers of traditional neural firing, calcium ions play a crucial role in this new signal, adding a layer of complexity to neural computations.
- **Dendritic Involvement**: The signal originates in the dendrites, the branched extensions of neurons that receive information from other cells. This emphasizes the dendrite's active role in information processing, rather than merely acting as a passive conduit.
- **Graded Nature**: Unlike the "all-or-nothing" nature of typical action potentials, this new signal appears to be graded, allowing for more nuanced computational processing.

How the Brain Transmits Information

To appreciate the significance of this discovery, it's essential to understand how neurons traditionally transmit information.

Neurons, the fundamental units of the brain, rely on both **electrical and chemical signals** to communicate.

1. **Electrical Signals (Action Potentials)**:
    - When a neuron receives a stimulus strong enough to cross its threshold, it generates an action potential.
    - This electrical impulse travels along the neuron's axon to its terminal, where it reaches the synapse.
2. **Chemical Signals (Neurotransmitters)**:
    - At the synapse, the action potential triggers the release of neurotransmitters.
    - These chemical messengers cross the synaptic gap and bind to receptors on the receiving neuron, potentially triggering a new action potential.

This seamless interaction of electrical and chemical signals forms the basis of neural communication, allowing the brain to perform complex tasks like thought, memory, emotion, and sensory perception.

Implications of the New Signal

The discovery of calcium-mediated dendritic action potentials could revolutionize our understanding of neural computation. Here's why:

1. **Enhanced Computational Power**:
    - Traditional models of the brain liken neurons to simple computational units that either fire or

remain silent. The graded nature of this new signal suggests a more nuanced system, akin to analog computing, where information processing can be far more sophisticated.

2. **Role of Dendrites**:
    - Dendrites were once thought to be passive structures that simply relay information to the neuron's body. This discovery highlights their active role in generating signals and processing information, potentially making each neuron a more powerful computational unit.
3. **Complex Neural Networks**:
    - Neurons don't work in isolation; they form vast, interconnected networks where each neuron communicates with thousands of others. The discovery of this new signal adds another layer of complexity to these networks, indicating that the brain's information-processing capabilities may be significantly underestimated.
4. **Bridging Biology and Technology**:
    - Both brains and computers rely on electrical voltage for operations. However, while computers use transistors, the brain uses action potentials and now, possibly, graded dendritic signals. This discovery could inspire advancements in artificial intelligence and neuromorphic computing, where systems are designed to mimic the brain's computational processes.

How This Discovery Differs from Traditional Brain Signals

Traditional action potentials operate on an **all-or-nothing principle**: a neuron either fires or it doesn't. This binary mode of operation is efficient for basic communication but limits the complexity of computations a single neuron can perform.

The newly discovered signal is **graded**, meaning it can vary in intensity, allowing for more intricate information processing. This is akin to comparing a digital system (binary) to an analog system (continuous). The analog nature of the new signal could enable neurons to perform more complex computations independently, reducing the reliance on larger networks for processing.

What This Means for Brain Research

The discovery of calcium-mediated dendritic action potentials opens several avenues for research and application:

1. **Advancing Neurological Studies**:
    - Understanding this new signal could provide insights into brain disorders where neural communication is disrupted, such as epilepsy, Alzheimer's disease, or autism.
2. **Improving AI and Neuromorphic Computing**:
    - By studying how neurons use this new signal, scientists can develop better algorithms and hardware for artificial intelligence, bringing it closer to mimicking human thought processes.
3. **Revisiting Neural Models**:

- Many existing models of brain function will need to be revised to incorporate this new form of neural signaling, potentially leading to a more accurate understanding of how the brain works.

A Deeper Look into Neural Networks

Neurons form intricate networks where a single neuron can send and receive signals from thousands of others. This creates a vast web of connections that allows the brain to process, store, and transmit information at astonishing speeds.

The discovery of calcium-mediated dendritic action potentials adds another layer to this complexity. It suggests that dendrites, which were previously thought to simply transmit incoming signals to the neuron's body, can independently generate their own signals. This could mean that the brain has an untapped reserve of computational power that researchers are only beginning to understand.

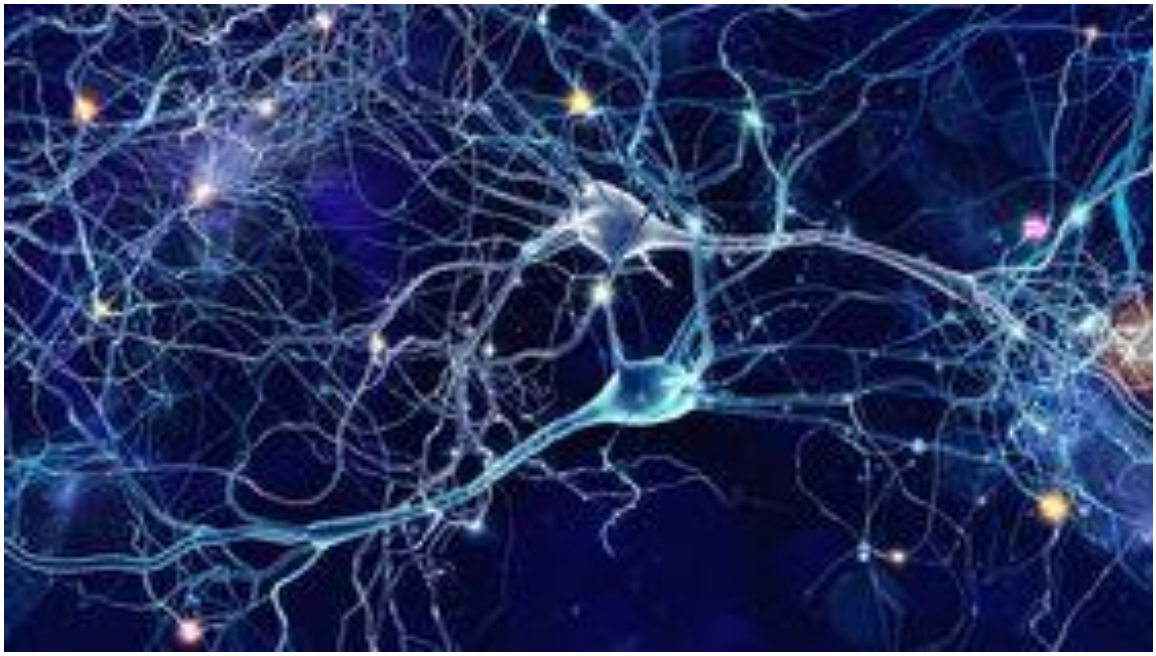
Conclusion: A New Frontier in Neuroscience

The discovery of a new type of signal in human brain cells is a monumental step forward in neuroscience. It challenges long-standing assumptions about how neurons function and opens up exciting possibilities for understanding the brain's true computational power. By shedding light on the role of dendrites and the graded nature of this new signal, scientists are uncovering

a more intricate picture of how our brains process and transmit information.

This breakthrough not only enhances our understanding of the human brain but also holds the potential to influence fields as diverse as medicine, artificial intelligence, and cognitive science. As research continues, this discovery may lead to new technologies, therapies, and insights into what makes the human brain the remarkable organ it is.

Scientists Have Discovered A New Kind of Signal in the Human Brain



Conceptual image of neurons firing in the brain. whitehoune/Shutterstock

Neuroscientists have discovered a new signal process in the brain that they suggest may be key to what makes us human.

The human brain is a complex system of cells called neurons that exchange information using electrical and chemical signals. In a new study published in Science, researchers found that certain cells in the human brain transmit signals in a way not seen in corresponding rodent cells.

Demonstrable differences in human compared to other mammal brains helps us pin down why human brains may be unique, which can lead to a better understanding, and even better modeling, of our brains.

The researchers looked at slices of brains from patients who had either epilepsy or tumors. They focused on the dendrites – the branch-like extensions of brain cells that connect to other brain cells, allowing information to be exchanged – in the second and third layers of the brain's cortex (outermost layer).

Most of what we know about dendrites is from rodent studies, so the opportunity to study human samples is vital. Patients with epilepsy were chosen due to epilepsy surgeries providing enough cortex tissue to analyze, and the tumor samples were to ensure that the results weren't only found in those with epilepsy.

Thanks to the human brain's expansion as we evolved, it has an unusually thick cortex (around 3 millimeters), which is disproportionately thick in the second and third layers, leading to large and elaborate dendrite trees.

The synapse is a structure that allows electric nerve impulses to be sent between two neurons. Neurons communicate via electrical events called "action potentials" – a burst of electrical activity when a neuron sends information away from the cell. Hundreds of synaptic inputs to a neuron determine whether an action potential results. The active electrical properties of these dendrites determine the numerous transformations from synaptic input to action potential, which means they are key to a neuron's computational power.

The team used a patch clamp to construct an electrical circuit for the cells and a fluorescing imaging technique to investigate the properties of the cells. They discovered previously unknown classes of action potentials in the dendrites of these neurons, which means their activity is much more complex than previously realized.

Among the revelations, the researchers noted that one of the new kinds of action potentials traveled using just calcium ions, instead of both calcium and sodium ions, something not seen before in mammal cortex cells.

They studied the behavior of the action potentials by creating a computer-simulated model and discovered another surprising aspect – they could perform a "computational" function that researchers had previously thought required an entire network of neurons, not just one. From muscle to memory: New research uses clues from the body to understand signaling in the brain February 9, 2025

Howard Hughes Medical Institute

New research shows that a network of subcellular structures similar to those responsible for propagating molecular signals that make muscles contract are also responsible for transmitting signals in the brain that may facilitate learning and memory.

Our biceps and our brain cells may have more in common than previously thought.

New research led by the Lippincott-Schwartz Lab shows that a network of subcellular structures similar to those responsible for propagating molecular signals that make muscles contract are also responsible for transmitting signals in the brain that may facilitate learning and memory.

"Einstein said that when he uses his brain, it is like he is using a muscle, and in that respect, there is some parallel here," says Janelia Senior Group Leader Jennifer Lippincott-Schwartz. "The same machinery is operating in both cases but with different readouts."

The first clue about the possible connection between brain and muscle cells came when Janelia scientists noticed something strange about the endoplasmic reticulum, or ER -- the membranous sheets and folds inside cells that are crucial for many cellular functions.

Lorena Benedetti, a research scientist in the Lippincott-Schwartz Lab, was tracking molecules at high resolution along the surface of the ER in mammalian neurons when she saw that the molecules were tracing a repeating, ladder-like pattern along the entire length of the dendrites -- the branch-like extensions on brain cells that receive incoming signals.

Around the same time, Senior Group Leader Stephan Saalfeld alerted Lippincott-Schwartz to high-resolution 3D electron microscopy images of neurons in the fly brain where the ER was also forming regularly spaced, transversal structures.

The ER normally appears like a huge, dynamic net, so as soon as Lippincott-Schwartz saw the structures, she knew her lab needed to figure out what they were for.

"In science, structure is function," says Lippincott-Schwartz, who also heads Janelia's 4D Cellular Physiology research area. "This is an unusual, beautiful structure that we are seeing throughout the whole dendrite, so we just had this feeling that it must have some important function."

The researchers, led by Benedetti, started by looking at the only other area of the body known to have similar, ladder-like ER structures: muscle tissue. In muscle cells, the ER and the plasma membrane -- the outer membrane of the cell -- meet at periodic contact sites, an arrangement controlled by a molecule called junctophilin.

Using high-resolution imaging, the researchers discovered that dendrites also contain a form of junctophilin that controls contact sites between their ER and plasma membrane. Further, the team found that the same molecular machinery controlling calcium release at muscle cells' contact sites -- where calcium drives muscle contraction -- was also present at dendrite contact sites -- where calcium regulates neuronal signaling.

Because of these clues, the researchers had a hunch that the molecular machinery at the dendritic contact sites must also be important for transmitting calcium signals, which cells use to communicate. They suspected that the contact sites along the dendrites might act like a

repeater on a telegraph machine: receiving, amplifying, and propagating signals over long distances. In neurons, this could explain how signals received at specific sites on dendrites are relayed to the cell body hundreds of micrometers away.

"How that information travels over long distances and how the calcium signal gets specifically amplified was not known," says Benedetti. "We thought that ER could play that role, and that these regularly distributed contact sites are spatially and temporally localized amplifiers: they can receive this calcium signal, locally amplify this calcium signal, and relay this calcium signal over a distance."

The researchers found that this process is triggered when a neuronal signal causes calcium to enter the dendrite through voltage-gated ion channel proteins, which are positioned at the contact sites. Although this initial calcium signal dissipates quickly, it triggers the release of additional calcium from the ER at the contact site. This influx of calcium at the contact site attracts and activates a kinase called CaMKII, a protein known to be important in memory. CaMKII alters the plasma membrane's biochemical properties, changing the strength of the signal that is passed down the plasma membrane. This process continues from contact site to contact site all along the dendrite to the cell body, where the neuron decides how it will communicate with other neurons.

The new research reveals a novel mechanism for signal transmission in brain cells and helps answer an open question in neuroscience about how intracellular signals travel over long distances in neurons, enabling information received at specific sites on dendrites to be processed in the brain.

It also sheds light on the molecular mechanisms underlying synaptic plasticity -- the strengthening or weakening of neuronal connections that enables learning and memory. Figuring out this process at the molecular level could increase understanding of how the brain works normally and in diseases where these processes go awry, like Alzheimer's. "We are showing that a structure -- a beautiful structure -- operating at a level of subcellular organization is having a huge effect on the way the entire neuronal system is operating vis-à-vis calcium signaling," Lippincott-Schwartz says. "This is a great example of how, in doing science, if you see a beautiful structure, it can take you into a whole new world."

Neuroscientists find evidence of an internal brain rhythm that orchestrates memory

Neuroscientists find evidence of an internal brain rhythm that orchestrates memory© PsyPost

A new study sheds light on how our brains rhythmically organize memories at the level of individual nerve cells. Researchers in Germany have found that single neurons in the human medial temporal lobe tend to synchronize their activity with slow brain waves, particularly during memory formation and retrieval. These patterns, known as theta-phase locking, appear to reflect an internal rhythm that helps structure cognitive processes. The findings were published in *Nature Communications*.

The study, led by neuroscientists at the University Hospital Bonn, the University of Bonn, and the University of Freiburg, examined how single-neuron activity in the human brain aligns with local electrical rhythms during memory tasks. The team focused on theta waves—slow oscillations typically occurring between 1 and 10 Hz—which have long been associated with memory processes in animal research. While studies in rodents have shown that hippocampal neurons often fire at specific phases of the theta rhythm, it has remained unclear whether similar dynamics hold in the human brain during real-time memory use.

"In the Bonn Spatial Memory Lab, we study how the brain forms memories about places and locations. Previous research showed that single brain cells can synchronize with rhythmic brain activity. We wanted to understand this synchronization more deeply and explored its role during both learning and remembering of spatial memories," explained Tim Guth of the University of Bonn. "Our goal is to understand the memory system of the brain at the level of individual cells and populations of cells. Ultimately, we hope to help improve the treatment of memory disorders."

The research took advantage of a unique clinical context. Patients with treatment-resistant epilepsy often undergo surgery to implant electrodes in the brain, helping doctors locate the source of seizures. With informed consent, these electrodes can also record brain activity at an extremely fine resolution—down to individual neurons. This setup allowed the researchers to directly observe how nerve cells behave as people form and recall memories.

Eighteen patients took part in a virtual navigation task called "Treasure Hunt," which asked them to explore a computer-generated beach and memorize the locations of hidden objects. During each trial, participants navigated to chests containing objects, learned their locations, and later recalled either the object associated with a specific location or the location linked to a particular object. Each session provided dozens of such encoding and retrieval episodes. The researchers recorded both behavioral accuracy and neural activity throughout.

Using advanced computational techniques, the research team analyzed how well individual neurons fired in sync with theta waves. To do this, they estimated the phase of the local field potential—a measure of the average electrical activity near each electrode—across a broad 1–10 Hz frequency range. This approach allowed them to track the timing of each spike relative to ongoing slow-wave activity.

The results provide evidence that neurons in the human medial temporal lobe—including the hippocampus, entorhinal cortex, and amygdala—frequently exhibit theta-phase locking. That is, neurons tended to fire at the same point in the theta cycle across time. About 86% of neurons showed significant phase locking across the full task, and many of them were aligned near the trough of the theta wave.

“Much like musicians in an orchestra follow a shared rhythm, many brain cells in the human memory system time their activity to the brain’s electrical oscillations,” Guth told PsyPost.

Importantly, the strength of this phase locking varied depending on the characteristics of the brain’s electrical background. Neurons were more tightly synchronized with theta waves during periods of high theta power and when the field potentials displayed steep aperiodic slopes—conditions thought to reflect greater neural inhibition. This suggests that theta-phase locking is not a fixed feature but is modulated by moment-to-moment changes in the local neural environment.

The presence of clear theta oscillations, identified using a cycle-by-cycle algorithm, also predicted stronger phase locking. Yet, even outside of these clearly rhythmic periods, neurons continued to show non-random firing relative to theta phase, suggesting that some degree of synchronization persists even when oscillations are less apparent.

“While most brain cells fired at the same point in the rhythm during both learning and recall, a small group shifted their timing,” Guth said. “This shift may help the brain separate the processes of storing new memories from retrieving old ones.”

The researchers also examined whether this rhythmic coordination related to successful memory performance. Surprisingly, they found no strong evidence that theta-phase locking was more pronounced during correctly remembered trials compared to forgotten ones. This held true during both the encoding and retrieval phases of the task. While previous studies using static images or verbal tasks have reported such effects, the dynamic and continuous nature of the spatial navigation task used here may have played a role in blunting phase-reset phenomena that typically enhance phase locking at stimulus onset.

Even though the overall strength of phase locking didn’t predict memory success, the study did find that a subset of neurons shifted their preferred firing phase between encoding and retrieval. About 9% of neurons exhibited such phase shifts, and these shifts tended to be slightly more common during successful memory trials. This observation lends partial support to theoretical models like the SPEAR (Separate Phases of Encoding And Retrieval) model, which proposes that encoding and retrieval occur at different points in the theta cycle to avoid interference.

The degree of phase shift between encoding and retrieval was relatively modest—typically around 40 to 90 degrees—falling short of the full 180-degree separation proposed in some models. Yet even smaller shifts may help segregate memory-related processes and reduce crosstalk between new learning and recall.

Theta-phase locking also varied by brain region. The parahippocampal cortex showed the highest percentage of neurons exhibiting phase locking, while the hippocampus had the lowest. This was not explained by differences in spike count or theta power across regions, indicating possible functional specialization in how different medial temporal areas contribute to rhythmic coordination.

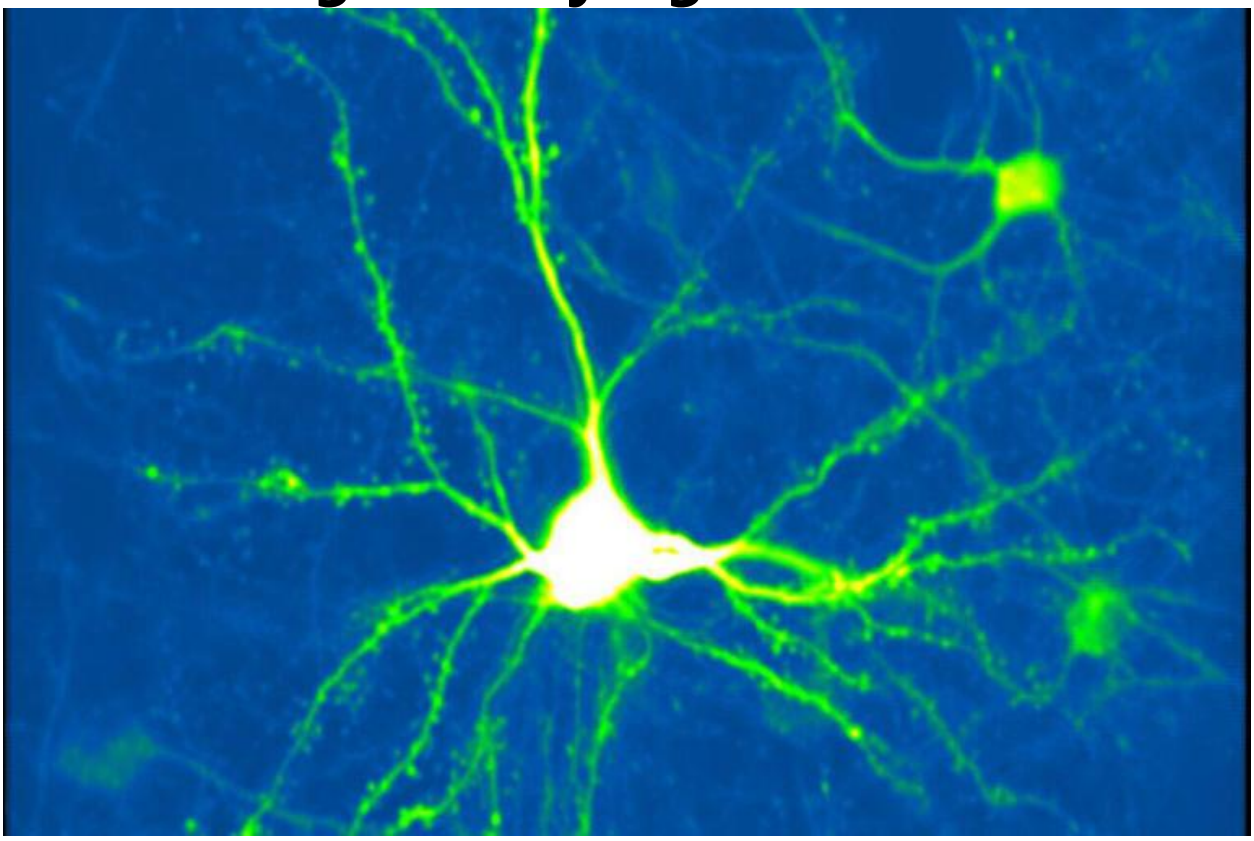
In their analysis, the researchers also explored how both oscillatory (periodic) and non-oscillatory (aperiodic) components of the local field potential shaped phase locking. Steeper aperiodic slopes—reflecting greater inhibition—were associated with stronger phase locking, while flatter slopes corresponded with reduced synchronization. The study used a method called SPRiNT to estimate these aperiodic features over time, offering a more granular view of how internal dynamics fluctuate during memory tasks.

Despite the robust evidence for theta-phase locking, the study has some limitations. The number of encoding and retrieval events may have been too small to detect subtle performance-related effects, and the lack of a sharply defined stimulus onset may have reduced the likelihood of observing phase resets. The wide frequency range used to define theta (1–10 Hz) may also differ from narrower bands used in other studies, potentially complicating comparisons. Additionally, while the presence of phase locking suggests temporal organization, the functional consequences for memory processing remain a topic for further research.

“Although this synchronization was active during memory processes, we still don’t know exactly why it happens or how essential it is for memory,” Guth said. “More research is needed to see how it changes across different memory tasks.”

The study, “Theta-phase locking of single neurons during human spatial memory,” was authored by Tim A. Guth, Armin Brandt, Peter C. Reinacher, Andreas Schulze-Bonhage, Joshua Jacobs, and Lukas Kunz.

How the brain shapes what we feel in real time: A new mechanism for modulating sensory signals



Cortical neuron expressing green fluorescent protein, imaged in the living mouse brain using two-photon microscopy. Credit: Ronan Chéreau

The cerebral cortex processes sensory information via a complex network of neural connections. How are these signals modulated to refine perception? A team from the University of Geneva (UNIGE) has identified a mechanism by which certain thalamic projections target neurons and modify their excitability.

The work, published in *Nature Communications*, reveals a previously unknown form of communication between two regions of the brain, the thalamus and the somatosensory cortex. It could explain why the same sensory stimulus does not always elicit the same sensation and open up new avenues for understanding certain mental disorders.

The same sensory stimulus can be perceived clearly at times, and remain vague at others. This phenomenon can be explained by the way the brain integrates stimuli. For example, touching an object outside our field of vision may be enough to identify it...or not.

These perceptual variations remain poorly understood, but may depend on factors such as attention or the disruptive presence of other stimuli. What is certain, according to neuroscientists, is that when we touch something, sensory signals from receptors in the skin are interpreted by a specialized region called the somatosensory cortex.

On their way to it, the signals pass through a complex network of neurons, including a crucial structure in the brain called the thalamus, which serves as a relay station. However, the process is not one-way.

A significant portion of the thalamus also receives feedback from the cortex, forming a loop of reciprocal communication. But the exact role and functioning of this feedback loop are still unclear. Could it play an active role in how we perceive sensory information?

A new modulatory pathway

To explore this question, neuroscientists at UNIGE studied a region at the top of pyramidal neurons of the somatosensory cortex, rich in dendrites—extensions that receive electrical signals from other neurons.

"Pyramidal neurons have rather strange shapes. They are asymmetrical, both in shape and function. What happens at the top of the neuron is different from what happens at the bottom," explains Anthony Holtmaat, full professor at the Department of Basic Neurosciences (NEUFO) and the Synapsy Center for Neuroscience Research for Mental Health at UNIGE's Faculty of Medicine, and director of the study.

His team focused on a pathway in which the top of pyramidal neurons in mice receives projections from a specific part of the thalamus. By stimulating the animal's whiskers—the equivalent of touch in humans—a precise dialogue between these projections and the dendrites of pyramidal neurons was revealed.

"What is remarkable, unlike the regular thalamic projections known to activate pyramidal neurons, is that the part of the thalamus providing feedback modulates their activity, in particular by making them more sensitive to stimuli," says Ronan Chéreau, senior researcher at NEUFO and co-author of the study.

An unexpected receptor

Using cutting-edge techniques—imaging, optogenetics, pharmacology and, above all, electrophysiology—the research team was able to record the electrical activity of tiny structures such as dendrites.

These approaches helped clarify how this modulation works at the synaptic level. Normally, the neurotransmitter glutamate acts as an activation signal. It helps neurons transmit sensory information by triggering an electrical response in the next neuron.

In this newly discovered mechanism, glutamate released from thalamic projections bindsto an alternative receptor located in a specific region of the cortical pyramidal neuron.

Rather than directly exciting the neuron, this interaction alters its state of responsiveness, effectively priming it for future sensory input. The neuron then becomes more easily activated, as if it were being conditioned to better respond to a future sensory stimulus.

"This is a previously unknown pathway for modulation. Usually, the modulation of pyramidal neurons is ensured by the balance between excitatory and inhibitory neurons, not by this type of mechanism," explains Ronan Chéreau.

Implications for perception and disorders

By demonstrating that a specific feedback loop between the somatosensory cortex and the thalamus can modulate the excitability of cortical neurons, the study suggests that thalamic pathways do not simply transmit sensory signals, but also act as selective amplifiers of cortical activity.

"In other words, our perception of touch is not only shaped by incoming sensory data, but also by dynamic interactions within the thalamocortical network," adds Anthony Holtmaat.

This mechanism could also contribute to understanding the perceptual flexibility observed in states of sleep or wakefulness, when sensory thresholds vary. Its alteration could also play a role in certain pathologies, such as autism spectrum disorders.

More information: Federico Brandalise et al, Thalamocortical feedback selectively controls pyramidal neuron excitability, *Nature Communications* (2025). DOI: 10.1038/s41467-025-60835-w

Provided by University of Geneva

Simple rhythmic sounds can reshape the brain’s entire network landscape, study finds

Simple rhythmic sounds can reshape the brain’s entire network landscape, study finds© PsyPost

Even a basic auditory rhythm can reconfigure how the brain organizes itself, according to a new study published in *Advanced Science*. The researchers introduced a new analytical tool called FREQ-NESS, which maps how brain networks operate simultaneously across different frequencies. The results suggest that hearing rhythmic tones engages the auditory cortex while also reorganizing the brain's broader network configuration, shifting dominant oscillations and enhancing communication between slower and faster brain rhythms.

Cognitive neuroscience aims to understand how the brain functions as a dynamic system that processes ongoing information from the environment. A key challenge has been accurately capturing how different brain networks—each with their own frequency and spatial characteristics—operate at the same time, particularly when the brain is responding to external stimuli like music or speech.

Previous research has tended to focus either on specific anatomical regions or on broad, canonical frequency bands such as alpha or gamma. These approaches have produced useful but fragmented insights. Another complication is that multiple brain processes overlap in time and space, which makes it hard to isolate individual networks using traditional methods.

To address this, the authors developed a method that could simultaneously capture frequency-specific brain networks with fine spatial and temporal resolution. Their approach also aimed to avoid the limitations of prior methods that depend on pre-defined anatomical regions or that group oscillations into broad frequency categories. The result was FREQ-NESS, short for FREQuency-resolved Network Estimation via Source Separation.

"We wanted a simple, transparent way to see how multiple brain networks operate at specific frequencies at the same time, and how this organization changes when the brain engages with sound and rhythm," said study author Mattia Rosso (@mattiarosso), a postdoctoral researcher at the Center for Music in the Brain at Aarhus University.

"Existing tools either predefine regions/bands or blur overlapping processes, so we built FREQ-NESS as an analytical pipeline to estimate frequency-resolved networks directly at the level of the brain's anatomical sources, facilitating the analysis of the temporal and spatial dynamics within the networks as well as the interactions across networks."

The researchers recruited 29 participants, mostly non-musicians, and recorded their brain activity using magnetoencephalography (MEG), which offers high temporal precision. Participants underwent two five-minute sessions: one where they sat in a resting state while watching a silent movie, and another where they passively listened to rhythmic tones while watching the same movie. The tones were presented at a steady rate of 2.4 Hz—about two beats per second.

When participants listened to the rhythmic tones, the brain's network landscape changed in three key ways.

First, the auditory stimulation caused the emergence of new networks that were sharply attuned to the stimulation frequency of 2.4 Hz and its harmonic at 4.8 Hz. These networks were concentrated in the auditory cortex, including Heschl's gyrus, and extended to medial temporal areas such as the hippocampus and insula. These regions are known to play roles in both early and higher-level auditory processing.

Second, existing brain networks shifted their frequency preferences and spatial configurations. For example, the peak alpha activity moved from 10.9 Hz to 12.1 Hz, and the spatial focus of alpha activity shifted from parieto-occipital regions to sensorimotor areas. This suggests that the alpha network, commonly involved in attention and inhibition, reorganized itself in response to rhythmic input—perhaps to support movement preparation or sensory prediction.

Third, some networks remained largely unchanged. The motor-related beta network, centered around 22.9 Hz and focused in the precentral gyrus, showed similar prominence and topography in both resting and listening conditions. This stability suggests that not all networks are influenced equally by external stimuli.

Beyond these shifts, the researchers also found that listening to rhythmic tones strengthened the coordination between slower and faster rhythms. Specifically, the phase of the auditory-attuned network at 2.4 Hz modulated the amplitude of gamma-band activity—fast oscillations above 60 Hz—in brain areas such as the insula and frontal operculum. This cross-frequency coupling was stronger during listening than at rest, suggesting that the brain ramps up coordination across timescales to process rhythmic stimuli.

Importantly, these gamma-band networks were not located in the auditory cortex itself, but appeared in broader associative regions. This indicates that the brain's

response to rhythm is not limited to processing sounds per se—it may also involve integrating sensory input with memory, attention, or predictive processes.

“While based on our simulations we expected the primary auditory network to attune to the auditory stimulus, we were surprised to find that the rest of the network landscape underwent a global reorganization,” Rosso told PsyPost. “In particular, we found that the alpha network not only sped up (from about 10.9 Hz to 12.1 Hz) but also shifted from occipital to sensorimotor regions, suggesting a preparatory state for action in response to rhythmic input.”

“We were also struck that the gamma effects weren’t centered in primary auditory cortex but in a broader network (insula, inferior temporal, hippocampal, frontal operculum/IFG), suggesting that fast activity is mediated by interactions with low-frequency auditory networks rather than originating locally. Finally, the robustness of the method with very short recordings (down to ~30 seconds) was a pleasant surprise, confirming that the method is both sensitive and feasible.”

The researchers confirmed the robustness of their method through several replication tests. They showed that similar network landscapes could be detected in independent datasets, that shorter recording durations still produced reliable patterns, and that using different sensor types (gradiometers vs. magnetometers) had little effect on the results.

To test the importance of the method’s spatial and temporal structure, they also introduced randomization procedures. When the spatial arrangement of voxels was scrambled, the frequency content of the data was preserved but the resulting networks were meaningless. When both space and time structure were disrupted, the method failed to isolate any coherent networks. This provided further evidence that the detected patterns were not artifacts but reflected genuine brain dynamics.

“Even during very simple listening, the brain reconfigures, attuning its internal dynamics to the external world in order to process information,” Rosso summarized. “It does so in three main ways: 1) it attunes its primary sensory networks to the stimulation frequency, 2) adapts the frequency and spatial arrangement of intrinsic oscillations, and 3) boosts communication between slow and faster internal rhythms. In short, rhythm doesn’t just ‘light up the auditory area’—it retunes the brain’s network landscape as a whole.”

While FREQ-NESS provides a powerful tool for mapping frequency-specific brain networks, the study had some limitations, including a modest sample size, the absence of baseline noise data from "empty-room" MEG recordings, and a minimalistic task design. The researchers suggest that future work could involve more ecologically valid stimuli and expand the method to support broadband and multimodal applications.

"The brain is an extremely complex system: frequency is only one criterion for its organization," Rosso noted. "FREQ-NESS is explicitly designed with the limited scope of separating brain networks and analyzing their interactions based on their frequency-specificity. We are very much aware of this, and so should be whoever is going to use the method for their investigation of the brain. Frequency alone does not 'solve the brain.'"

"Our study presents the frequency-resolved variant of the broader NESS framework, which stands for 'Network Estimation via Source Separation'. In a research landscape moving toward overly complex or AI-based 'black box' approaches, our aim is to extract insights from simple and interpretable linear decomposition methods, with very few degrees of freedom."

"The two major directions now are: making the method multimodal (e.g., applicable to data of different nature), and developing a broadband variant, which is already in press in a high-impact journal. In the meantime, we and collaborators across European centers are applying the pipeline to study how the brain's network landscape changes across states of consciousness, during psychedelic intake, with aging, in musicians, and other experimental conditions and populations."

"The FREQ-NESS toolbox and full pipeline are openly available on our GitHub repository (https://github.com/mattiaRosso92/Frequency-resolved_brain_network_estimation_via_source_separation_FREQ-NESS/tree/main/FREQNESS_Toolbox), and the paper is open access," he added. "We hope others will use it to probe how ongoing brain rhythms shape perception and action. We are also very open to providing guidance and setting up collaborations, so please do not hesitate to reach out."

The study, "FREQ-NESS Reveals the Dynamic Reconfiguration of Frequency-Resolved Brain Networks During Auditory Stimulation," was authored by Mattia

Rosso, Gemma Fernández-Rubio, Peter Erik Keller, Elvira Brattico, Peter Vuust, Morten L. Kringelbach, and Leonardo Bonetti.

Neuroscientist just discovered a fascinating fact about the grooves in your brain

A new study published in *The Journal of Neuroscience* offers insight into how small grooves in the brain's surface — known as tertiary sulci — might help explain individual differences in reasoning ability. Researchers from the University of California, Berkeley, found that the depth of these tiny brain folds in children and adolescents was linked to stronger communication between two areas known to support higher-order thinking: the lateral prefrontal cortex and the lateral parietal cortex. The findings suggest that subtle anatomical variations in these brain folds could influence how efficiently different parts of the brain work together during complex thinking.

The brain's outer layer, called the cerebral cortex, is characterized by a folded structure with ridges (gyri) and grooves (sulci). These folds allow the large surface area of the cortex to fit within the confines of the skull. While most neuroscience research has focused on the major sulci that are present in nearly everyone, the current study focused on smaller and shallower grooves called tertiary sulci.

These sulci tend to vary more across individuals and are found in association areas of the brain — regions that have expanded significantly over the course of human evolution and are involved in advanced cognitive processes.

The rationale for the study stemmed from earlier findings showing that certain sulci in the lateral prefrontal cortex were associated with better reasoning skills in children and adolescents. Building on this, the researchers hypothesized that these small grooves might support reasoning by affecting the structure and function of brain networks. More specifically, they proposed that deeper tertiary sulci might bring distant brain regions closer together, enabling more efficient communication — a concept known as network centrality.

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To examine this, the researchers recruited 43 neurotypical children and adolescents between the ages of 7 and 18 from a larger developmental study of reasoning ability. All participants were right-handed native English speakers and underwent detailed brain imaging while performing a task designed to measure abstract reasoning. The task required them to examine patterns among simple shapes and determine relationships, both at a basic level (e.g., comparing shapes) and at a more complex level (e.g., identifying consistent rules across pairs of shapes). The researchers also collected structural MRI scans to precisely map the sulci in each participant's brain.

A total of 42 sulci were manually identified in each hemisphere of the brain, focusing on the lateral prefrontal and parietal cortices. The researchers then used functional MRI data collected during the reasoning task to assess how strongly each sulcus was connected to others, essentially building a map of functional connectivity across these grooves. Advanced data analysis techniques were employed to measure three aspects of each sulcus's role within the brain network: how many other sulci it was connected to (degree), how often it served as a bridge between other connections (betweenness), and how broadly its connections spanned across different functional clusters (participation coefficient).

One key question was whether the sulci had unique patterns of connectivity that distinguished them from one another. The answer was yes. Using machine learning techniques, the team showed that each sulcus had a highly distinctive "connectivity fingerprint," allowing for 96% accuracy in distinguishing one sulcus from another based on its pattern of functional connections. This high discriminability supported the idea that sulci are not just random anatomical wrinkles but may correspond to functionally meaningful brain units.

Next, the researchers grouped sulci based on the similarity of their connectivity patterns. They found five major clusters, with some groups containing both prefrontal and parietal sulci and others consisting solely of prefrontal sulci previously linked to reasoning.

Interestingly, some of these clusters were distinct from the large-scale brain networks typically identified in studies of resting-state brain activity. This suggests that using sulci as the basic unit of analysis could offer a more personalized and anatomically grounded approach to studying brain function.

A key finding was that in several specific tertiary sulci — including the right pmfs-a, left pmfs-i, and left pimfs — greater depth was linked to higher network centrality. In other words, these deeper sulci tended to have stronger and more distributed connections across the brain network, particularly with other important sulci involved in vision and attention.

These relationships held even after accounting for age and head motion, and were not driven solely by proximity to other sulci. The findings support the idea that deeper tertiary sulci may promote greater neural efficiency by facilitating shorter and more direct communication between key brain areas.

“For some specific sulci, the deeper the sulcus the better integrated it was within the network of prefrontal and parietal sulci we examined — i.e., the more tightly coupled its activation was with a number of other sulci.”

These associations were not uniform across all sulci. Some sulci with large surface areas, such as those in the intraparietal sulcus, had higher centrality measures overall, but did not show the same depth-dependent variation as the tertiary sulci. The researchers also found that the link between sulcal depth and network connectivity was not confined to sulci previously linked to reasoning ability. For instance, similar relationships were observed in a sulcus called aipsJ, which is sometimes used as a neurosurgical corridor, and a newly identified sulcus in the parietal lobe known as slocs-v.

These results lend support to a long-standing hypothesis that individual variability in sulcal anatomy could play a functional role in cognition. The idea dates back to the 1960s, when neuroanatomist Sanides proposed that subtle differences in sulcal structure might reflect and even shape cognitive abilities. The current findings suggest that sulcal depth, especially in regions that develop late in gestation and continue to change during childhood, could serve as a biomarker for individual differences in reasoning and possibly other mental functions.

“Sulcal patterns are not random, and are linked with cognition and functional organization. Fine-grained individual anatomy may help in developing tools and grounded hypotheses to better account the high individual variability between people,” said co-author Suvi Häkkinen, an assistant project scientist.

“We went into the study with the hypothesis that functional connectivity (that is, patterns of coordinated brain activation across a set of regions) could be the missing link explaining relationships between sulcal depth and cognition that we had previously found,” Bunge added, “but it was a wide open question as to whether we’d find any relation between depth and functional connectivity. It was a nice surprise that it turned out as it did!”

“I was surprised to learn from Suvi’s analyses just how well a pattern classifier could distinguish sulci — even small, neighboring sulci — from one another based on their connectivity fingerprints (i.e., their patterns of coupling to other regions). I was also surprised that some of the prefrontal sulci had more similar functional fingerprints to certain parietal sulci than to other prefrontal sulci.”

Despite its strengths, the study has several limitations. The amount of functional MRI data collected per participant was relatively modest, which may limit the precision of individual-level network measures. The sample was also restricted to children and adolescents, leaving open the question of whether similar patterns exist in adults or in individuals with neurodevelopmental disorders. In addition, while the study used rigorous control analyses to address confounds like head motion and spatial proximity, some residual effects cannot be ruled out.

Future research could expand this approach to different brain regions, age groups, and cognitive domains. The method of defining brain networks based on sulcal anatomy — rather than predefined regions or general brain atlases — may offer a more individualized way to study brain function. It could also help identify early anatomical markers for cognitive strengths and vulnerabilities, potentially informing education, clinical interventions, and neuroscience-based diagnostics.

The study, "Anchoring functional connectivity to individual sulcal morphology yields insights in a pediatric study of reasoning," was authored by Suvi Häkkinen, Willa I. Voorhies, Ethan H. Willbrand, Yi-Heng Tsai, Thomas Gagnant, Jewelia K. Yao, Kevin S. Weiner, and Silvia A. Bunge.

The Glymphatic System: A Novel Component of Fundamental Neurobiology

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Abstract

Throughout the body, lymphatic fluid movement supports critical functions including clearance of excess fluid and metabolic waste. The glymphatic system is the analog of the lymphatic system in the CNS. As such, the glymphatic system plays a key role in regulating directional interstitial fluid movement, waste clearance, and, potentially, brain immunity. The glymphatic system enables bulk movement of CSF from the subarachnoid space along periarterial spaces, where it mixes with interstitial fluid within the parenchyma before ultimately exiting from the parenchyma via perivenous spaces. This review focuses on important questions about the structure of this system, why the brain needs a fluid transport system, and unexplored aspects of brain fluid transport. We provide evidence that astrocytes and blood vessels determine the shape of the perivascular space, ultimately controlling the movement of perivascular fluid. Glymphatic fluid movement has the potential to alter local as well as global transport of

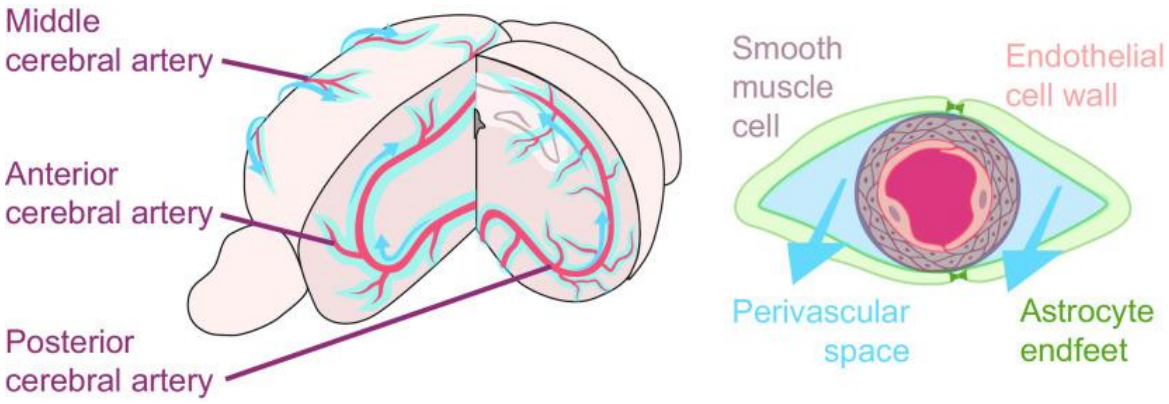
signaling molecules and metabolites. We also highlight the evidence for cross talk among the glymphatic system, cardiovascular system, gastrointestinal tract, and lymphatic system. Much remains to be studied, but we propose that the glymphatic/lymphatic system acts as a cornerstone in signaling between the brain and body.
Keywords: astrocyte, cerebrospinal fluid, choroid plexus, glymphatic, peptides, perivascular space

Introduction

The awake, active brain builds up metabolic waste such as amyloid-β, which negatively affects neural functions if not removed. The glymphatic hypothesis postulates that the restorative function of sleep is a consequence of basic housekeeping whereby the glymphatic system “sweeps” the brain clear of waste by providing a continuous flow of fluid across the brain and out to the periphery, thereby counteracting protein accumulation and the development of neurodegenerative diseases such as Alzheimer's disease (Nedergaard and Goldman, 2020).

The glymphatic system enables bulk movement of CSF from the subarachnoid space along periarterial spaces, where it mixes with interstitial fluid (ISF) within the parenchyma before ultimately exiting from the parenchyma via perivenous spaces (Fig. 1) and drains into the peripheral lymphatic system (Iliff et al., 2012). This fluid movement occurs through a mixture of advection and diffusion, and enables rapid exchange of fluid between the tissue and the perivascular space with a net directionality toward the venous system (Thomas, 2019). Physiologic drivers such as arterial pulsatility, vasomotion, and respiration establish this directionality (Rennels et al., 1985; Iliff et al., 2013b; Kiviniemi et al., 2016; Mestre et al., 2018a; Fultz et al., 2019; van Veluw et al., 2020).

Figure 1.



The vascular network is a scaffold for glymphatic fluid transport along the perivascular spaces. Glymphatic fluid (light blue) enters the brain via the perivascular space of the major arteries (red; left). Arteries and veins are lined by perivascular spaces, where

astrocyte end feet (green) cover smooth muscle cells (gray) and the endothelial wall of the vasculature (pink; right). This perivascular unit is a critical component of the glymphatic system, and its geometry is biologically optimized to promote fluid movement (blue arrows).

Evidence of CSF entering the periarterial space is not novel (Brierley, 1950; Rennels et al., 1985; Hadaczek et al., 2006); nor is the concept that CSF mixes with interstitial fluid (Levin et al., 1970; Kimelberg et al., 1978; Vladić et al., 2009) or even the hypothesis that this pathway may be a waste removal system (Lewis, 1877; Obersteiner, 1890), although the concept was insufficiently tested and thus prematurely discounted (Woollam and Millen, 1954). The features unique to discovery of the glymphatic system were as follows: (1) the direction of fluid transport, starting with entry of CSF at the periarterial space followed by exit of “dirty” interstitial fluid along the perivenous spaces; (2) a metabolic waste product, amyloid-β, was shown to be exported via the glymphatic system; (3) the fluid transport depends on polarized expression of the water channel aquaporin 4 (AQP4) in the vascular end feet of astrocytes; and (4) fluid transport and CSF entry into the neuropil exhibit a striking upregulation during sleep, paralleled by an increase in the clearance of metabolic waste (Iliff et al., 2012; Xie et al., 2013; Kress et al., 2014; Lundgaard et al., 2017, 2018; Mestre et al., 2018b).

Since its elucidation in 2012, the glymphatic system has provoked controversy, primarily because of a lack of data and adequate tools to characterize noninvasively a low-pressure fluid transport system residing in an electrically active organ encased within the rigid walls of the skull (Mestre et al., 2020a). Procedures associated with acute injection of tracers, such as opening the skull and insertion of a cannula, will effectively inactivate the glymphatic system (Mestre et al., 2018b; Plog et al., 2019). An equally troublesome issue is presented by the postmortem disappearance of fluid-filled CSF perivascular spaces and the reallocation of CSF tracers to other compartments, which invalidates the use of histology for characterization of brain fluid transport (Mestre et al., 2018a; Ma et al., 2019). Similar phenomena are quite commonly reported in the broader literature pertaining to interstitial fluid movement. For example, lymphatic capillaries were thought to be absent from skeletal muscle be cause the capillaries fully collapse in tissue preparations (Schmid-Schönbein, 1990). The more recent discovery of prelymphatic chambers in peripheral human interstitium demonstrates that large interstitial fluid compartments exist in all organs, but disappear during histologic procedures, highlighting the necessity of microscopy *in vivo* for the study of basic fluid transport (Benias et al., 2018). Finally, the new knowledge that bulk fluid movement through the brain is actively regulated by sleep (Xie et al., 2013; Lundgaard et al., 2017), anesthesia (Benveniste et al., 2017; Gakuba et al., 2018; Hablitz et al., 2019; Lilius et al., 2019), and time of day (Taoka et al., 2018; Cai et al., 2020; Hablitz et al., 2020) makes it inappropriate to compare previous studies in anesthetized animals with current work that includes the state of brain activity and time of day as important variables.

Caveats and controversies of the glymphatic hypothesis, along with their implications for neuropathology, have been reviewed at length in the recent literature (Kent and Mistlberger, 2017; Abbott et al., 2018; Rasmussen et al., 2018; Sun et al., 2018; Thomas, 2019; Nedergaard and Goldman, 2020; Troili et al., 2020; Wardlaw et al., 2020; Mestre et al., 2020a). For this reason, we place more focus in this review on important questions that remain to be addressed, fundamental questions about why the brain needs a fluid transport system, and the most exciting unexplored aspects of brain fluid transport.

In this review, we first compare and contrast the glymphatic system in the brain with the more traditional lymphatic system of the periphery, as both systems are essential for tissue homeostasis, interstitial fluid movement, immune function, and waste clearance. From there, we focus our efforts onto the perivascular space: what is known and unknown about the anatomy, and how differential regulation of the astrocytes and vasculature might alter global and local waste clearance. Then, we expand our questions beyond the perivascular space to how neuronal activity, intrinsically unique to the CNS, may alter interstitial fluid flow. In the final sections of this review, we ask the following question: does the glymphatic system have a role beyond simply “cleaning the brain”? We provide potential mechanisms of glymphatic bulk fluid signaling, whereby CSF may carry neuromodulators and/or vasoactive compounds to the perivascular space, changing interstitial fluid dynamics and brain activity. Finally, it has become increasingly clear that the brain does not work in isolation. We discuss known models of cross talk among the CNS, circulatory system, gastrointestinal (GI) track, and immune system, and how the glymphatic system may be a cornerstone in the communication between the brain and the body.

Comparison of glymphatic and lymphatic functions

Throughout the body, lymphatic fluid movement supports critical functions including clearance of excess fluid and metabolic waste and regulating tissue immunity (Miteva et al., 2010; Petrova and Koh, 2020). Tissues lacking traditional lymphatic capillaries develop alternative means of waste clearance and immune surveillance. In the eye, for example, Schlemm's canal, an endothelial-lined compartment that exhibits lymphatic markers (Grüntzig and Hollmann, 2019), drains fluid from the cornea (Petrova and Koh, 2018). Additionally, there is a glymphatic clearance system that exports intraocular fluid along the optic nerve (Wang et al., 2020). Both pathways ultimately convey fluid to the traditional lymphatic system. Based on the physiological requirements for local distribution of blood-derived nutrients and removal of metabolic waste, it is not surprising that the brain has compensated for its lack of lymphatic capillaries by developing an analogous perivascular perfusion system to maintain tissue homeostasis.

In peripheral tissue, blood capillaries provide a constant influx of an ultrafiltrate of plasma. The inflowing fluid percolates around the cells, dispersing glucose and other nutrients, while removing waste products via drainage to lymphatic vessels, which ultimately return the fluid to the venous circulation (Scallan et al., 2016; Breslin et al.,

2018). In the brain, the blood–brain barrier (BBB) restricts ultrafiltration of plasma in most regions. Likely as a compensation, the CNS produces its own fluid, CSF, in the choroid plexus (Redzic et al., 2005). However, a portion of CSF production may still occur via the influx of plasma across the vast surface area of the microvasculature (Rasmussen et al., 2021). After production, CSF is, in part, circulated into the brain parenchyma along the periarterial spaces (Fig. 1; Mestre, 2018a). Thus, both in peripheral tissues and CNS, fluid entry occurs at the arterial segment of the microvascular bed, likely driven by arterial pulsatility that propels fluid influx into the tissue. However, the efflux routes of interstitial fluid differ. While lymphatic capillaries are the primary drainage path in peripheral tissues, interstitial fluid exits the CNS along the perivenous spaces and cranial/spinal nerves (Iliff et al., 2012, 2013b; Rangroo Thrane et al., 2013). CSF and interstitial fluid eventually drain from the CNS via a traditional lymphatic network located in the meninges (Aspelund et al., 2015; Louveau et al., 2015, 2018; Da Mesquita et al., 2018a; Ahn et al., 2019), as well as along nerve sheaths in the cribriform plate, which lead to cervical lymphatic vessels (Kida et al., 1993), with both pathways leading to the superficial and deep cervical lymphatic nodes (Raper et al., 2016; Cao et al., 2018; Zou et al., 2019). Ultimately, all CSF drains into the venous circulatory system, either indirectly through the lymphatic system or directly via the arachnoid villi. Thus, interstitial fluid transport is directional, with distinct influx and efflux routes both in peripheral tissues and the CNS. Lymphatic vessels are endowed with valves to prevent fluid backflow (Breslin, 2014). Whether the glymphatic system exhibits analogous one-way gating mechanisms within the neuropil to support directional fluid movement remains to be explored.

The glymphatic system can clear potassium, waste metabolites such as lactate, and peptides/proteins including amyloid-β and tau, along with a variety of contrast agents and tracers (Iliff et al., 2012, 2013a; Xie et al., 2013; Kress et al., 2014; Lundgaard et al., 2017; Eide and Ringstad, 2019; Monai et al., 2019). Clearance kinetics of tracer injected into the neuropil depends on the injection site (Cserr et al., 1981; Szentistványi et al., 1984), suggesting the existence of regional differences in waste clearance. Also, in support of regional differences in glymphatic function, Alzheimer's disease model mice exhibited lower CSF/ISF exchange in the rostral cortex compared with caudal cortex, which favored tau protein deposition in posterior regions (Harrison et al., 2020). These findings in brain parallel results in the gastrointestinal tract, where lymph composition and immune function differ between anatomic regions of the organ (Esterházy et al., 2019). Glymphatic clearance kinetics also vary across sleep state (Xie et al., 2013) as well as time of day (Hablitz et al., 2020), with increased clearance during both sleep and the inactive phase, demonstrating that the mechanism and routes of fluid efflux from brain are more complex than a simple plumbing system.

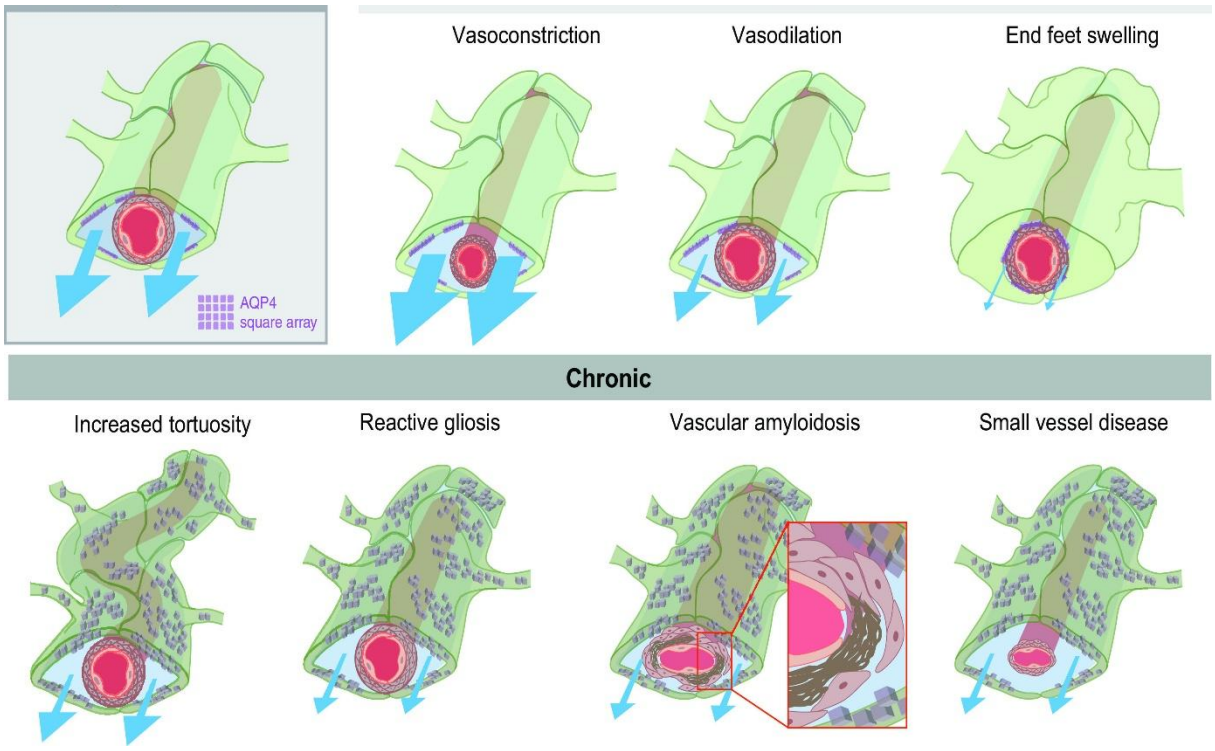
We still do not know precisely where in the brain interstitial fluid moves, and whether there are areas of pooling or slow flow within the brain. Additionally, details of where and how the glymphatic system connects to the lymphatic system remain unknown. Indeed, our current knowledge of the anatomic pathways of interstitial fluid clearance

within the brain is rudimentary, relying on experimental procedures with invasive intracranial injections, sampling at discrete time points, and bulk transfer of tracers.

Open questions about the perivascular space, astrocytes, and flow

The perivascular space (PVS) is distinct from the highly complex and convoluted interstitial space of the brain parenchyma, and is a critical feature of the glymphatic system. The PVS surrounds the cerebral vasculature and is lined by astrocyte end-feet plastered alongside the pericytes and endothelial cells that form the BBB (Figs. 1, 2; Simard et al., 2003; Troili et al., 2020). Estimates of the extent of astrocytic coverage around capillaries range from 64% to 100% (Sasaki and Mannen, 1981; Bertossi et al., 1993; Simard et al., 2003; Oberheim et al., 2009; Korogod et al., 2015; Munk et al., 2019), likely because of the variable effects of postmortem histologic analysis and low-resolution microscopy. For example, three-dimensional electron microscopy reconstruction of a single hippocampal capillary showed tiled and interlocking end feet covering blood vessels and pericytes (Mathiisen et al., 2010), whereas another study based on cryofixation reported a coverage of only 64% in transversely sectioned capillaries (Korogod et al., 2015). Recent work has demonstrated a better correlation between astrocyte end foot size and vessel diameter in arteries than for veins, though these correlations were evaluated only in penetrating and ascending vessels (Wang et al., 2021). However, the thickness of the astrocyte ensheathment of the vasculature is relatively consistent in arteries, capillaries, and veins (McCaslin et al., 2011). Beyond these few studies limited to cerebral cortex, there is very little detailed information on end foot coverage of the arterial or venous walls, or how this may relate to the perivascular space. This lack of quantitative information about the PVS directly limits the applicability of models of glymphatic fluid flow, in which the geometry of the perivascular space dictates the calculated rate of CSF influx into the neuropil (Schain et al., 2017; Mestre et al., 2018a; Tithof et al., 2019).

Figure 2.



The perivascular space (PVS) can be modulated by changes to both astrocytes and the vasculature. The normal perivascular unit is composed of astrocyte end feet (green) covering smooth muscle cells (tan) and endothelial cell walls (pink) of the vascular network, promoting CSF (blue) movement along these channels. AQP4 (purple) is located in square arrays on the vascular-adjacent end feet of astrocytes. Acute changes to either the vasculature or astrocyte end feet can alter glymphatic fluid movement. Vasoconstriction increases the PVS, increasing flow (indicated by blue arrows; Mestre et al., 2020b). This is in contrast to vasodilation that is expected to decrease flow. Swelling of astrocytic end feet can alter the size of the PVS space in the setting of pathology (e.g., spreading depression; Schain et al., 2017), but it is possible that changes in the vascular end feet of astrocytes are a physiological mechanism by which glymphatic function is controlled. Chronic pathologic changes may also impair CSF influx (bottom). We hypothesize that vasculature changes such as increased tortuosity with aging alter fluid flow. Reactive gliosis (shown as a dark green color and mislocalized AQP4), is a common hallmark of neuropathology (Ikeshima-Kataoka, 2016; Verkhratsky et al., 2016; Wang and Parpura, 2016; Kovacs et al., 2018), which will, most likely decrease flow. Vascular amyloidosis, characterized by amyloid-β plaques (brown) accumulating between the smooth muscle cells and the endothelial cell wall, and small-vessel disease, characterized by altered vascular shape and enlarged perivascular spaces, both decrease glymphatic flow.

Acute changes in the geometry of the PVS on vasoconstriction, vasodilation, or astrocyte swelling have the potential to affect the movement of glymphatic fluid (Fig. 2). Evidence for this hypothesis comes from mouse disease models. For example, in a model of acute stroke, ischemic spreading depolarization triggers the constriction of

blood vessels, thus widening the PVS (Fig. 2) and enabling a rapid influx of CSF to the parenchyma (Mestre et al., 2020b). In cortical spreading depression, a rapid neuronal depolarization event that is frequently associated with migraine aura, the PVS closes, resulting in reduced interstitial clearance of tracer (Schain et al., 2017). Spreading depression causes rapid vasodilation followed by vasoconstriction. It has been proposed that the vasoconstriction phase correlates with astrocytic end foot swelling, and underlies a reduction of flow in the PVS (Rosic et al., 2019). However, the magnitude of vasoreactivity or end foot swelling necessary to perturb glymphatic flow is unknown.

The PVS and vascular compartments are dynamic volume spaces that provide the brain with a mechanism to couple hyperemia and waste clearance. In humans, neuronal slow waves occurring during sleep are coupled to hemodynamic oscillations, which in turn are coupled to CSF flow. Specifically, a nocturnal peak in 0.2–4 Hz neuronal activity triggers increased cerebral blood flow, which reduces the amount of CSF movement in the ventricle and the brain parenchyma, directly supporting the concept of blood/CSF volume switching (Fultz et al., 2019). Acute hypertension increases the stiffness of the arterial wall, resulting in decreased pulsatility and PVS fluid flow by as much as 50% (Mestre et al., 2018a), highlighting the interplay between the vasculature and the PVS in glymphatic function. There is also evidence that posture can effect glymphatic flow (Lee et al., 2015), but whether this is related to known changes in cerebral blood flow (Foley et al., 2005; Kose and Hatipoglu, 2012), changes in intracranial pressure (Andresen et al., 2015), and/or changes in sympathetic noradrenergic tone (Stewart, 2012) remain unknown. Given that astrocytes are a critical component of the neurovascular unit and participate directly in the regulation of cerebral blood flow (Iadecola and Nedergaard, 2007), it seems likely that astrocytes regulate volume dynamics between the vasculature and perivascular spaces.

The primary evidence for astrocytic regulation of glymphatic fluid movement, beyond the spatial organization of the PVS, is that AQP4 facilitates glymphatic fluid transport (Iliff et al., 2012; Mestre et al., 2018b). The expression of AQP4 is normally highly polarized toward the plasma membrane of the astrocytic end feet facing the PVS (Fig. 2) and is anchored by the dystrophin-associated complex (Waite et al., 2012; Zhang et al., 2014). Mislocalization of AQP4 from astrocytic end feet has been linked to glymphatic malfunction in multiple lines of work (Kress et al., 2014; Ren et al., 2017; Lundgaard et al., 2018; Mestre et al., 2018b; Ohene et al., 2019; Wei et al., 2019; Harrison et al., 2020; Xue et al., 2020; Liu et al., 2020b). Though AQP4 polarization toward the vascular end feet constitutes a key regulatory mechanism, it is likely that astrocytes can alter glymphatic function by additional mechanisms.

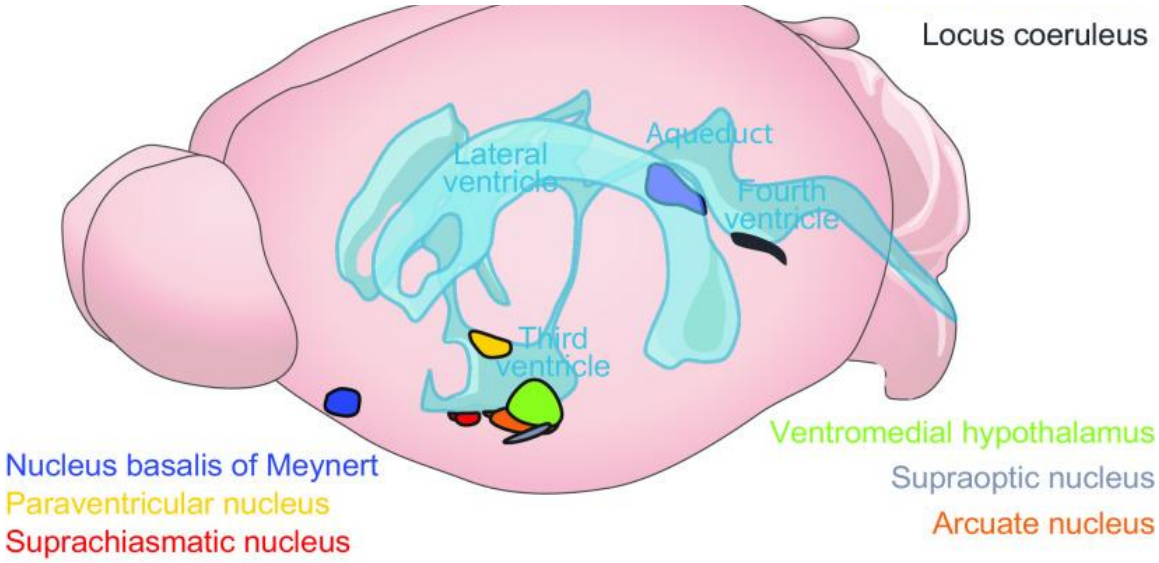
It is clear that glymphatic function is highly dependent on optimized perivascular spaces with low resistance to fluid flow, yet very few studies have tested whether long-term remodeling of the shape, permeability, and patency of the PVS is linked to glymphatic dysfunction. With increased age, glymphatic flow decreases, alongside increased reactive gliosis and a reduction in the polarized expression of AQP4 toward the vascular

end feet of astrocytes (Fig. 2; Kress et al., 2014). Reactive gliosis, particularly manifested by increased GFAP and mislocalized AQP4, is a hallmark of neuropathology (Ikeshima-Kataoka, 2016; Verkhratsky et al., 2016; Wang and Parpura, 2016; Kovacs et al., 2018) and could potentially indicate alterations to astrocytic end foot morphology, although this conjecture has not been tested. Additionally, the tortuosity of the brain vasculature increases with aging (Fig. 2; Thore et al., 2007), which likely presents an impediment to fluid flow.

The functionality and shape of the cerebrovasculature also changes in disease. In cerebral amyloid angiopathy, $A\beta_{1\text{-}40}$ accumulates in vessel walls, causing vessel weakening and collapse of the perivascular space (Smith, 2018; Gatti et al., 2020). In small-vessel disease, which is a frequent complication of hypertension and diabetes, arterial stiffening and remodeling of the cerebral arteries cause enlargement of the PVS (Fig. 2; Mestre et al., 2017; Lerman et al., 2019). The functional importance of this chronic PVS remodeling has yet to be established, leading to several unresolved questions, including the following. If the curvature and surface area of the vasculature increases, can the astrocytic end feet compensate? Would changes in astrocyte volume or morphology, such as occurring during reactive gliosis, directly impact local glymphatic flow? And, finally, do the borders of the PVS ever break down, and how would this effect polarized fluid flow?

Astrocytes are morphologically complex cells. In addition to the vascular end feet, astrocytes extend innumerable fine, irregular processes that exhibit local Ca^{2+} signaling independent of the soma and other processes (Tong et al., 2013; Shigetomi et al., 2016; Verkhratsky and Nedergaard, 2018). The astrocyte and its processes can change rapidly in volume, within a matter of seconds (Takano et al., 2005; Risher et al., 2009; Sherpa et al., 2016). This process appears to be independent of AQP4 and occurs under conditions of high K^+ such as during increased neuronal activity (Walch et al., 2020). This is consistent with reduced interstitial space measurements during wakefulness (Xie et al., 2013), and most likely is a prime mechanism for rapid alterations in glymphatic flow. More chronic changes to astrocyte morphology have been found in the hypothalamus. Astrocytes can extend and retract fine processes across sleep states (Bellesi et al., 2015), hydration state (Hawrylak et al., 1998), during pregnancy and lactation (Theodosis, 2002; Theodosis et al., 2008), and in response to the light/dark cycle (Lavialle and Servière, 1993; Lavialle et al., 2001, 2011). Such morphologic changes can last from several hours to days and are generally reversible. In the case of lactation, glial processes retract from the supraoptic nucleus (SON) of the hypothalamus (Fig. 3) and remain retracted until pup weaning, when the glial processes extend back into the region (Hatton, 1997). It is unknown whether astrocytic morphologic changes alter glymphatic function.

Figure 3.



Anatomical localization of key brain regions is strategically placed around CSF reservoirs. The hypothalamus is located along the third ventricle and base of the brain above the basal cisterns, a prime position for CSF signaling. It contains the suprachiasmatic nucleus (red), arcuate nucleus (orange), paraventricular nucleus (yellow), ventromedial hypothalamus (green), and the supraoptic nucleus (navy), which is a hub of peptidergic signaling that controls basic biological functions such as circadian timing, reproduction, feeding, hydration, and more. The nucleus basalis of Meynert (blue), dorsal raphe nucleus (purple), and locus coeruleus (gray) are also primed for brain-wide CSF-mediated cholinergic, serotonergic, and noradrenaline signaling.

Glymphatic–neuronal interactions

The brain is a unique organ because of the complexity of its neuronal networks, which signal to execute complex behaviors, tasks, and thought. Neural signaling (i.e., synaptic transmission and propagation of action potentials) requires tight regulation over interstitial ion concentrations (Rasmussen et al., 2020). For example, simply changing the interstitial ion concentration can induce arousal state changes, with lower interstitial Ca^{2+}, Mg^{2+}, and H^{+}, and higher K^{+} inducing awake brain activity (Ding et al., 2016). Astrocytes are highly dynamic regulators of interstitial K^{+} concentrations and pH, and likely influence the concentrations of several other ions, as well as the interstitial space volume (Verkhratsky and Nedergaard, 2018). It is presently unknown whether the glymphatic system independently contributes to state-dependent changes in interstitial ion concentrations, but there are grounds to speculate that fluid transport and the ion composition of the interstitial fluid are intimately linked.

The strongest evidence linking brain-wide neuronal activity and glymphatic activity is derived from sleep studies. Non-rapid eye movement (NREM) sleep consists of four stages with different patterns of brain electrical activity including the phase dominated

by large amplitude, synchronous, slow oscillations of 1–4 Hz. There is strong evidence that glymphatic flow increases to promote the delivery of larger CSF volumes to the brain during NREM sleep (Xie et al., 2013), and under anesthetic regimens characterized by a high prevalence of 1–4 Hz activity (Hablitz et al., 2019). In humans, the volume of CSF movement within the fourth ventricle is also increased during NREM sleep (Fultz et al., 2019), and AQP4 haplotype is associated with changes in NREM sleep architecture (Ulv Larsen et al., 2020), suggesting a link between fluid movement within the brain and neuronal activity. During REM sleep, the cortex exhibits low-voltage, rapid desynchronized neuronal activity that superficially resembles the pattern during wakefulness. It remains to be established whether glymphatic flow declines during REM sleep, as during waking. Mechanisms mediating the functional relationship between neuronal activity and bulk fluid movement have not been tested; nor have the above observations been applied to investigations of sleep deprivation or insomnia.

Synchronizing distinct populations of neurons may influence local hemodynamics and fluid flow to ultimately change local solute transport. This phenomenon has been shown for CSF dynamics across the brain as a whole (Fultz et al., 2019), but has yet to be demonstrated for regional brain or perivascular-localized waste clearance. Sensory manipulations targeted to network activity that were reduced in different pathologic states including aging and neurodegeneration, such as gamma oscillations (~40 Hz) seen during response to external stimuli (Jafari et al., 2020), may improve glymphatic clearance. Support for this hypothesis is found in mouse models of Alzheimer's disease, where the stimulation of sensory systems can alter local waste buildup. Inducing synchronous gamma oscillations via 40 Hz light flickers is reported to decrease amyloid burden in the visual cortex (Iaccarino et al., 2016). Entraining the auditory system via 40 Hz tone evokes decreased amyloid protein buildup across the entire cortex via a glia- and vascular-dependent mechanism (Martorell et al., 2019).

Glymphatic bulk fluid signaling

To this point, we have focused on CSF/ISF exchange and waste clearance, which is likely the primary function of the glymphatic system. Albeit less studied, glymphatic transport may fulfill additional roles, including the widespread distribution of signaling molecules within brain. CSF may also play a largely unacknowledged role in transporting CNS signaling molecules to peripheral tissues, which we shall discuss here.

CSF is primarily produced by the choroid plexus located in the ventricles and then undergoes directional transport from the lateral ventricle to the third ventricle, through the aqueduct of Sylvius, and finally to the fourth ventricle, where it exits the brain via the foramina of Magendie and Luschka. From the foramina, CSF enters the cisterna magna and is either shunted out of CNS via the meningeal lymphatic system or redirected back into the brain by the glymphatic system. The entry path for the glymphatic system is via the pontine cistern, where CSF moves along the perivascular space surrounding the basilar artery to the circle of Willis. CSF then ascends along the perivascular spaces of

the anterior, middle, and posterior cerebral arteries. Rodent studies have demonstrated that during sleep, CSF transport along the glymphatic system is fast (minutes to hours; Xie et al., 2013) compared with during the active phase, where CSF redistributes to the mandibular lymph nodes in an equally fast manner (Hablitz et al., 2020).

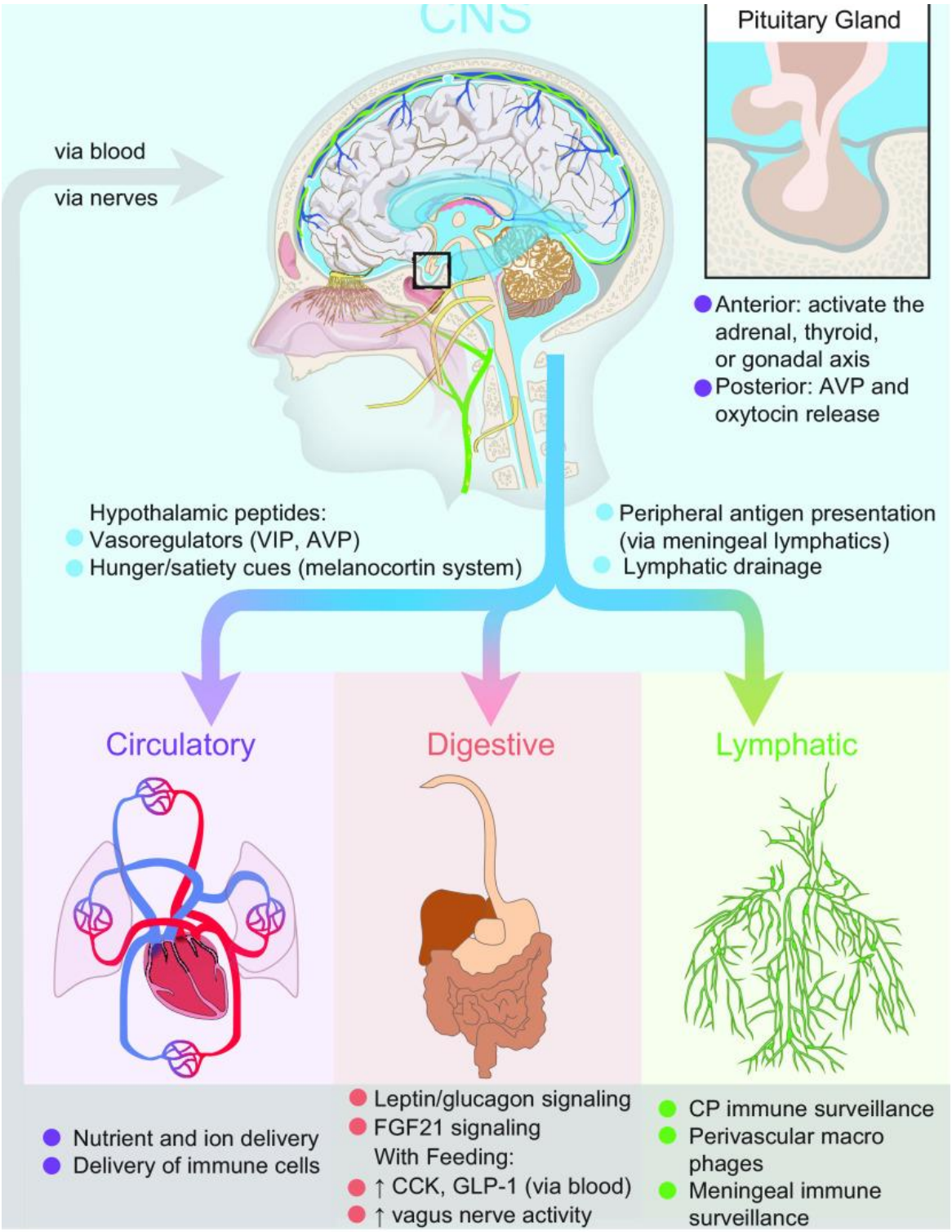
The choroid plexus secretes or transfers from blood a host of solutes, metabolites, vitamins, and carrier proteins to CSF (please see next section for details). Remarkably, most of the brain regions that produce signaling molecules with the potential for long-range volume transmission are located along the ventricular path of CSF transport (Fig. 3). The classical neuromodulators such as acetylcholine, noradrenaline, and serotonin, which are thought to participate in volume transmission, are produced by cell groups generally lying just below the ependymal lining of the ventricles or the pial lining of the ventral brain surface. The nucleus basalis of Meynert, which contains most of the cholinergic neurons in the CNS, is located in the basal forebrain region just on top of the pial membrane facing the optic nerve, while its medial boundary meets the wall of the lateral ventricle (Liu et al., 2015). The locus coeruleus, which is the main source of norepinephrine in the CNS, is located along the lateral wall of the fourth ventricle. The serotonergic dorsal raphé nucleus is positioned centrally in the midline of the brainstem, but sends extensive ascending projections to the wall of the lateral, third, and fourth ventricles (Simpson et al., 1998). Although it remains to be demonstrated, the spatial distribution of acetylcholine-, norepinephrine-, and serotonin-secreting neurons or their projections suggests that these neuromodulators may be distributed by CSF transport following their release from the axonal terminals (Agnati et al., 1986; Vizi et al., 2010; Taber and Hurley, 2014; Veening and Barendregt, 2015). In contrast, the dopamine neurons of the substantia nigra are located in the ventral midbrain posterior to the cerebral peduncle and are thus not in direct contact with the brain surfaces. Neither do projections from these dopaminergic neurons terminate at the ventricular surface. This may reflect a necessity for local, tightly regulated dopaminergic signaling and/or a mechanism to protect the rest of the brain from CSF-driven global dopaminergic neuromodulation.

Perhaps the most important example of brain–CSF communication is presented by the hypothalamus. The hypothalamus controls fundamental physiological and behavioral functions such as eating, drinking, regulating body temperature, neuroendocrine release, and internal circadian timing. Anatomically, the hypothalamus is in prime position to access the CSF, as it surrounds the third ventricle, is ventrally in contact with the CSF pool of the basal cisterns (Fig. 3), and has specialized cells, tanycytes, that can directly interact with the CSF pool in the ventricles (Rodríguez et al., 2010). The hypothalamus is a hub of peptidergic signaling, including neuropeptide Y and pro-opiomelanocortin in the arcuate nucleus, arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP) in the suprachiasmatic nucleus, and oxytocin and AVP in the supraoptic and paraventricular nuclei. Biomarkers of the melanocortin system are implicated in appetite suppression, and melanin-concentrating hormone, which is important for initiation of feeding, is present in the CSF, enabling brain- and body-wide signaling (Kim et al., 2014; Page-Wilson et al., 2017; Noble et al., 2018). Hypothalamic

peptides such as VIP and AVP are present in CSF (Burbach, 1982) and can directly alter vascular tone (Henning and Sawmiller, 2001; Pelletier et al., 2014), thus potentially regulating glymphatic flow through the neuropil. The plethora of peptidergic neuromodulators that participate in CSF/ISF-mediated volume transmission, and the vasoreactive action of many of these peptides, support a model of hypothalamic bulk fluid signaling.

Nuclei of hypothalamus densely innervate the pituitary gland, an endocrine organ at the base of the brain (Spencer and Deak, 2017; Qin et al., 2018). A distinct feature of the pituitary is its localization outside the CNS barriers of the brain, spinal cord, and CSF. The pituitary gland is positioned in an indentation of the skull known as the sella turcica and is separated from the subarachnoid space by the diaphragma sellae, a dural membrane that prevents CSF from accessing the gland (Fig. 4). As a consequence of this arrangement, pituitary hormones are secreted directly into the blood, avoiding CSF transport within CNS (Patel et al., 2021; Rawindraraj et al., 2021).

Figure 4.



The glymphatic system as an interface between the brain and body. The brain can communicate to the circulatory, digestive, and lymphatic systems by secreting signaling molecules to CSF, driving fluid to the meningeal lymphatics for antigen presentation, and ultimately draining to the lymph nodes of the lymphatic system. A unique feature of the brain is the hypothalamic signaling to the pituitary gland, where neurons can either

induce neuroendocrine signaling in the anterior pituitary, or directly release peptides such as AVP and oxytocin into the blood vessels of the posterior pituitary. Feedback between these systems is potentially bidirectional when considering blood composition, feeding and fasting metabolites, and immune surveillance. The pituitary gland is uniquely shielded from direct interaction with the CSF pool and direct CNS signaling by its location in an indentation of the skull covered by the diaphragma sellae, a dural membrane.

The pituitary is divided into two lobes that are functionally and developmentally distinct (Kiecker, 2018). The posterior pituitary gland contains axonal projections from magnocellular neurosecretory neurons located in the SON and paraventricular nucleus (PVN) of hypothalamus. These neurons secrete oxytocin and AVP, which are stored in the posterior pituitary and then are released into the blood. In addition, the magnocellular neurosecretory cells can release oxytocin and AVP from their dendrites within the parenchyma of the hypothalamus. Dendrites from the SON form a plexus lying just below the pial surface of the SON, whereas the dendrites from PVN form a bundle at the subependymal region of the third ventricle, suggesting that oxytocin and vasopressin are distributed via CSF transport within the CNS (Heimer-McGinn et al., 2013), in addition to their peripheral actions. Cortical signaling by oxytocin and AVP alter social and cognitive function without having direct synaptic pathways (Ludwig and Leng, 2006; Meyer-Lindenberg et al., 2011; Abramova et al., 2020). The organization of the hypothalamus and posterior pituitary suggests that oxytocin and AVP could exert their effects on social and cognitive function through a combination of central and peripheral regulation of glymphatic function, where blood-borne peptides change vascular tone and interstitial fluid-mediated peptidergic signaling changes synaptic tone.

Parvocellular hypothalamic neurons secrete neurohormones that are exported to the anterior pituitary via hypophyseal portal blood vessels, thus avoiding CSF transport and distribution within CNS. These neurohormones control the release of growth hormone, prolactin, adrenocorticotropin, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone, ultimately inducing body-wide stress, sex, and metabolic responses. Exploration of neuroendocrine regulation of glymphatic flow is in its infancy. Activation of the adrenal gland is a classic example of a hypothalamic–pituitary cascade, which initiates a concerted, body-wide stress response. Chronic stress can cause glymphatic malfunction (Wei et al., 2019; Liu et al., 2020b), but it remains unknown how acute stress changes brain fluid movement.

Text box 1:

Bringing it all together with the circadian system

Sleep and circadian rhythmicity are evolutionarily conserved behavioral and physiological states, serving to restore bodily functions and, in the case of circadian rhythms, predicting changes in food availability, temperature, predation, and other factors essential to ensure survival. Sleep and circadian timing both regulate glymphatic function. In this section, we summarize the relevant literature and generate new

hypotheses about the mechanisms and function of circadian rhythms and sleep in glymphatic function.

In humans, most brain regions exhibit endogenous rhythms in neuronal excitability, with peaks in excitability occurring within a 4 h window across the brain (Muto et al., 2016). This synchronized daily activity enables complex behaviors to have phase-appropriate performance peaks, such that learning and memory capacities peak during the active phase (Iyer et al., 2014; Smarr et al., 2014). Recent work has shown that, in addition to synchronized neuronal excitability, the distribution of CSF in the brain and lymph nodes is under circadian control, with increased glymphatic influx and clearance from the brain during the rest phase, and increased lymphatic drainage during the active phase (Hablitz et al., 2020). If we consider rhythmic, synchronized neuronal activity, the circadian cycle of glymphatic flow, and the earlier hypothesis that synchronized neuronal activity can drive fluid flow, one might suppose that fluid flow in the brain may act as a circadian entrainment mediator, whereby individual brain oscillators drive rhythmic fluid flow to the next oscillator in line, thus synchronizing brain activity across the 24 h cycle.

We have already introduced the hypothesis that the hypothalamus is a key signaling area for bulk flow, based on its anatomic location and highly enriched concentrations of neuropeptides. The mammalian suprachiasmatic nucleus (SCN) of the hypothalamus is considered the circadian pacemaker; synchronizing and driving daily rhythms in gene expression, metabolism, physiology, and behavior (Mohawk and Takahashi, 2011). Classic work demonstrated that grafting SCNs of rhythmic animals into the third ventricle of SCN-lesioned animals restores behavioral rhythmicity (Silver and LeSauter, 1993) and showed that this effect was dependent on a diffusible factor (Silver et al., 1996), suggesting that the SCN may directly communicate with the CSF pool to synchronize circadian rhythms. The SCN has a very distinct localization of neuropeptides (Morin et al., 2006). VIP neurons, which are responsible for maintaining intrinsic rhythmicity (Aton et al., 2005; Todd et al., 2020), are located in the ventral portion above the optic chiasm. AVP neurons, which regulate plasticity of rhythms (Mieda, 2019; Rohr et al., 2020), are found in the dorsal portion of the SCN along the third ventricle. Although both VIP and AVP are present within CSF (Burbach, 1982), how these enter the CSF pool remains unknown. Based on the localization of these vasoactive peptides (Henning and Sawmiller, 2001; Pelletier et al., 2014), it is tempting to hypothesize that the mechanism of action whereby VIP released near the basal cisterns impacts vascular tone of the glymphatic system, whereas AVP release into the ventricles—upstream in the CSF pathway—is able to tune fluid flow in “downstream” areas. Whether AVP is released by the SCN into the ventricles, perhaps via direct innervation (Taub et al., 2021), a small portion of tanycytes within the SCN (Wen et al., 2020), or a separate transependymal method, remains unknown.

One such downstream area may be the pineal gland, which sits near the center of the parenchyma, is highly vascularized, and is bathed in CSF. CSF tracers accumulate in the pineal gland of mice and rats (Iliff et al., 2013a; Benveniste et al., 2017). In rodents,

the SCN triggers a multisynaptic pathway that activates sympathetic preganglionic neurons in the spinal cord to release norepinephrine from terminals innervating the pineal gland, thus inducing nighttime melatonin production on demand for immediate release (Ganguly et al., 2002; Saper et al., 2005). The primary function of melatonin is to entrain circadian rhythms and improve sleep timing, along with a host of other beneficial pleiotropic effects (Aulinas, 2000; Pandi-Perumal et al., 2006). Nightly melatonin secretion is the primary biomarker for the circadian phase in humans and declines with aging, leading to alterations in sleep timing (Pace-Schott and Spencer, 2011). Though it is widely accepted that melatonin is secreted directly to the blood, this is primarily because of early literature reports correlating melatonin production in the pineal gland to blood plasma levels (Hedlund et al., 1977; Illnerova et al., 1978). Anatomical studies of the pineal gland give conflicting results, suggesting an intact BBB in fetal tissue (Moller, 1974) and, perhaps, subregion differences in pineal BBB permeability in adults (Duvernoy and Risold, 2007). Although melatonin is found in the CSF (Bruce et al., 1991; Skinner and Malpaux, 1999), it is unclear whether this melatonin pool derives from rapid diffusion from blood across lipid membranes, or whether the pineal gland directly secretes this powerful chronobiotic molecule directly to the CSF to entrain the brain.

The circadian system is just one example of several concerted biological responses that might be controlled by glymphatic—and CSF—movement. We highlight the circadian system as an example of how neuronal activity, CSF movement, and bulk fluid signaling might interact to impact neuronal function, physiology, and, ultimately, behavior.

CSF goes to glymphatics and beyond

Above, we discussed how the glymphatic system can be regulated by the cardiovascular system, neuronal function, and, perhaps, by CSF/ISF bulk flow of neurotransmitters and peptides. We have discussed the pathway of fluid movement from the ventricles to the glymphatic and lymphatic systems of the body. Finally, we hypothesized that the while the glymphatic system clears waste, it is also a mechanism of brain-wide signaling that could dramatically impact the survival of an organism adapting to a new environment. Here, we expand on known CSF-localized signaling cues originating from the blood, gut, or immune system. We discuss how they impact glymphatic function and highlight new questions in brain–body interactions.

Cross talk between the blood and the brain

The vascular system carries oxygen and glucose along with other nutrients and immune cells throughout the body and is critical to maintain tissue homeostasis. As discussed above, the BBB limits the transfer of solutes between blood and brain. The brain obtains an ultrafiltrate of blood via the choroid plexus, a highly vascularized ependymal cell layer lining the ventricles, which is responsible for the bulk of CSF production (Redzic et al., 2005). As such, the choroid plexus may be considered the “starting point” of the glymphatic system. In addition to transferring signaling molecules like growth factors

from the blood into the brain, the choroid plexus can also contribute to brain homeostasis by producing and secreting into the CSF a host of enzymes and growth factors such as transthyretin, insulin-like growth factor II, and interleukin-1β (Benarroch, 2016). Interestingly, the choroid plexus also expresses receptors for norepinephrine, melatonin, and AVP, among other hypothalamic-derived neuropeptides, which, on activation, can modulate the rate of CSF production (Nilsson et al., 1992). How CSF production rates impact glymphatic flow in rodent brain remains unexplored because of a lack of proper techniques (Orešković et al., 2003; Orešković and Klarica, 2014). Recent work has shown that physiological processes that reduce glymphatic function like age and wakefulness also suppress CSF production, yet other manipulations showed that there is no correlation between CSF production and glymphatic fluid transport (Liu et al., 2020a). It thus remains unknown whether CSF production and glymphatic function are interdependent, but it is clear that the choroid plexus is a key player in the feedback loop among the brain, glymphatic system, and CSF production from the blood (Fig. 4).

It is important to note that BBB restriction to influx of an ultrafiltrate of plasma is not consistent across brain regions, specifically in the circumventricular organs in the hypothalamus where the interstitial fluid of these regions are barricaded by defined glial borders from the rest of the brain (Rodríguez et al., 2010). Also, the BBB does not necessarily preclude the regulation of transport between the blood and parenchyma of ions, oxygen, molecules, and even cells under pathologic conditions (Daneman and Prat, 2015). Although the BBB is clearly necessary for brain health, how the BBB alters or interacts with glymphatic fluid flow is at this point completely unknown.

There is bidirectional signaling between the vascular compartment and the brain. As discussed above, the vascular compartment uses the choroid plexus as a gateway to provide access for signaling molecules to the CNS. The brain communicates directly with the vascular compartment via the posterior pituitary gland (Fig. 4). Peptidergic neuronal projections in the posterior pituitary from oxytocin and AVPergic neurons arising from the paraventricular nucleus (Fig. 3) release neuropeptides directly into the vascular system, with downstream effects on cardiovascular tone, kidney function, and other functions (Palkovits, 1984). Feedback from this peripheral pathway can alter cerebral blood flow at a more global level compared with local/bulk hypothalamic signaling, perhaps altering PVS volume to increase or decrease glymphatic flow depending on brain state.

The gut–brain axis

Above, we have discussed how the glymphatic system clears metabolic waste from the brain. One might anticipate that this fluid pathway would be receptive to satiety and hunger cues. We now discuss modes of cross talk between the gastrointestinal tract itself and the brain (Fig. 4). Intravenous administration of triglycerides alters the composition of CSF lipids (Hanson et al., 2019), and glymphatic transport can distribute lipids across the brain (Rangroo Thrane et al., 2013). Alterations in the blood/brain/CSF

distribution of triglycerides also cause a dysregulation of central leptin signaling (Banks et al., 2018), which controls appetitive behavior. These pathways support the hypothesis that after absorption in the GI tract and passage through the liver, food-derived lipids and other hydrophobic molecules can enter the CSF from the blood via the choroid plexus and across the BBB. In the brain, the major carrier of cholesterol is apolipoprotein E (APOE), which is abundantly produced by astrocytes as well as by the choroid plexus (Xu et al., 2006), suggesting that APOE might be distributed in brain by fluid movement in the perivascular space. APOE isoforms, when delivered by the CSF, enter periarterial spaces with different efficacy (Achariyar et al., 2016). Interestingly, APOE genotype is the strongest genetic risk factor known for late-onset sporadic Alzheimer's disease (Belloy et al., 2019), and glymphatic function is reduced in mouse models of AD (Peng et al., 2016; Da Mesquita et al., 2018b; Harrison et al., 2020). Thus, it is possible that differential distribution of lipids derived from the gut along the perivascular spaces directly contributes to brain pathology.

The vagus nerve provides a direct neural connection from the gut to the brain. Upon feeding, the visceral branches of the vagus nerve are activated by a variety of neuroendocrine cues, including cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1), that are produced by enteroendocrine cells of the gut (Dockray, 2009; Krieger et al., 2015). Upon food deprivation, grehlin, orexin-a, and reduced CCK levels can reduce firing and retrograde signaling by the vagus nerve (Dockray, 2009). Vagus nerve stimulation leads to increased glymphatic influx (Cheng et al., 2020). Feeding may inherently increase glymphatic function to promote the intracerebral circulation of hypothalamic satiety cues. The gut peptide GLP-1 has also gained significant attention in neurodegeneration research (Grieco et al., 2019), and it is a matter of interest that treatment with a GLP-1 receptor agonist reduced CSF production (Botfield et al., 2017). Similarly, fibroblast growth factor 21 (FGF21) is produced by the liver (von Holstein-Rathlou and Gillum, 2019) and, when injected into the CSF, can inhibit sugar and alcohol appetite (von Holstein-Rathlou et al., 2016) via activation of the paraventricular nucleus and regulation of the hypothalamic–pituitary–thyroid axis (Yilmaz et al., 2018).

Vagal nerve stimulation, GLP-1 signaling, and CSF-derived FGF21 regulation of the hypothalamic–pituitary–thyroid access provide additional putative links among gut, brain, and glymphatic function extending beyond traditional cues such as insulin, leptin, and glucagon signaling.

When discussing the gut–brain axis, it would be impossible not to mention the microbiome, which is the host of bacteria present within the digestive tract that is essential not only for digestion, but can also impact neuroendocrine and inflammatory responses in the gut. The microbiome has been implicated in almost every disease of the nervous system, although the mechanisms of action are relatively unclear (Martin et al., 2018). There is some evidence that APOE isoform influences microbiota composition (Parikh et al., 2020). The microbiota can regulate factors such as GLP-1, thus ultimately changing vagus nerve activity (Everard and Cani, 2014; Martin et al., 2018). Finally, microbiota composition can somehow alter tight junctions at the BBB

(Braniste et al., 2014), potentially dysregulating the composition of CSF/ISF that the choroid plexus normally maintains. Presently available evidence makes it tempting to suggest a link between the microbiome and glymphatic function, though we cannot yet propose a pathway other than via vagal retrograde signaling or BBB–glymphatic interactions.

Immune surveillance between the brain and the CNS

A primary feature of the traditional lymphatic system is to monitor the state of tissue inflammation. It is thus no surprise that immune surveillance is at play at every point of the pathway for CSF production and movement (Fig. 4). The choroid plexus is host to numerous leukocyte immune cells residing in the space between the leaky vessels and the ependymal cells, which provide continuous immune surveillance and enable macrophage invasion to the CSF after brain tissue damage (Shechter et al., 2013; Schwartz and Baruch, 2014). Perivascular macrophages associated with large brain vessels clear cellular debris and monitor CSF (Faraco et al., 2017). In the interstitial space, microglia and astrocytes clear debris locally, and can secrete chemokines and cytokines to the ISF/CSF (Pranzatelli, 2018; Afridi et al., 2020). Ultimately, CSF drains to the cervical lymphatic vessels and nodes (Kida et al., 1993; Louveau et al., 2018; Da Mesquita et al., 2018a; Ahn et al., 2019), which modulate the recruitment of immune cells from the blood (Breslin et al., 2018). In addition to blood-derived immune cells, the meninges contain a pool of myeloid immune cells from the bone marrow in the skull and vertebra (Cugurra et al., 2021), and this new discovery may alter our understanding of immune cell recruitment to perivascular spaces during CNS repair.

Though immune cross talk between the brain and periphery is anatomically possible, it remains completely unexplored whether/how glymphatic function may change systemic immune responses. Immune challenges, such as acute lipopolysaccharide injections, impairs glymphatic function (Manouchehrian et al., 2021). There is evidence that CSF distribution between the lymph nodes and brain is dependent on AQP4 and is controlled by circadian timing (Hablitz et al., 2020). Perhaps such a pathway for increasing CSF delivery directly to the lymph nodes is the key to mobilization of immune responses during the day (Haspel et al., 2020). Additionally, we note that AQP4 knock-out mice have reduced neuroimmune pathology in models of meningitis and experimental autoimmune encephalomyelitis (Papadopoulos and Verkman, 2005; Li et al., 2009). Glymphatic–lymphatic cross talk may also occur in the dural sinuses, where brain-derived antigens accumulate in the dural sinuses, are captured by antigen precursor cells, and trigger a local immune response (Rustenhoven et al., 2021). Recent work in meningeal lymphatic biology has demonstrated that γδ17 T cells can regulate anxiety-like behavior, while $CD4^+$ T-cell signaling alters learning (Radjavi et al., 2014; Alves de Lima et al., 2020), suggesting that cross talk among the lymphatic system, the glymphatic system, and the brain as a whole may do more than simply protect against disease, but may also regulate fundamental animal behaviors.

Closing remarks

We have shown how the glymphatic system is the lymphatic analog of the brain, with a central role in regulating directional interstitial fluid movement, waste clearance, and potentially brain immunity. Astrocytes and blood vessels determine the shape of the PVS, ultimately controlling the movement of perivascular fluid. Glymphatic fluid movement has the potential to alter local (within a brain region) as well as global (brain-wide) transport of signaling molecules as well as metabolites known to be implicated in homeostasis and specific behaviors. There is a potential for cross talk among the glymphatic system, cardiovascular system, gastrointestinal tract, and the lymphatic system. Much remains to be studied, but we are of the opinion that the glymphatic/lymphatic system acts as a cornerstone in the architecture of the brain and body signaling.

To harness therapeutically the glymphatic system to reduce disease burden, we must first better understand the full range of biological functions of CSF flow. Specifically, future work should focus on how to manipulate the availability of CSF to supply the glymphatic system, and to elucidate the underlying physiological mechanisms that redistribute CSF between the brain and body.