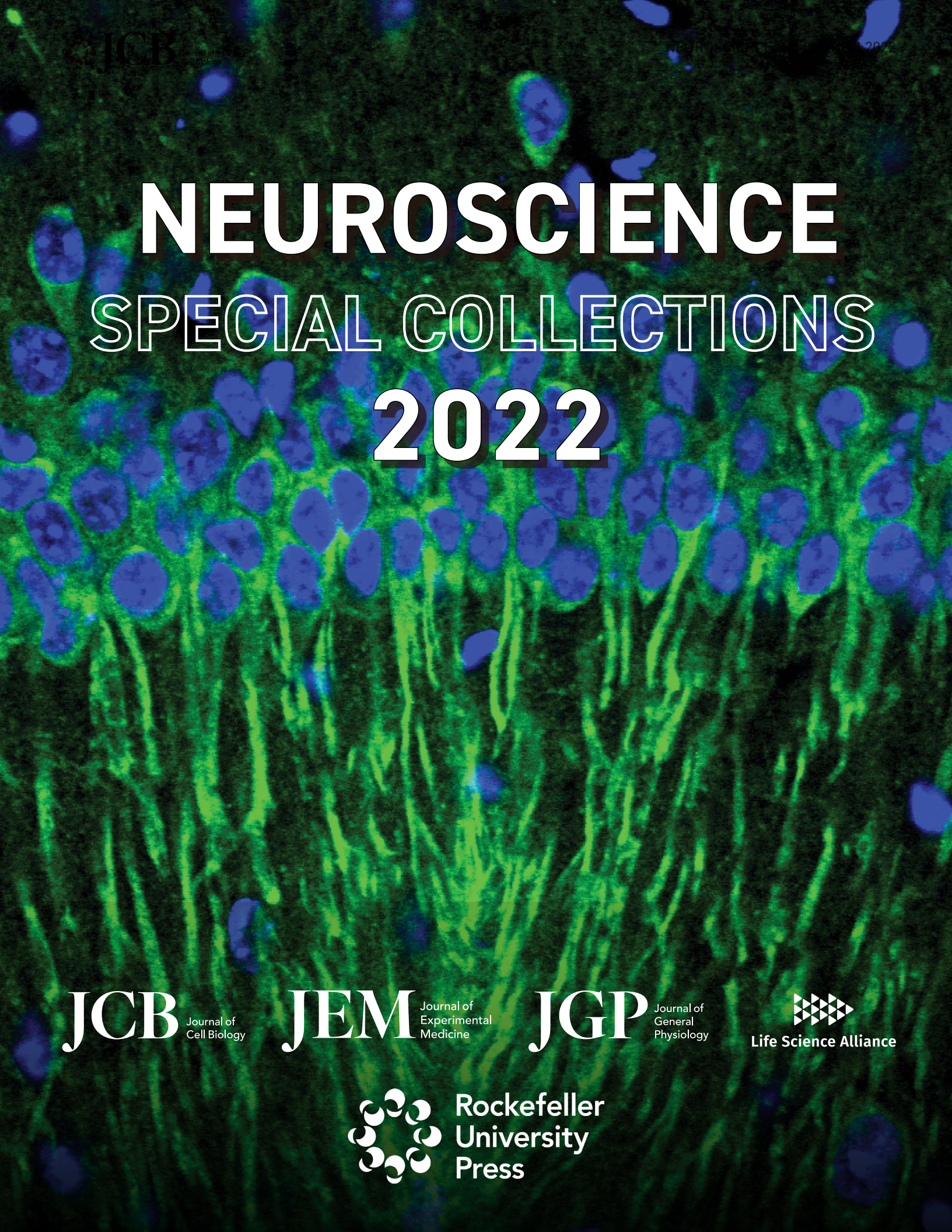
NEUROSCIENCE
SPECIAL COLLECTIONS
2022
JCB Journal of Cell Biology
JEM Journal of Experimental Medicine
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Rockefeller University Press

JCB Journal of Cell Biology

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Brochure articles by Ben Short, PhD

Design by Yuko Tonohira

On the cover: Confocal image of 7-mo-old PS19 hippocampus stained for phosto-tau (pS199/202 tau, green).

Rockefeller University Press

NEUROSCIENCE SPECIAL COLLECTIONS 2022

The editors of the *Journal of Cell Biology* (*JCB*), *Journal of Experimental Medicine* (*JEM*), *Journal of General Physiology* (*JGP*), and *Life Science Alliance* (*LSA*) are pleased to present special collections of recently published articles that elucidate new advances within the field of neuroscience. If you enjoy these highlights, we encourage you to scan the QR codes that follow to view the full collections online.

A CRYO-ET SURVEY OF THE MAMMALIAN AXOPLASM

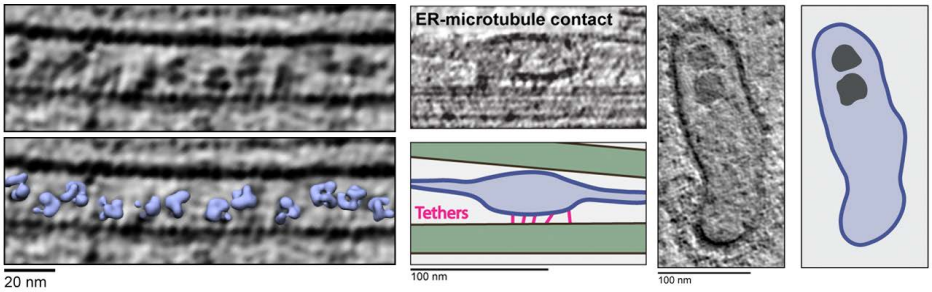
An ultrastructural study reveals surprising new features of the cytoskeleton and intracellular compartments in mouse sensory axons.

Neuronal axons are packed with cytoskeletal filaments, as well as organelles and protein assemblies that are being transported to the synaptic terminals or recycled back to the neuronal cell body. These intracellular structures have been closely studied for many decades, but recent developments in cryo-electron tomography (cryo-ET) are enabling researchers to examine cellular ultrastructure in more detail than ever before.

"Cryo-ET can provide subnanometer-resolution structural information in situ," explains Helen Foster, a former PhD student in Andrew Carter's group at the MRC Laboratory of Molecular Biology, Cambridge, UK. "Previous descriptions of axonal ultrastructure using cryo-ET mainly focused on synaptic regions. In our study, we characterized the axon shaft."

Foster, together with fellow PhD student Camilla Ventura Santos, cultured mouse dorsal root ganglion sensory neurons on EM grids and acquired tomograms of their axonal processes. The researchers observed numerous cytoskeletal filaments that all ran roughly parallel with each other along the axon shaft. These filaments included microtubules, actin, and intermediate filaments, but Foster and colleagues also saw a fourth class of filaments, thinner and shorter than the others, whose identity remains to be determined.

As expected, the vast majority of microtubules showed the same polarity as each other, with their stable minus ends oriented toward the cell body and their more dynamic plus ends pointed to the axon terminals. Surprisingly, however, Foster and colleagues found that the minus and plus ends of axonal microtubules have similar morphologies to each other. The g-tubulin ring complex, which nucleates axonal microtubules, does not appear to remain bound to the minus ends.



Among other new features, cryo-ET reveals ring-like MIPs in the lumen of axonal microtubules (left), tethers connecting the smooth ER to microtubules (center), and the presence of granules within membrane-bound vesicles (right). © 2021 MRC Laboratory of Molecular Biology

The researchers saw numerous globular particles, known as microtubule inner proteins (MIPs), in the lumen of axonal microtubules. These particles, which are thought to promote microtubule stability, had a defined, ring-like structure, even though they likely contain a disordered protein called MAP6.

The smooth ER forms a network of tubules throughout the axoplasm to facilitate lipid metabolism and calcium homeostasis. Foster and colleagues found that the ER tubules are connected to axonal microtubules by multiple, short tethers of unknown identity.

The researchers saw many other types of membrane-bound organelles associated with microtubules. Some of these organelles, which are transported along the microtubules by molecular motors, contained unexpected content in their lumens, such as granules and membrane sheets. Finally, Foster and colleagues also saw non-membrane-bound compartments, including known large ribonucleoprotein complexes called vaults and virus-like capsid particles of unknown identity and function.

"Our work demonstrates that cryo-ET can uncover new features even in a well-studied system like neuronal axons," says Carter. "It also presents the challenge of identifying the components that make up these novel structures."

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ORIGINAL PAPER

Foster, H.E., C. Ventura Santos, and A.P. Carter. 2022. A cryo-ET survey of microtubules and intracellular compartments in mammalian axons. *J. Cell Biol.* 221 (2): e202103154.

https://doi.org/10.1083/jcb.202103154

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JCB Journal of Cell Biology

NEURONAL STIMULATION REGULATES THE NUCLEAR PROTEOME

Study shows that neuronal activity triggers degradation of the tumor suppressor PDCD4, altering the expression of genes involved in synapse formation and remodeling.

Activity-dependent changes in gene expression are crucial for the formation and plasticity of neuronal circuits. Researchers have identified many of the transcriptional regulators responsible for these changes, along with the genes that they control. But relatively little is known about the mechanisms that link neuronal stimulation at synapses to transcriptional changes within the nucleus.

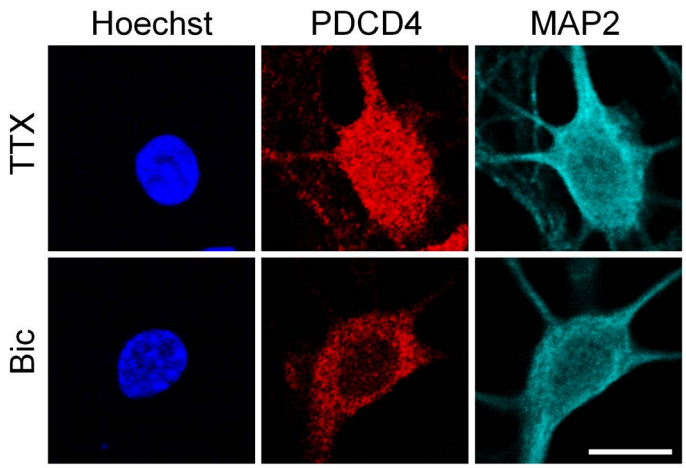
"To better understand how neuronal activity is coupled with changes in transcription, we developed an assay to systematically identify activity-dependent changes in the nuclear proteome of neurons," explains Kelsey Martin, of the University of California, Los Angeles.

Martin and colleagues, including project scientist Jennifer Achiro and graduate student Wendy Herbst, employed a proximity ligation method, in which a nuclear-localized version of the peroxidase APEX2 is expressed in rat forebrain neurons and allowed to biotinylate nuclear proteins following a period of either neuronal silencing or stimulation. The researchers then purified biotinylated proteins and analyzed them by mass spectrometry to examine how neuronal activity alters the protein composition of the nucleus.

Using this approach, Martin and colleagues discovered that neuronal activity dramatically decreases the nuclear abundance of a protein called PDCD4, a tumor suppressor previously shown to repress translation in the cytoplasm. Further experiments revealed that neuronal stimulation triggers the rapid, proteasome-mediated degradation of PDCD4 within the nucleus. "Our data suggests that, in response to neuronal activity, Protein Kinase C phosphorylates PDCD4 at Serine 71, which allows the ubiquitin ligase βTRCP1/2 to bind and promote PDCD4 degradation," Herbst explains.

The researchers determined that the degradation of nuclear PDCD4 enables the activity-dependent transcription of a subset of genes involved in synapse formation, remodeling, and transmission. Blocking PDCD4 degradation prevented changes in the expression of these genes in response to neuronal stimulation.

"We propose that PDCD4 has a direct role in regulating activity-dependent transcription in the nucleus, in addition to its well-characterized role as a translational repressor in the cytoplasm," Martin says. "Future investigation of the mechanisms by which PDCD4 regulates transcription of these genes will provide further insight into the understudied role of PDCD4 as a transcriptional regulator, as well as the cell biological mechanisms by which experience alters neuronal gene expression to enable the formation and function of neural circuits."



The abundance of PDCD4 (red) is reduced in the nucleus (blue) of neurons (cyan) stimulated by treatment with the GABA receptor antagonist bicuculline (bottom row), compared with neurons silenced by treatment with the sodium channel blocker tetrodotoxin (top row). © 2021 Herbst et al.

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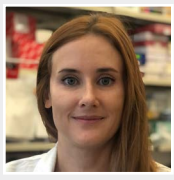

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ORIGINAL PAPER

Herbst, W.A., W. Deng, J.A. Wohlschlegel, J.M. Achiro, and K.C. Martin. 2021. Neuronal activity regulates the nuclear proteome to promote activity-dependent transcription. *J. Cell Biol.* 220 (12): e202103087.

https://doi.org/10.1083/jcb.202103087

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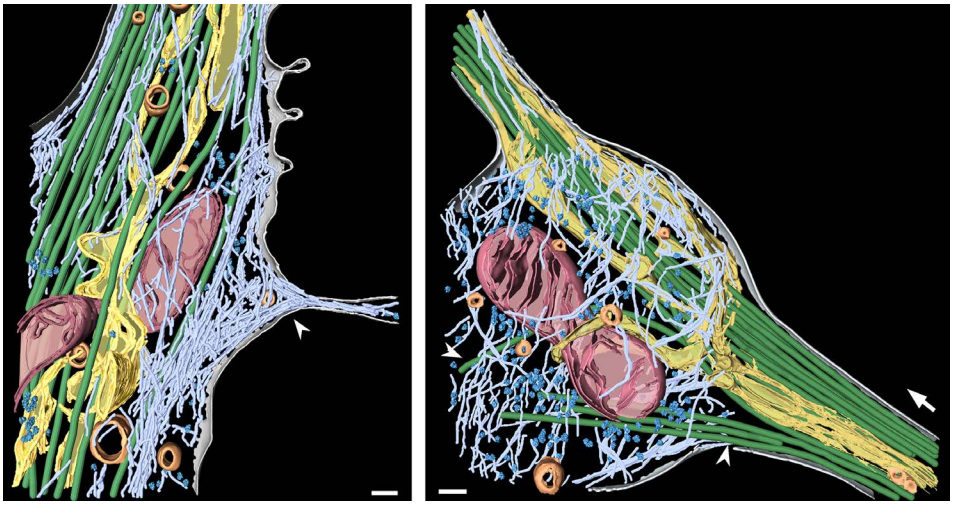
CRYO-ET REVEALS CELLULAR MACHINERY AT AXON BRANCHES

Ultrastructural analysis identifies cytoskeletal reorganization and accumulation of organelles at axonal branch points.



Cryo-ET of a premature (left) and mature (right) axon branch, showing the cellular membrane (gray), microtubules (green), actin (light blue), mitochondria (pink), ER (yellow), ribosomes (dark blue), and vesicles (orange). © 2022 Nedozralova et al.

Axon branching is a key process in the formation of complex neural circuits. The process begins with the formation of actin-rich filopodial protrusions, followed by the entry of microtubules into the maturing branches. In addition to these cytoskeletal rearrangements, the formation of new axon branches requires large amounts of proteins and lipids as well as cellular energy in the form of ATP. However, it was unclear how these cellular components were supplied for axon branching and outgrowth.

"To understand the organization of the key players for axon branching, we directly visualized the molecular organization of both premature and mature axon branching sites of mouse primary neurons by cryo-electron tomography," explains Naoko Mizuno, a senior investigator at the National Institutes of Health.

Mizuno and colleagues, including co-first authors Hana Nedozralova and Nirakar Basnet, observed a branching site as a hot spot for dynamic cellular activities with a unique organization of the cytoskeleton. At nascent branches lacking microtubules, numerous short, unaligned actin filaments accumulate at the base of the dense actin bundles that form filopodia. Mature branches, in contrast, contain loosely packed microtubules surrounded by short, unorganized actin filaments.

Compared with the tightly packed microtubule bundles that fill the axon shaft, the looser packing of microtubules at axon branch points provides space for other organelles. Mizuno and colleagues found that mitochondria, the supplier of chemical energy, accumulate at these critical sites for growth and that the ER, which likely provides the lipids required for membrane expansion at growing branches, adopts a spread-out, mesh-like morphology at axon branch points and appears to co-migrate into branches with microtubules.

Interestingly, Mizuno and colleagues discovered that ribosomes were also enriched at branch sites, which have previously been observed only in the soma and at the synapses. They were mostly arranged in polysomes as they synthesize multiple copies of the same protein. In contrast, Mizuno and colleagues rarely saw any ribosomes along the axon shaft. "Our visualization of ribosomes provides direct evidence that proteins are synthesized locally at axon branches, sites of dynamic cellular activities," says Mizuno. "Our data on the colocalization of short actin filaments and active polysomes supports the hypothesis that local actin synthesis is a crucial part of the machinery to build up filopodia, which is a central process for axon branch formation."

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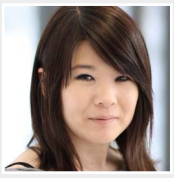

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ORIGINAL PAPER

Nedozralova, H., N. Basnet, I. Ibiricu, S. Bodakuntla, C. Biertümpfel, and N. Mizuno. 2022. In situ cryo-electron tomography reveals local cellular machineries for axon branch development. *J. Cell Biol.* 221 (4): e202106086.

https://doi.org/10.1083/jcb.202106086

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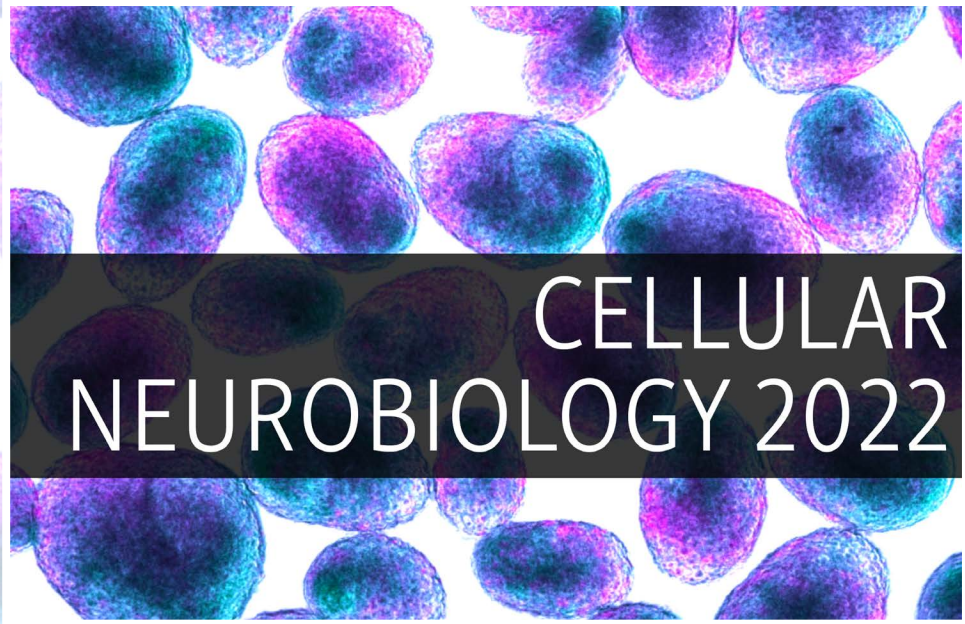

We are proud to offer a special collection of recent work on neurobiology, covering the cell biology of neurons, glia, and neuronal development and function. Work in this collection advances our understanding of axon branching, lysosome activity and regulation in neurons, and chemotherapy-induced peripheral neuropathy. Readers will also find work touching on transcriptional regulation in neurons by liquid–liquid phase separation, a novel neuron/astrocyte organoid co-culture system, and a cryo-electron tomography survey of axonal structure.

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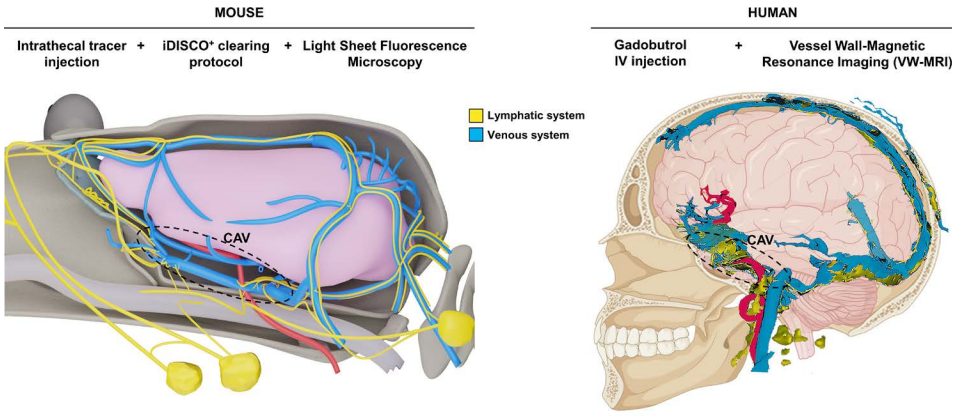
3D MAPS OF THE MENINGEAL LYMPHATIC NETWORK

Comprehensive imaging study reveals that network of lymphatic vessels draining cerebrospinal fluid from the CNS is well conserved between mice and humans.

Over the last few years, researchers have demonstrated the existence of lymphatic vessels in the dura mater, the outermost layer of the meningeal membranes that envelop the brain and spinal cord. These meningeal lymphatic vessels (MLVs) help drain cerebrospinal fluid (CSF) into collecting lymph nodes within the neck, aiding the clearance of toxic waste products from the central nervous system, and enabling immunosurveillance of brain tissues. MLVs may therefore influence the progression of a range of neurological disorders, including multiple sclerosis, Alzheimer's disease, and brain tumors, and are considered potential therapeutic targets for many of these pathologies.

To date, MLV drainage pathways have been identified in the dorsal and caudobasal regions of the dura mater, but whether they exist in other parts of the skull is less well established. "We wanted to investigate CSF lymphatic drainage with submillimeter resolution by large-field imaging of the whole head in both mice and humans," explains Jean-Leon Thomas, who co-led the study with Anne Eichmann.

To follow lymphatic drainage in mice, the researchers, including first author Laurent Jacob, injected a fluorescent tracer into the animals' CSF and then imaged cleared whole-head preparations using light-sheet fluorescence microscopy. Over time, the fluorescent tracer was taken up by MLVs before draining into the collecting lymph nodes of the neck. These MLVs were closely associated with the venous sinuses of the dura mater, which are key sites of neuroimmune communication. Notably, Thomas and Eichmann's teams observed an extensive network of MLVs surrounding the cavernous sinus in the anterior region of the brain, in addition to the MLVs previously characterized in the dorsal and caudobasal regions.



A schematic representation of the meningeal lymphatic networks (yellow) in mice and humans, showing their close association with dural venous sinuses (blue), including the cavernous sinus (CAV). © 2022 Jacob et al.

The researchers saw a highly similar pattern of MLVs in humans, too. Using a novel real-time vessel-wall magnetic resonance imaging protocol, the teams generated 3D maps of the blood and lymphatic vasculature in the meninges of 11 neurology patients systemically injected with the contrast agent gadobutrol. "The procedure allows quantitative mapping of human intracranial MLVs and may be relevant for diagnostic imaging of patients with CSF drainage defects and neurological diseases," Eichmann says.

One patient with a rare disorder known as Gorham-Stout disease showed a dramatic overgrowth of MLVs. No obvious abnormalities were observed in the other 10 patients, but, intriguingly, female patients had a reduced MLV volume compared with male patients. Though the sample number is small, the researchers speculate that this could have implications for the increased prevalence of diseases such as multiple sclerosis and idiopathic intracranial hypertension in females.

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ORIGINAL PAPER

Jacob, L., J. de Brito Neto, S. Lenck, C. Corcy, F. Benbelkacem, L.H. Geraldo, Y. Xu, J.-M. Thomas, M.-R. El Kamouh, M. Spajer, M.-C. Potier, S. Haik, M. Kalamarides, B. Stankoff, S. Lehericy, A. Eichmann, and J.-L. Thomas. 2022. Conserved meningeal lymphatic drainage circuits in mice and humans. *J. Exp. Med.* 219 (8): e20220035.

https://doi.org/10.1084/jem.20220035

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JEM Journal of Experimental Medicine

BOOSTING NEUROGENESIS RESCUES MEMORY DEFECTS IN MICE WITH ALZHEIMER'S DISEASE BY RESTORING THE ENGRAM

Study shows that new neurons can incorporate into memory-storing neural circuits and restore their normal function.

New neurons are produced from neural stem cells via a process known as neurogenesis. Previous studies have shown that neurogenesis is impaired in both Alzheimer's disease (AD) patients and laboratory mice carrying genetic mutations linked to AD, particularly in the hippocampus, a region of the brain that is crucial for memory acquisition and retrieval.

"However, whether defects in neurogenesis contribute to the cognitive impairments associated with AD is unclear," explains Professor Orly Lazarov of the Department of Anatomy and Cell Biology in the University of Illinois Chicago College of Medicine.

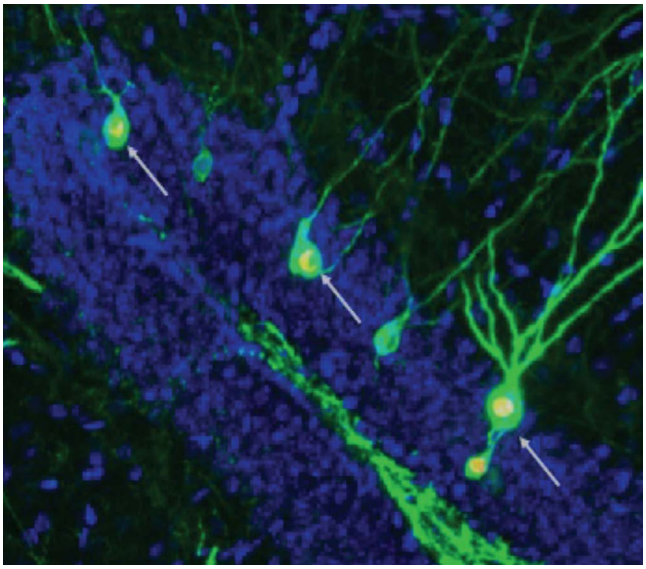
Lazarov and colleagues, including co-first authors Rachana Mishra, Trongha Phan, Pavan Kumar, and Zachery Morrissey, boosted neurogenesis in AD model mice by genetically enhancing the survival of neuronal stem cells. The researchers deleted *Bax*, a gene that plays a major role in neuronal stem cell death, ultimately leading to the maturation of more new neurons. Increasing the production of new neurons in this way restored the animals' performance in two different tests measuring spatial recognition and contextual memory.

By fluorescently labeling neurons activated during memory acquisition and retrieval, the researchers determined that, in the brains of healthy mice, neural circuits involved in storing memories include many newly formed neurons alongside older, more mature neurons. These memory-storing circuits contain fewer new neurons in AD mice, but the integration of newly formed neurons was restored when neurogenesis was enhanced.

Further analyses of the neurons forming the memory-storing circuits revealed that boosting neurogenesis also increases the number of dendritic spines, which are structures in synapses known to be critical for memory formation, and restores a normal pattern of neuronal gene expression.

Lazarov and colleagues confirmed the importance of newly formed neurons for memory formation by specifically inactivating them in the brains of AD mice. This reversed the benefits of boosting neurogenesis, preventing any improvement in the animals' memory.

"Our study is the first to show that impairments in hippocampal neurogenesis play a role in the memory deficits associated with AD by decreasing the availability of immature neurons for memory formation," Lazarov says. "Taken together, our results suggest that augmenting neurogenesis may be of therapeutic value in AD patients."



In the hippocampus of mice with AD, boosting neurogenesis increases the number of newly formed neurons involved in storing and retrieving memories (arrows).

RESEARCHER DETAILS

{Left to right: Orly Lazarov, Trongha Phan, Zach Morrissey, Pavan Kumar}

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Mishra, R., T. Phan, P. Kumar, Z. Morrissey, M. Gupta, C. Hollands, A. Shetti, K.L. Lopez, M. Maienschein-Cline, H. Suh, R. Hen, and O. Lazarov. 2022. Augmenting neurogenesis rescues memory impairments in Alzheimer's disease by restoring the memory-storing neurons. *J. Exp. Med.* 219 (9): e20220391.

https://doi.org/10.1084/jem.20220391

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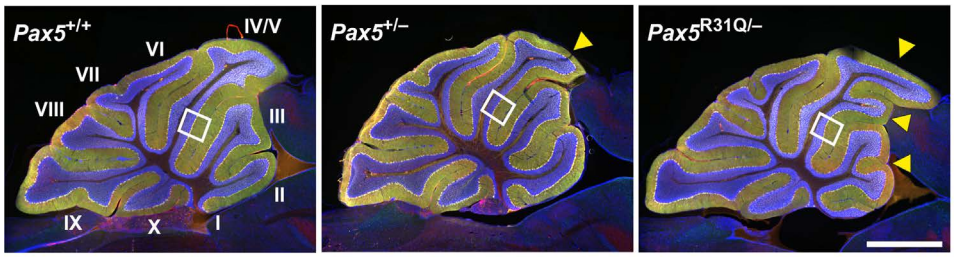
PAX5 DEFICIENCY CAUSES AUTISM SPECTRUM DISORDER AND HYPOGAMMAGLOBULINEMIA

Study identifies patient with biallelic mutations in the PAX5 gene that block B cell development and cause a range of neurodevelopmental deficits.

The transcription factor PAX5 is a critical regulator of B cell development and mature B cell function. It is also expressed in the developing brain—particularly in a region called the isthmic organizer that is located at the midbrain–hindbrain boundary—and has been flagged as a candidate risk gene for autism spectrum disorder (ASD).

Fabian Kaiser and colleagues at Erasmus University Medical Center (Erasmus MC) in Rotterdam identified a pediatric patient with recurrent infections and low serum antibody levels who was also diagnosed with ASD combined with sensorimotor and cognitive deficits. Whole-exome sequencing revealed that the patient had biallelic mutations in the *PAX5* gene: one allele contained a hypomorphic mutation (R31Q) of a conserved arginine residue in the transcription factor's DNA-binding domain, while the other allele contained a premature stop codon (E242*) that likely leads to nonsense-mediated mRNA decay.

Kaiser worked with Aleksandra Badura's group at Erasmus MC and Meinrad Busslinger's laboratory at the Research Institute of Molecular Pathology in Vienna to explore how these *PAX5* mutations might cause the patient's B cell and neurodevelopmental defects. The researchers generated mice carrying both the R31Q and E242* mutations, and found that the animals showed remarkably similar phenotypes to the original patient.



Yellow arrowheads indicate the abnormal folding of the cerebellar vermis in *Pax5*$^{+/-}$ (center) and *Pax5*$^{R31Q/-}$ (right) mice, compared with wild-type (*Pax5*$^{+/+}$) controls (left). © 2022 Kaiser et al.

For example, the mice lacked mature B cells due to a partial developmental arrest in the transition from pro-B to pre-B cells, and those B cells that did manage to mature showed a reduced response to immunization. ChIP- and RNA-sequencing revealed that the R31Q mutation disrupts Pax5's ability to bind and regulate a subset of its usual targets, dysregulating their expression and impairing B cell development.

Pax5 mutant mice also displayed neurological phenotypes similar to those seen in the pediatric patient, including hypersociability, hyperactivity, and repetitive behaviors, as well as motor control and cognitive impairments.

"We also observed the same neuroanatomic defects in both the patient and mouse model," explains Kaiser. *PAX5* mutations caused abnormal folding of the cerebellum, as well as hypoplasia of two midbrain regions, the substantia nigra and ventral tegmental area, that control motor function and reward processing, respectively. This hypoplasia was associated with a loss of inhibitory GABAergic neurons.

Lineage tracing experiments revealed that Pax5 is normally expressed in the progenitors of both cerebellar cells and GABAergic midbrain neurons. "Taken together, our results support an important developmental role of Pax5 in midbrain and cerebellar morphogenesis, including the differentiation of midbrain GABAergic neurons, and thus provide a mechanistic basis for understanding why the loss of Pax5 leads to neurodevelopmental abnormalities in mice and humans," Busslinger says.

"Disruption of the excitatory/inhibitory balance has become a dominant theory concerning the pathogenesis of ASD," adds Badura. "While most studies have focused on the neocortex and hippocampus, our data imply that the inhibitory circuitry of the midbrain also needs to be addressed in future studies."

RESEARCHER DETAILS

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https://doi.org/10.1084/jem.20220498

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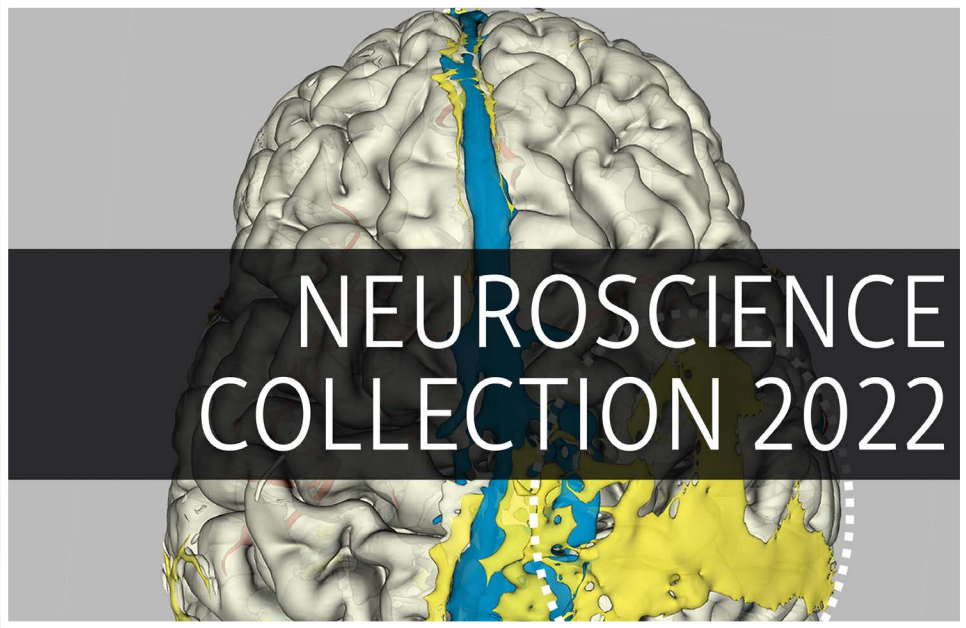

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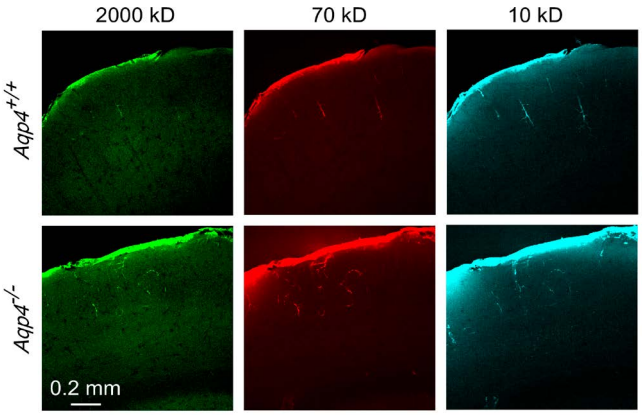
AQUAPORIN-4-INDEPENDENT SOLUTE UPTAKE IN THE BRAIN

Researchers develop new method to better address the controversial role of astrocyte water channels in solute exchange between the cerebrospinal fluid and interstitial space.



Fluorescent dextrans of different molecular weights show similar degrees of uptake into the brains of wild-type (top) and AQP4 knockout (bottom) mice, after direct surface application under the same, constant pressure.

The exchange of solutes between the cerebrospinal fluid (CSF) and brain interstitial fluid via the perivascular spaces surrounding blood vessels is vital for maintaining tissue homeostasis and removing waste products from the brain. Almost 10 years ago, a "glymphatic" system was hypothesized to induce a "cleansing flow" of fluid and solutes through the brain interstitium from periarterial to perivenular spaces. This directional flow was proposed to be driven by aquaporin-4 (AQP4) water channels present in the endfeet of astrocytes that encircle the outer surface of the brain's blood vessels.

However, the existence of the glymphatic system, and the role of AQP4 in solute transport, has been highly controversial. "Experimental studies investigating the contribution of AQP4 to solute exchange between the CSF and brain interstitium following solute injection into the cisterna magna of mice have generated results that diverge widely in both the basal extent of solute uptake and the effect of AQP4 deletion," explains Alex Smith of the University of California, San Francisco.

Smith and colleagues determined that at least some of this experimental variability is due to the fact that the transport of solutes from the CSF to the brain interstitium is highly sensitive to injection conditions. Compared with slow, low-volume injections into the cisterna magna, rapid, high-volume injections enhanced solute uptake into the brain, causing increased dispersal of solutes within the subarachnoid space and greater entry into perivascular compartments. Notably, rapid, high-volume injections elevated intracranial pressure, which could increase solute uptake in a number of ways.

"To avoid these confounding sources of variability in cisternal injection experiments, we developed a constant pressure and concentration approach to investigate solute transfer from CSF to the interstitial compartment in mice," Smith says. After opening up the skull and removing a small area of the dura mater, fluorescent solutes dissolved in a small volume of artificial CSF can be applied directly to the surface of the brain at a controlled, constant pressure, leading to their transport into perivascular compartments and brain interstitium.

Using this approach, Smith and colleagues confirmed that higher pressures increase the uptake of solutes into perivascular compartments. However, when the researchers applied solutes directly to the brains of wild type and AQP4-knockout mice under the same pressure conditions, they saw no differences in the uptake of solutes into the perivascular or interstitial spaces.

"Taken together, our results offer an explanation for the variability in cisternal injection studies and demonstrate that AQP4 is not required for solute transfer from the CSF to the interstitial space in mouse brain," Smith says.

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ORIGINAL PAPER

Smith, A.J., G. Akdemir, M. Wadhwa, D. Song, and A.S. Verkman. 2021. Application of fluorescent dextrans to the brain surface under constant pressure reveals AQP4-independent solute uptake. *J. Gen. Physiol.* 153 (8): e202112898.

https://doi.org/10.1085/jgp.202112898

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JGP Journal of General Physiology

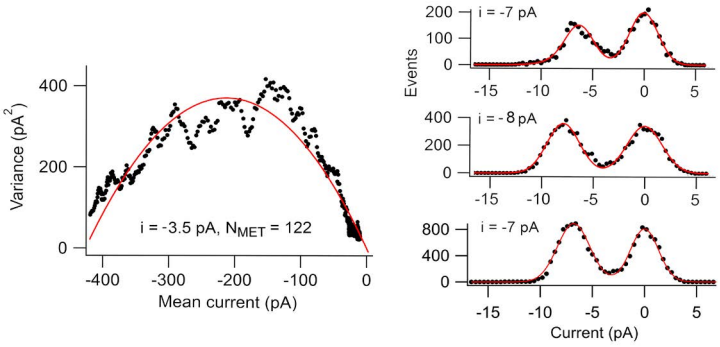
FLUCTUATION ANALYSIS REVEALS FAST ACTIVATION OF HAIR CELL MECHANOTRANSDUCER CHANNELS

Analyzing current fluctuations in outer hair cells underestimates the conductance of mechanoelectrical transducer channels, but can provide an estimate of the speed of channel gating.

Hair cells in the cochlea convert sound vibrations into electrical analog signals. The initial step in this process is the sound-induced movement of stereocilia on the hair cell surface, which activates mechanoelectrical transducer (MET) channels in the stereociliary membrane. Though MET channels are thought to be composed of the ion channel TMC1, the molecular structure of the channels, and many of their key properties, remains unclear.

This is due, in part, to the difficulties involved in measuring the properties of single MET channels. One commonly used method requires treating hair cells with a calcium buffer to disrupt most of the tip links that connect neighboring stereocilia, reducing MET channel activation so that the conductance of individual channels can be recorded. A second method infers single-channel conductance by analyzing fluctuations in the macroscopic current. "We compared the two methods in order to assess the error introduced by the filtering out of rapid current transients during the fluctuation analysis," explains Robert Fettiplace of the University of Wisconsin School of Medicine and Public Health.

Fettiplace and colleagues, including first author Maryline Beurg, used the two methods to determine the conductance of MET channels in mouse apical outer hair cells, sometimes applying both approaches to the very same cell. The researchers found that fluctuation analysis provides conductance values half the size of those determined by recording single channel events after tip link destruction.



Fluctuation analysis of the current variation in an apical outer hair cell (left) gives rise to an estimated single-channel current of −3.5 pA. Recordings of single-channel events in the same cell after tip link destruction (right) show an average current amplitude of −7.3 pA. © 2021 Beurg et al.

Fluctuation analysis may underestimate channel conductance if channel gating is so fast that instances of channel opening are filtered out during the analysis. Indeed, mouse MET channels are expected to have extremely rapid activation kinetics so that they can encode sound frequencies of up to 70 kHz.

To test this possibility, Fettiplace and Beurg collaborated with Jong-Hoon Nam at the University of Rochester to simulate the gating of MET channels with different activation time constants and perform fluctuation analyses on the output using various filter cutoffs. Their modeling showed that rapid channel activation does cause fluctuation analysis to underestimate MET channel conductance, and, by comparing their simulated and experimental data, the researchers were able to estimate that the MET channels in apical outer hair cells have an activation time constant of 10 µs.

"This activation rate is faster than any other known channel," says Fettiplace. "However, our experiments were performed at room temperature and it will be even faster at mouse body temperature." The researchers estimate that the activation time constant of MET channels would be ~3 µs at 37°C, sufficiently high to enable the faithful encoding of high frequency sounds at the edge of the murine auditory range.

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ORIGINAL PAPER

Beurg, M., J.-H. Nam, and R. Fettiplace. 2021. The speed of the hair cell mechanotransducer channel revealed by fluctuation analysis. *J. Gen. Physiol.* 153 (10): e202112959.

https://doi.org/10.1085/jgp.202112959

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GAP JUNCTIONS AND HEMICHANNELS KEEP THE RPE CONNECTED

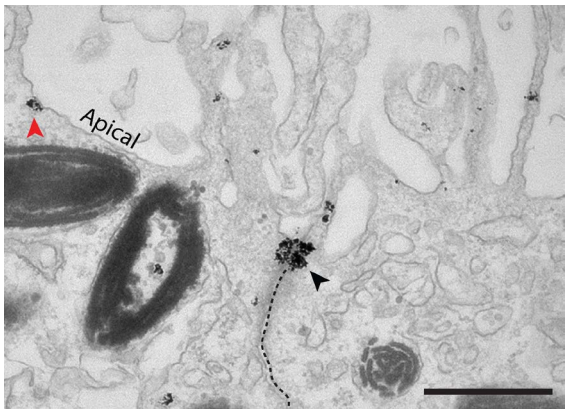
Study shows that the electrical properties of the retinal pigment epithelium are influenced by connexin-based gap junctions and hemichannels.

Gap junctions are composed of connexin proteins, which form hexameric hemichannels in the membranes of many cell types. Hemichannels in neighboring cells can align to create a gap junction that allows small molecules and ions to pass between cells. But individual hemichannels can have gap junction–independent functions as well.

Gap junctions are particularly prominent in the retinal pigment epithelium (RPE), a layer of densely pigmented cells that underlies the retina and supports the function of photoreceptors. Many of these support functions are regulated by voltage-gated ion channels, and gap junctions could potentially help synchronize the RPE's functions by facilitating electrical coupling between cells.

Electrical conductance through gap junctions is likely to lower a cell's input resistance, thereby reducing its electrical excitability. Soile Nymark and colleagues at Tampere University, Finland, conducted a study where they measured the input resistance of RPE cells in monolayer cultures derived from human embryonic stem cells, and also in intact RPE isolated from mouse eyes. In both cases, treating cells with the gap junction blocking agent meclofenamic acid (MFA) dramatically increased input resistance, and this effect was reversed when the drug was washed out.

The researchers, including first author Julia Fadjukov, then switched to a dual patch clamp configuration to measure the extent of electrical coupling between cells. Neighboring RPE cells were, indeed, highly coupled and this connectivity was abolished by the addition of MFA. Coupling between non-adjacent cells was significantly smaller, however, suggesting that, at least under baseline conditions, the connectivity of mammalian RPE monolayers is relatively low. "However, we think this can be dynamically regulated to allow fast spreading of ions and other signaling molecules across the epithelium," Nymark says.



Immuno-EM of human embryonic stem cell–derived RPE shows the presence of connexin 43 in both gap junctions (black arrowhead) and apical hemichannels (red arrowhead). © 2022 Fadjukov et al.

Based on their measurements, Nymark and colleagues worked with Sophia Wienbar and Gregory Schwartz at Northwestern University to construct a computational model of an RPE cell network. The model revealed that inhibiting gap junctions cannot be the only mechanism by which MFA increases input resistance. Indeed, MFA also blocks individual hemichannels, and Fadjukov et al. determined that there are functional hemichannels in the apical membrane of RPE cells.

To test whether these hemichannels contribute to input resistance, the researchers used TAT-Gap19, a specific inhibitor of hemichannels formed by connexin 43, the most prominent connexin in RPE cells. Sure enough, TAT-Gap19 treatment increased input resistance, indicating that hemichannels, as well as gap junctions, influence the electrical properties of the RPE.

Nymark and colleagues now want to investigate whether the connectivity provided by gap junctions helps the physiological processes in the RPE that require precise synchronization, as well as the intriguing possibility that the apical hemichannels facilitate signaling between the RPE and the retina.

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ORIGINAL PAPER

Fadjukov, J., S. Wienbar, S. Hakanen, V. Aho, M. Vihinen-Ranta, T.O. Ihalainen, G.W. Schwartz, and S. Nymark. 2022. Gap junctions and connexin hemichannels both contribute to the electrical properties of retinal pigment epithelium. *J. Gen. Physiol.* 154 (4): e202112916.

https://doi.org/10.1085/jgp.202112916

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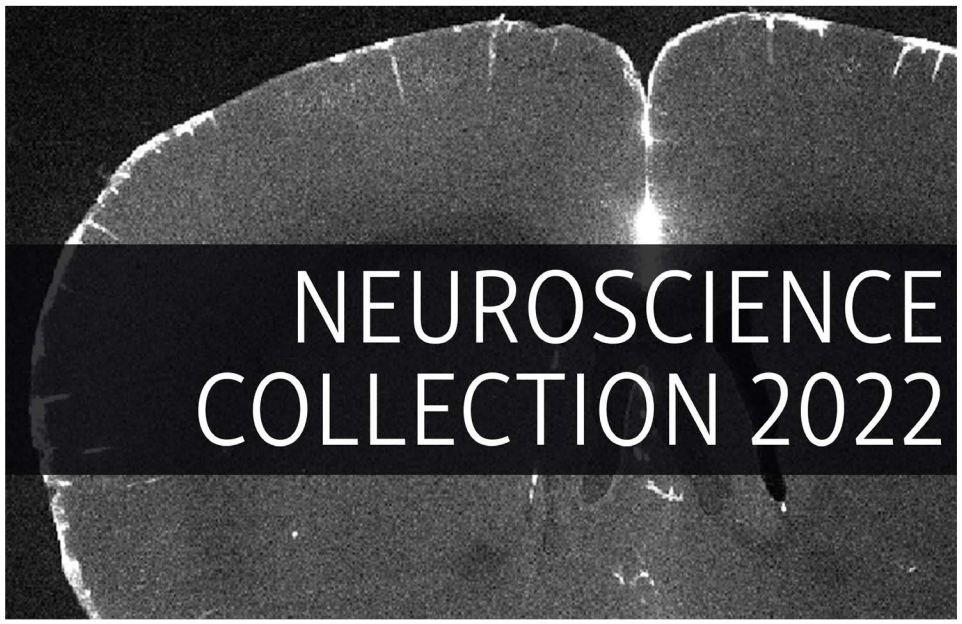

We hope you enjoyed these excerpts, and we invite you to explore the complete collection at the *JGP* website. Subjects range from the action of sea snail toxins on AMPA receptors to the role of gap junctions and connexin hemichannels in retinal pigment epithelium. The collection features studies that analyze mechanotransduction in cochlear outer hair cells, model the relationship between dendritic spine morphology and synaptic strength, and link Huntington's disease to altered T-tubule structure in skeletal muscle. It also highlights new methods to monitor solute uptake from the cerebrospinal fluid and study conformational changes in endogenous voltage-gated ion channels. The collection has been curated to showcase the diversity of topics and the quality of research published in *JGP*.

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TOXIC PEPTIDES SPREAD FROM ASTROCYTES TO MOTOR NEURONS

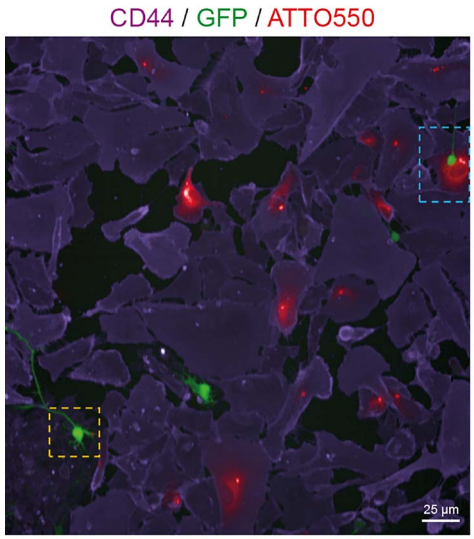
Astrocytes can take up dipeptide repeat proteins linked to neurodegenerative disease and transmit them to motor neurons, where they may impair lysosomal function.

Expansion of a hexanucleotide repeat in the first intron of the *C9ORF72* gene is the main genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Unconventional translation of this repeat results in the production of a number of toxic dipeptide repeat (DPR) proteins, which are thought to contribute to pathogenesis in a variety of ways.

Poly-GA DPRs are the most abundant of these toxic protein species. "Numerous cell culture and in vitro studies have suggested that aggregates of poly-GA DPRs induce proteasome impairment, DNA damage, cognitive disability, motor deficits, pro-inflammatory responses, and neurodegeneration," says Mimoun Azzouz of the University of Sheffield.

To learn more about the role of poly-GA DPRs in disease spread, Azzouz and colleagues, including first author Paolo Marchi and co-corresponding author Ronald Melki, expressed the proteins in *E. coli* and analyzed their aggregation in vitro. Poly-GA DPRs oligomerized in a time- and concentration-dependent manner, forming compact, solid-like aggregates (in contrast to another ALS-linked DPR, poly-PA, which formed spherical, liquid-like droplets). After 15 days, poly-GA oligomers developed into β-sheet-containing fibrillar structures.

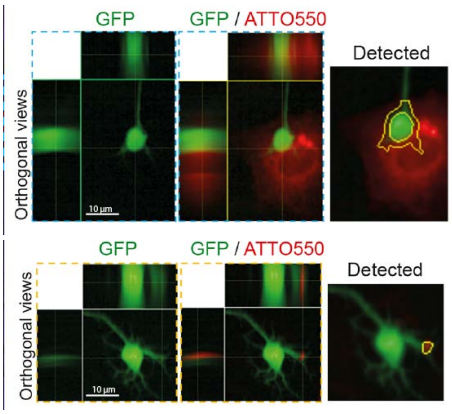
The researchers found that poly-GA



oligomers and fibrils are taken up by a variety of cell types in culture, including human astrocytes derived from induced neural progenitor cells. Oligomers are taken up efficiently via a dynamin-independent mode of endocytosis. The internalization of fibrils, in contrast, depended upon dynamin-mediated endocytosis.

In cortical neurons, internalized poly-GA oligomers and fibrils accumulate in lysosomes within large axonal swellings. Poly-GA-containing lysosomes are enlarged and show decreased motility, raising the possibility that poly-GA aggregates impair lysosomal function.

DPRs, including poly-GA, are thought



Confocal imaging of a co-culture experiment shows that astrocytes (violet) take up poly-GA aggregates (red) and transmit them to motor neurons (green). © 2022 Marchi et al.

to spread through the brain via cell-to-cell transmission; the role of astrocytes and other glial cells in disease propagation is unclear. In a series of co-culture experiments, Azzouz and colleagues determined that astrocytes can take up poly-GA aggregates and transmit them to motor neurons.

"Taken together, our results shed light on the mechanisms of poly-GA aggregation, cellular uptake, and cell-to-cell propagation, as well as suggesting lysosomal impairment as a possible feature underlying the cellular pathogenicity of these DPR species," Azzouz says.

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ORIGINAL PAPER

Marchi, P.M., L. Marrone, L. Brasseur, A. Coens, C.P. Webster, L. Bousset, M. Destro, E.F. Smith, C.G. Walther, V. Alfred, R. Marroccella, E.J. Graves, D. Robinson, A.C. Shaw, L.M. Wan, A.J. Grierson, S.J. Ebbens, K.J. De Vos, G.M. Hautbergue, L. Ferraiuolo, R. Melki, and M. Azzouz. 2022. C9ORF72-derived poly-GA DPRs undergo endocytic uptake in iAstrocytes and spread to motor neurons. *Life Science Alliance.* 5 (9): e202101276.

https://doi.org/10.26508/lsa.202101276

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ASSESSING THE SAFETY AND STABILITY OF COCHLEAR OPTOGENETICS

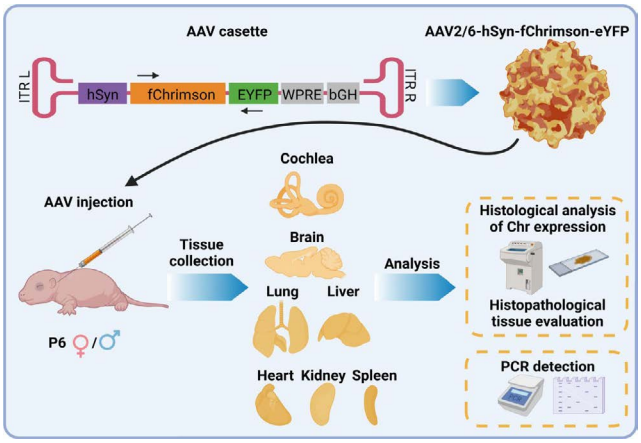
Study analyzes the long-term effects of AAV-mediated channelrhodopsin expression in mice.

Disabling hearing loss is estimated to affect ~5% of the world's population. Cochlear implants can partially restore hearing by electrically stimulating the auditory nerve, but their ability to resolve multiple sound frequencies is limited, making it difficult, for example, to understand speech in noisy environments.

Optogenetics may provide an alternative approach to hearing restoration. Adenoassociated virus (AAV)-based gene delivery could potentially be used to express light-activated bacterial channelrhodopsins in the spiral ganglion neurons (SGNs) that make up the auditory nerve. Optical cochlear implants might then be designed to stimulate these neurons in a spatiotemporally precise manner, thereby improving sound frequency resolution.

The optogenetic manipulation of SGNs using the AAV-mediated expression of channelrhodopsins has been shown to activate the auditory pathway and restore hearing responses in rodents. "However, for future clinical applications, this genetic manipulation must be safe, efficient, and stable to provide users reliable optogenetic hearing for years after gene therapy," says Tobias Moser on the Göttingen Campus.

Moser and colleagues, including co-corresponding author Vladan Rankovic and co-first authors Burak Bali and Eva Gruber-Dujardin, therefore studied the long-term effects of AAV-based delivery of channelrhodopsins into SGNs. The researchers injected the cochleae of newborn mice with AAV carrying the coding sequence for a fast gating channelrhodopsin under the control of a neuron-specific promoter and followed the animals over the course of their ~2-yr lifespan.



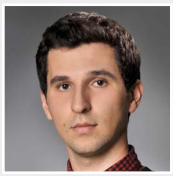
The researchers the long-term effects of injecting AAV encoding the channelrhodopsin fChrimson into the cochleae of newborn mice. © 2022 Bali et al.

Moser and colleagues determined that a large fraction of SGNs continued to express the channelrhodopsin 2 yr after gene delivery. Though the number of SGNs naturally declines with age, the researchers found that this decline is slightly accelerated in AAV-injected cochleae, particularly in the apical and middle parts of the structure. "Thus, a potential negative, long-term effect of AAV-mediated optogenetic manipulation on SGNs cannot be ruled out, and needs further investigation," Moser cautions.

To rule out potential negative effects on other cell types, Moser and colleagues examined whether AAV-mediated channelrhodopsin expression spread to other parts of the body. The researchers were unable to detect the virus in a number of peripheral organs, but AAV-mediated expression of channelrhodopsin did spread to other neurons throughout the brain after postnatal administration. However, this did not appear to drive any serious histopathological changes, such as excessive neurodegeneration or inflammation, in the brains of 2-yr-old mice.

"Nonetheless, future work employing routes of virus administration with potential of clinical translation, should aim to restrict AAV-mediated gene expression to SGNs," says Moser. "Overall, our observation of long-term and relatively stable channelrhodopsin expression with rather mild adverse effects suggests the feasibility of late preclinical studies of optogenetic hearing restoration, including in non-human primates."

RESEARCHER DETAILS



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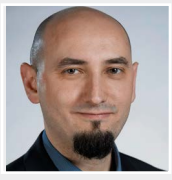

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ORIGINAL PAPER

Bali, B., E. Gruber-Dujardin, K. Kusch, V. Rankovic, and T. Moser. 2022. Analyzing efficacy, stability, and safety of AAV-mediated optogenetic hearing restoration in mice. *Life Science Alliance.* 5 (8): e202101338.
https://doi.org/10.26508/lsa.202101338

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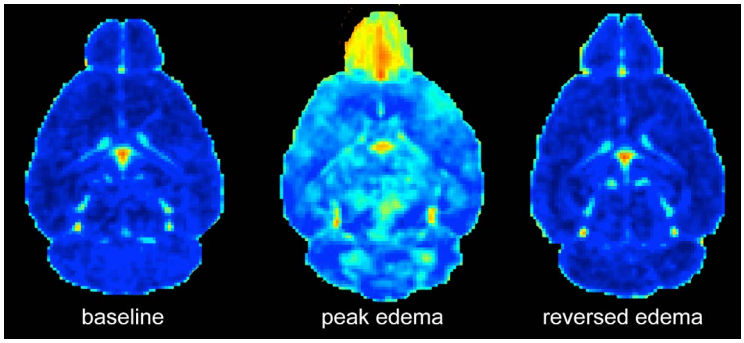
A RELIABLE MOUSE MODEL FOR REVERSIBLE CEREBRAL MALARIA

New experimental model indicates key role for transcellular blood–brain barrier disruption in malaria-induced reversible brain swelling.



MRI shows the malaria-induced development, and subsequent reversal, of edema in the brain of a mouse previously vaccinated with radiation-attenuated sporozoites. © 2022 Jin et al.

Cerebral malaria is the most severe complication of *Plasmodium falciparum* infection. Most common in children, cerebral malaria is associated with the accumulation of parasite-infected erythrocytes in the brain's microvasculature, leading to brain edema. This swelling is often fatal, but is reversible in many patients, although up to one third of these survivors suffer long-term neurocognitive impairments.

"Investigating the mechanisms underlying reversible edema is necessary to establish new therapeutic approaches and to eventually reduce permanent brain damage in cerebral malaria," explains Angelika Hoffmann of the University Hospital Bern. "However, pathophysiological studies have been hampered by the lack of animal models that reliably show reversible edema."

Hoffmann and colleagues, including first author Jessica Lin, developed a new experimental cerebral malaria model by vaccinating mice with radiation-attenuated *Plasmodium berghei* sporozoites. Unvaccinated mice generally develop fatal brain edema when infected with active sporozoites, but vaccinated animals reliably developed reversible brain edema and survived. This allowed the researchers to study the acute stage of reversible edema, as well as its after effects on the brain.

Mice with reversible edema showed significantly less blood–brain barrier disruption and brain swelling than mice with fatal cerebral malaria. When the researchers examined the disrupted vasculature by electron microscopy, they found that, in mice with reversible edema, the tight junctions between endothelial cells remain intact at the acute stage of the disease. Instead, the endothelial cells contain increased numbers of intracellular vesicles that can transport fluid from the vessel lumen to the brain parenchyma in a process known as transcellular blood–brain barrier disruption. The number of vesicles then declined after edema reversal.

After edema reversal, however, the mice continued to develop permanent brain damage in the form of microhemorrhages, particularly in the region of the brain—the olfactory bulb—that showed the highest degree of blood–brain barrier disruption during the acute stage of edema. Microhemorrhages have been linked with long-term neurological impairments in a number of diseases and Hoffmann and colleagues identified similar microvascular damage in a third of adult cerebral malaria survivors.

"Taken together, our results suggest a potential association between the degree of initial transcellular blood–brain barrier disruption, consecutive brain swelling and microhemorrhages," Hoffmann says. "This association suggests that adjuvant drug treatment targeting transcellular blood–brain barrier disruption could reduce microvascular damage and protect from long-term neurocognitive impairment."

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ORIGINAL PAPER

Jin, J., M.A. Ba, C.H. Wai, S. Mohanty, P.K. Sahu, R. Pattnaik, L. Pirpamer, M. Fischer, S. Heiland, M. Lanzer, F. Frischknecht, A.-K. Mueller, J. Pfeil, M. Majhi, M. Cyrklaff, S.C. Wassmer, M. Bendszus, and A. Hoffmann. 2022. Transcellular blood–brain barrier disruption in malaria-induced reversible brain edema. *Life Science Alliance.* 5 (6): e202201402.

https://doi.org/10.26508/lsa.202201402

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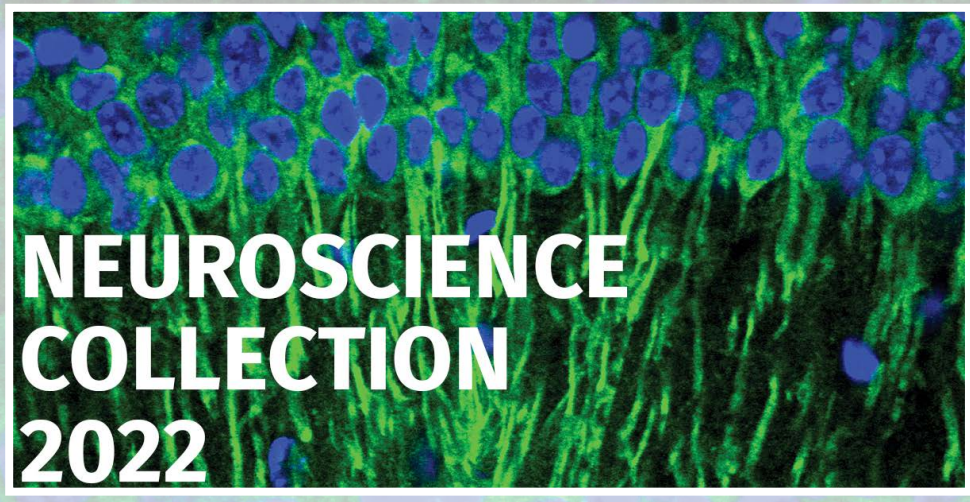

We are pleased to present this special collection of articles recently published in *LSA* highlighting some of the latest advances in neuroscience. Articles featured in the collection were published within the past 12 months and include original findings on optogenetic hearing restoration, the identification of a mutation underlying peripheral neuropathy, and pathogenic mechanisms that underlie neurodegenerative diseases such as Alzheimer's disease.

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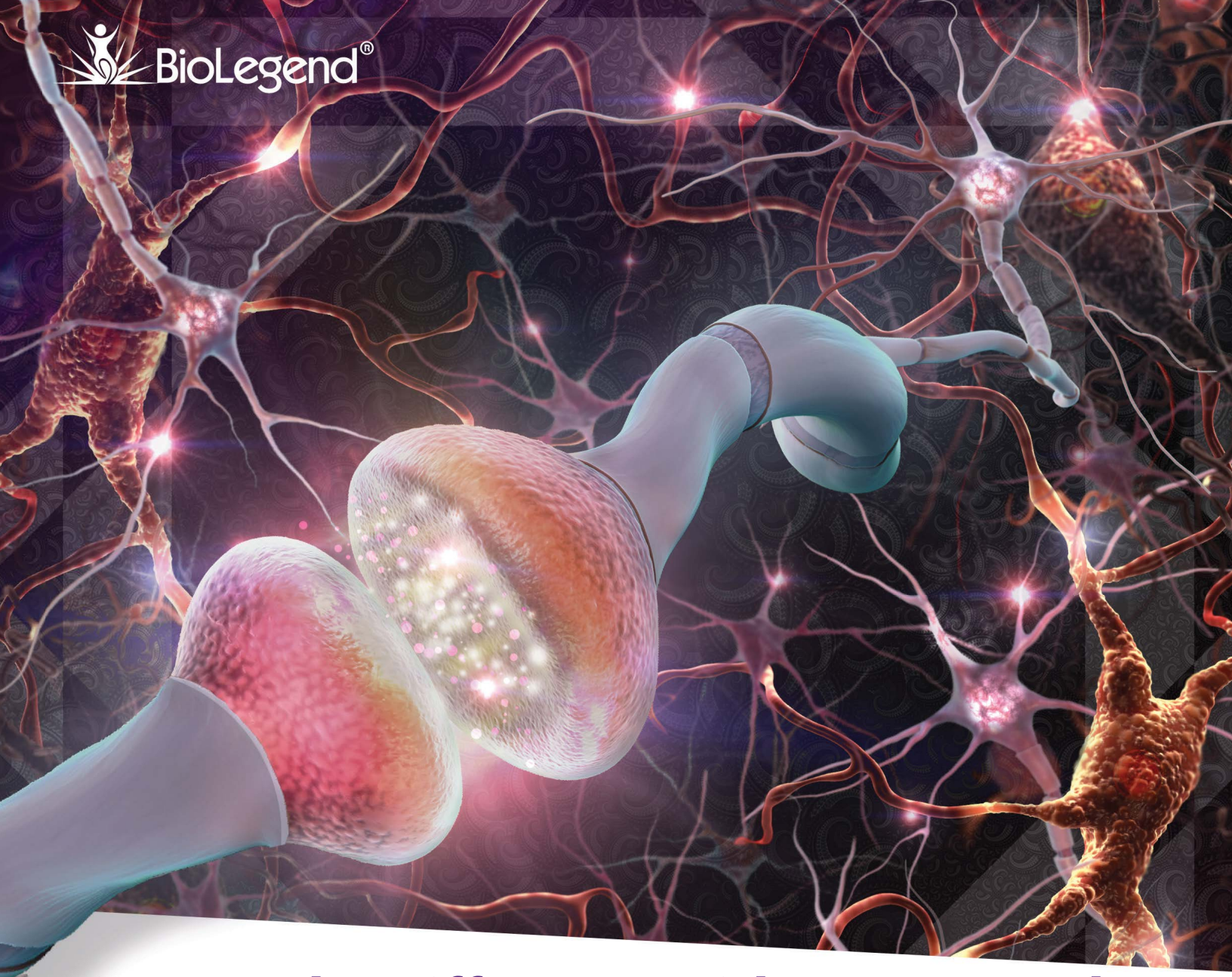

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