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Brochure articles by Ben Short, PhD and Christina Szalinski, PhD

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On the cover: Vascular graph of a mouse brain 7d after infection with SARS-CoV-2, as featured in the article "SARS-CoV-2 can infect neurons and damage brain tissue" on page 17. Original paper: Song, E., C. Zhang, B. Israelow, A. Lu-Culligan, A.V. Prado, S. Skriabine, P. Lu, O.-E. Weizman, F. Liu, Y. Dai, K. Szigeti-Buck, Y. Yasumoto, G. Wang, C. Castaldi, J. Heltke, E. Ng, J. Wheeler, M.M. Alfajaro, E. Levavasseur, B. Fontes, N.G. Ravindra, D. Van Dijk, S. Mane, M. Gunel, A. Ring, S.A.J. Kazmi, K. Zhang, C.B. Wilen, T.L. Horvath, I. Plu, S. Haik, J.-L. Thomas, A. Louvi, S.F. Farhadian, A. Huttner, D. Seilhean, N. Renier, K. Bilguvar, and Akiko Iwasaki. 2021. Neuroinvasion of SARS-CoV-2 in human and mouse brain. *J. Exp. Med.* 218 (3): e20202135.

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NEUROSCIENCE COLLECTION 2021

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 a special combined collection of recently published articles that elucidate new advances within the field of neuroscience. If you enjoy this collection, we encourage you to sign up for email alerts from *JCB*, *JEM*, and *JGP* here, and *LSA* here.

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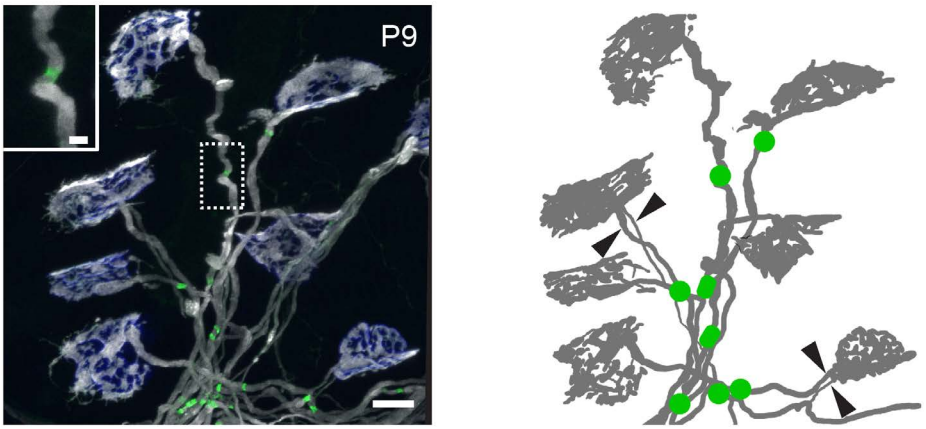
REMODELING MOTOR AXONS WAIT TO MYELINATE

Study shows that myelination of terminal axon branches is delayed until competing branches have been eliminated from neuromuscular junctions

In newborn mice, multiple motor axon branches innervate the same postsynaptic site. The different branches compete for territory at the neuromuscular junction (NMJ) until, about two weeks after birth, a single "winner" remains, and the other branches have been removed. Around the same time, glial Schwann cells wrap the terminal axon branch in a myelin sheath to facilitate rapid and synchronous neurotransmission toward the NMJ. But whether neuronal remodeling and myelination influence each other's progress is unclear.

"We wanted to ask how axonal competition and axon–glial differentiation are coordinated at the single-branch level and what signaling mechanisms are involved," explains Monika Brill, who, together with Thomas Misgeld led this study at Technische Universität München in Germany.

Brill and colleagues, including first author Mengzhe Wang, examined mouse NMJs at different stages of development and found that myelination onset is delayed until neuronal modeling is completed; myelination markers were elevated at singly innervated NMJs compared with doubly innervated NMJs, where the competition between branches was still ongoing. In contrast, myelination has no influence on neuronal remodeling; axon branches that begin to myelinate while still competing with other branches can lose that competition and be eliminated.



In the triangularis sterni muscles of a P9 mouse, axons (white) that are singly innervating postsynaptic sites (blue) have paranodal junctions (green), indicating the onset of myelination. In contrast, axons that are doubly innervating postsynaptic sites (highlighted by arrowheads in the schematic) show no markers of paranodal junctions or myelination. © 2021 Wang et al.

The outcome of neuronal remodeling is regulated by synaptic activity. While losing branches are removed by cytoskeletal degradation, the winning branch is stabilized by the maturation of its microtubule network. Wang et al. found that these processes are tightly coupled to myelination. Synaptic activity appears to promote microtubule maturation in the winning axon branch, enabling pro-myelination signaling factors, such as neuregulin, to be delivered to the axon terminal, where they can induce the differentiation of neighboring Schwann cells.

"Together, our experiments reveal an intercellular signaling mechanism that regulates myelination on a branch-to-branch level in the developing peripheral nervous system," Misgeld says.

Similar signaling mechanisms may exist in the central nervous system, where local myelination patterns can change and fine-tune neural circuits in response to neuronal activity and synaptic remodeling. "When disturbed, such signaling pathways could contribute to the disrupted timing of developmental events characteristic of some neuropsychiatric disorders, where axonal transport, neuronal remodeling, and myelination all show subtle defects," Brill suggests.

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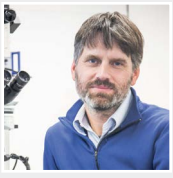

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Wang, M., T. Kleele, Y. Xiao, G. Plucinska, P. Avramopoulos, S. Engelhardt, M.H. Schwab, M. Kneussel, T. Czopka, D.L. Sherman, P.J. Brophy, T. Misgeld, and M.S. Brill. 2021. Completion of neuronal remodeling prompts myelination along developing motor axon branches. *J. Cell Biol.* 220 (4): e201911114.

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ACTIVATED MICROGLIA LIMIT Aβ-LINKED TAUOPATHY

Study suggests that the TREM2-dependent activation of microglia may delay the development of a key pathological hallmark of Alzheimer's disease

Alzheimer's disease (AD) is characterized by abnormal accumulations of both amyloid-β (Aβ) peptides and phosphorylated tau protein. Aβ peptides aggregate to form extracellular neuritic plaques (NPs) that promote the accumulation of tau aggregates in surrounding dystrophic neurites (NP-tau). This is thought to be a critical step in AD pathogenesis that drives neurodegeneration. But how the formation of Aβ and NP-tau aggregates are related to another pathological hallmark of AD—the activation of brain-resident phagocytic cells known as microglia—remains unclear, with studies in mice producing a variety of conflicting results.

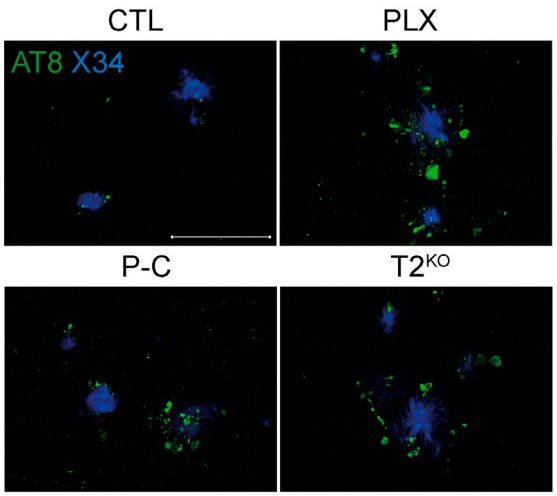
"Thus far, most studies have evaluated the role of microglia in AD in the context of Aβ or tau pathologies separately," explains David Holtzman from the Washington University School of Medicine in St. Louis. "We wanted to examine the role of microglia in mitigating against Aβ-driven tau seeding and spreading."

To address this question, Holtzman and colleagues, including first author Maud Gratuze, examined 5XFAD mice, which form Aβ plaques capable of promoting the accumulation of NP-tau after tau aggregates are injected into their brains. The researchers found that depleting microglia from the brains of these animals—using a pharmacological inhibitor of the CSF1 receptor—enhanced the seeding and spreading of NP-tau, and increased the number of plaque-associated dystrophic neurites. Similarly, 5XFAD mice lacking TREM2, an AD-linked gene involved in microglial activation, also showed accelerated NP-tau accumulation and increased neuritic dystrophy, suggesting that TREM2-expressing microglia are required to slow the spread of Aβ-induced NP-tau.

Surprisingly, however, Holtzman and colleagues also found that NP-tau accumulation was also enhanced in 5XFAD mice whose microglia had been temporarily depleted before being allowed to repopulate the brain. Neuritic dystrophy was also increased in these animals. "This suggests that the repopulated microglia are deficient in mitigating against plaque-associated toxicity and, importantly, Aβ-mediated tau seeding and spreading," Holtzman says.

Holtzman and colleagues determined that the repopulated microglia had yet to acquire an activated phenotype, showing reduced expression of various proinflammatory and disease-associated genes, including TREM2. The cells also showed lower levels of lysosomal function, suggesting they might fail to limit the spread of NP-tau because they are less able to degrade protein aggregates such as tau. Accordingly, Holtzman and colleagues found that TREM2-deficient phagocytes degrade tau aggregates more slowly than wild-type cells.

"Our data support the idea that the TREM2-dependent activation of microglia is essential to limit Aβ plaque–mediated tau pathogenesis in AD," Holtzman says. "Manipulating microglial function to decrease plaque-associated tauopathy may therefore be a potential therapeutic strategy to slow the early progression of AD pathology."



Compared with the brain of a control 5XFAD mouse injected with tau (CTL, top left), the accumulation of NP-tau (green) around Aβ plaques (blue) is enhanced in mice depleted of microglia (PLX, top right), mice with repopulated microglia (P-C, bottom left) and mice lacking TREM2 ($T2^{KO}$, bottom right). © 2021 Gratuze et al.

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Gratuze, M., Y. Chen, S. Parhizkar, N. Jain, M.R. Strickland, J.R. Serrano, M. Colonna, J.D. Ulrich, and D.M. Holtzman. 2021. Activated microglia mitigate Aβ-associated tau seeding and spreading. *J. Exp. Med.* 218 (8): e20210542.

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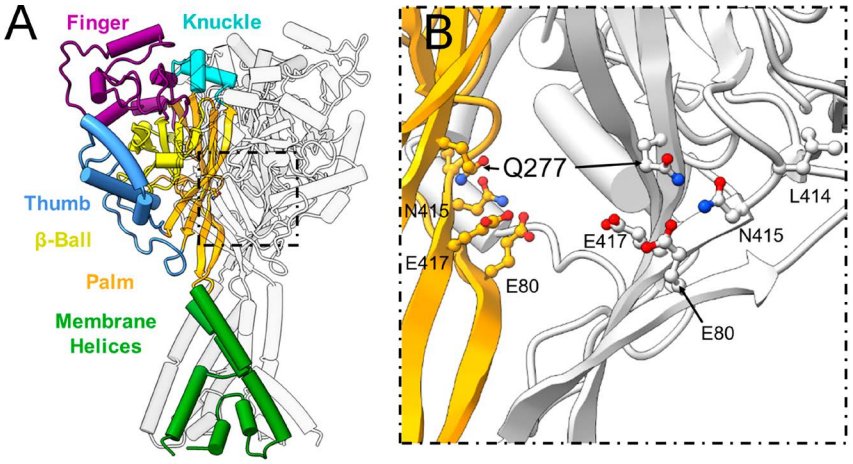
ANALYZING ASIC DESENSITIZATION

Research indicates that, contrary to a previous report, a mutation in acid-sensing ion channel 1 does not completely abolish desensitization



Structure of chicken ASIC1 in the resting state, with a close up view showing the position of Q277, the site of a glycine mutation that reduces desensitization in slightly acidic pH conditions. © 2021 Rook et al.

Acid-sensing ion channels (ASICs) play a role in fear and anxiety, learning and memory, pain, muscle fatigue, migraines, ischemic brain injury, inflammation, and cancer. They are found in various tissues, including the nervous system, and when they are activated by low pH, they allow sodium to go through the channel. Like other ion channels, ASICs undergo a period of desensitization after activation, during which time they cannot be activated. Identifying mutations that prevent desensitization can help researchers better understand the mechanism of desensitization. Though a mutation in ASIC1 was reported to prevent desensitization, a team led by Matthew Rook and David MacLean at the University of Rochester Medical Center found that its effects are not as clear-cut.

"Mutating Gln276 to a glycine (Q276G) in human ASIC1a was reported to mostly abolish desensitization at both the macroscopic and the single channel levels, potentially providing a valuable tool for subsequent studies," MacLean says. The research team studied the equivalent mutation (Q277G) in chicken ASIC1, because it has been the focus of other studies. Using fast perfusion electrophysiology, they found that at slightly acidic pH Q277G shows slightly reduced desensitization, but under more acidic conditions it behaves like a wild-type channel.

Using molecular dynamics simulations, the team found that the Gln277 side chain participates in a hydrogen bond network that might stabilize the desensitized conformation, suggesting an alternate mechanism to a previous study that proposed Gln277 worked as a valve to enable or restrict part of ASIC1 from rotating. Using mutations to disrupt a potential hydrogen bond network without having much impact on structure rotation, MacLean and colleagues observed a much faster recovery from desensitization in functional experiments, indicating that Gln277 coordinates a hydrogen bond network and doesn't act as a valve.

In whole-cell recordings with human ASIC1a, they found that the Q276G mutation reduces desensitization, but not to the extent reported previously. Furthermore, the impacts of Q276G on desensitization depended on the human variant used—in the common G212 variant, the Q276G mutation slows desensitization, but in the rare D212 variant desensitization accelerates.

MacLean explained, "our data reveal that while the Q/G mutation does not abolish desensitization as previously reported, it does point to unexpected differences between chicken and human ASICs and the need for careful scrutiny before using this mutation in future studies."

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ORIGINAL PAPER

Rook, M.L., M. Miaro, T. Couch, D.L. Kneisley, M. Musgaard, and D.M. MacLean. 2021. Mutation of a conserved glutamine residue does not abolish desensitization of acid-sensing ion channel. *J. Gen. Physiol.* 153 (8): e202012855.

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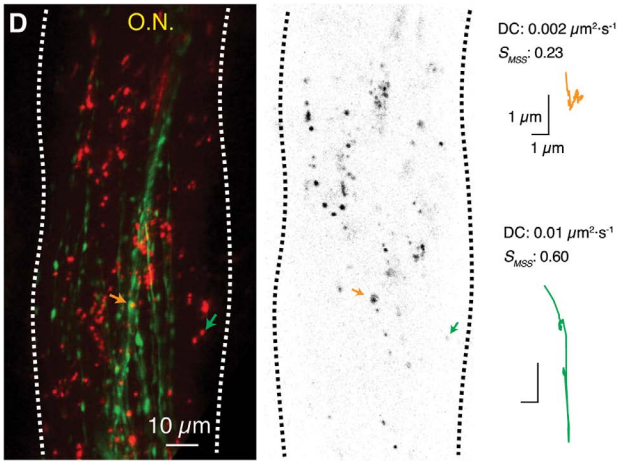
DIFFUSION OF ENDOLYSOSOMES CHANGES DURING SYNAPTIC REMODELING

Study shows that endolysosomes in live neurons diffuse differently under normal conditions compared with when remodeling is induced

Neurons pass information via neurotransmitters, which are released from vesicles within the axon terminals' presynaptic compartment. There is strict regulation of the number, location, and composition of these vesicles. Also, the mobility of these vesicles is higher in synapses that have higher neurotransmitter release rates, like those in the retina, compared to neurons with lower neurotransmitter release rates. However, it was not known whether other organelles, such as endolysosomes, which contribute to synaptic stability and the maintenance of vesicle pools, also adapt their mobility to meet changes in synaptic demand.

Endolysosomes are thought to have two roles in the cell: one that is considered to be homeostatic—maintaining a proper protein composition of the presynaptic terminal—and another that is considered to be degradative—eliminating proteins and macromolecular complexes. Beatrice Terni and Artur Llobet at the Institute of Neurosciences of the University of Barcelona and the Bellvitge Biomedical Research Institute in Barcelona, Spain, sought to determine whether a shift between endolysosome's homeostatic and degradative roles is linked to changes in their mobility.



A maximum intensity projection of a tadpole with olfactory neurons genetically labeled with GFP (green) and endolysosomes labeled in red. In the center is a confocal section to track endolysosomes, which showed both confined diffusion (orange trace) and directed motion (green trace).

The duo used *Xenopus tropicalis* tadpoles, which they say "offer a unique experimental platform to address this question because they allow the in vivo visualization of the entire morphology of some neuronal types, as, for example, olfactory sensory neurons (OSNs)." These OSNs can be genetically labeled, and the team followed endolysosomes in live tadpoles using a molecule called lysotracker. They measured particle mobility using an established algorithm, called the moment scaling spectrum, which indicates whether a particle has free diffusion, confined diffusion, or directed motion.

In presynaptic terminals of OSNs under normal, homeostatic conditions, the team determined that F-actin confines diffusion of endolysosomes. They induced degradative conditions by administering SPARC, a protein produced by some glial cells of the nervous system that can cause synaptic elimination in neurons, disrupting the tadpoles' ability to detect odorant molecules in the water. After adding SPARC, the team observed remodeling of synaptic F-actin patches coinciding with increased motion of endolysosomes in the presynaptic compartment.

Terni says their results "show that the diffusion of presynaptic endolysosomes increases during conditions of synaptic remodeling to support their local degradative activity."

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ORIGINAL PAPER

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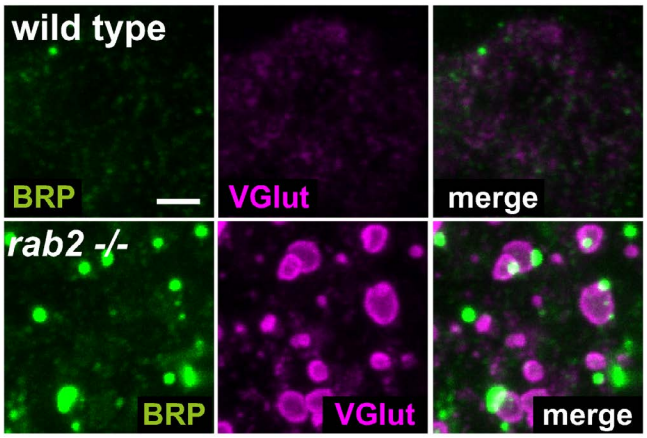
RAB2 REGULATES PRESYNAPTIC PRECURSOR FORMATION

Study shows that the small GTPase Rab2 controls the export and sorting of presynaptic proteins at the trans-Golgi



Compared with wild-type motoneurons (top), the active zone protein Bruchpilot (green) and the synaptic vesicle protein VGlut (magenta) accumulate in the cell bodies of neurons lacking Rab2 (bottom). © 2021 Götz et al.

The assembly of new synapses requires the delivery of numerous presynaptic proteins from the neuronal cell body, where they are synthesized, to the synaptic terminal. Active zone scaffold proteins, voltage-gated ion channels, and synaptic vesicle proteins are thought to assemble together in defined ratios before being transported along the axon on presynaptic precursor vesicles. But how these vesicles are formed remains unclear.

After their formation, presynaptic precursor vesicles mature into lysosome-related organelles and acquire the small GTPase Arl8, which recruits kinesin motor proteins so that the vesicles can be transported along axonal microtubules to synaptic terminals.

"We wanted to identify novel regulators of precursor biosynthesis," explains Petzoldt, a senior researcher in Stephan Sigrist's laboratory at Freie Universität Berlin. "We hypothesized that precursor biogenesis requires membrane remodeling enzymes, such as Rab proteins, small GTPases that control vesicle budding and fusion by recruiting effector proteins."

Petzoldt and colleagues, including first author Torsten Götz, therefore depleted various Rab proteins from *Drosophila* motoneurons and found that removing Rab2 caused numerous presynaptic proteins to accumulate in the cell body. Their levels were correspondingly reduced at synaptic terminals, impairing neurotransmission.

Further analyses using both electron and super-resolution stimulated emission depletion (STED) microscopy revealed that, in the absence of Rab2, presynaptic proteins accumulate at the trans-Golgi in small tubular shaped vesicles that have a different size and shape than the mature, lysosome-like precursor vesicles previously identified. Moreover, mature precursor vesicles contain both active zone and synaptic vesicle proteins but, in the absence of Rab2, active zone and synaptic vesicle proteins accumulate in separate vesicles.

Taken together, the researchers' observations suggest that Rab2 regulates two independent pathways at the trans-Golgi that sort and export active zone and synaptic vesicle proteins into separate, immature presynaptic precursor vesicles. These vesicles subsequently mature and come together to form the lysosome-like precursor vesicles that recruit Arl8 and are delivered to synaptic terminals to promote synaptogenesis. Indeed, genetic epistasis experiments confirmed that Rab2 acts upstream of Arl8 in presynaptic precursor biogenesis.

"Our study contributes to a comprehensive model of the biosynthetic pathway underlying presynaptic precursor biogenesis and could support future research by connecting neurodevelopmental defects, such as autism spectrum disorders and schizophrenia, that are associated with *rab2* mutations, with Golgi pathway–related neurodegenerative diseases," Petzoldt says.

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PRECLINICAL VALIDATION OF A POTENT γ-SECRETASE MODULATOR

Study shows that new drug is safe and effective in animals, paving the way for future clinical trials to prevent Alzheimer's disease in humans

The formation of amyloid plaques composed of amyloid-β (Aβ) peptides is a key pathological hallmark of Alzheimer's disease. Aβ peptides are generated by enzymes called β-secretase and γ-secretase, which sequentially cleave amyloid precursor protein on the surface of neurons to release Aβ fragments of varying lengths. Some of these fragments, such as Aβ42, are particularly prone to forming amyloid plaques, and their production is elevated in patients with mutations predisposing them to early-onset Alzheimer's disease.

Several attempts have been made to treat or prevent Alzheimer's disease using drugs that inhibit either β-secretase or γ-secretase. But many of these drugs have proved to be unsafe in humans, likely because β-secretase and γ-secretase are required to cleave additional proteins in the brain and other organs. A better approach could involve drugs known as γ-secretase modulators (GSMs), which, instead of inhibiting the γ-secretase enzyme, slightly alter its activity so that it produces fewer Aβ peptides that are prone to form plaques while continuing to cleave its other protein targets.

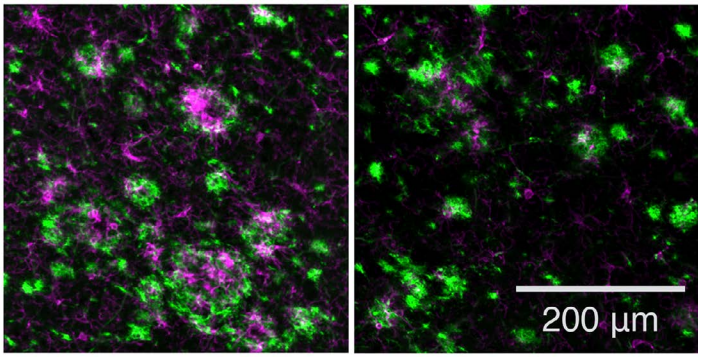
"GSMs therefore offer the ability to mitigate mechanism-based toxicities associated with γ-secretase inhibitors," says Steven L. Wagner, a professor in the Department of Neurosciences at the University of California, San Diego School of Medicine.

Wagner and colleagues, including first author Kevin D. Rynearson, developed a novel GSM and tested it on mice, rats, and macaques. Repeated low doses of the drug completely eliminated Aβ42 production in mice and rats without causing any toxic side effects. The drug was also safe and effective in macaques, reducing Aβ42 levels by up to 70%.

The researchers then tested the drug in a mouse model of early-onset Alzheimer's disease, treating the animals either before or shortly after they began to form amyloid plaques. In both cases, the novel GSM decreased plaque formation and reduced plaque-associated inflammation, which is thought to contribute to the development of disease.

This suggests that the drug could be used prophylactically to prevent Alzheimer's disease, either in patients with genetic mutations that increase susceptibility to the disease or in cases where amyloid plaques have been detected by brain scans.

"In this study, we have pharmacologically characterized a potent GSM that, based on its preclinical attributes, appears to equal or exceed the potency of any previously tested GSMs," adds Dr. Rudolph E. Tanzi, Professor of Neurology at Harvard and Massachusetts General Hospital, who collaborated with Wagner's team on the project. "Future clinical trials will determine whether this promising GSM is safe in humans and could be used to effectively treat or prevent Alzheimer's disease."



Compared with a control (left), treatment with the novel GSM (right) reduces the number of amyloid plaques (green) and proinflammatory microglia (magenta) in the brains of mice carrying mutations linked to early-onset Alzheimer's disease. © 2021 Rynearson et al.

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Rynearson, K.D., M. Ponnusamy, O. Prikhodko, Y. Xie, C. Zhang, P. Nguyen, B. Hug, M. Sawa, A. Becker, B. Spencer, J. Florio, M. Mante, B. Salehi, C. Arias, D. Galasko, B.P. Head, G. Johnson, J.H. Lin, S.K. Duddy, R.A. Rissman, W.C. Mobley, G. Thinakaran, R.E. Tanzi, and S.L. Wagner. 2021. Preclinical validation of a potent γ-secretase modulator for Alzheimer's disease prevention. *J. Exp. Med.* 218 (4): e20202560.

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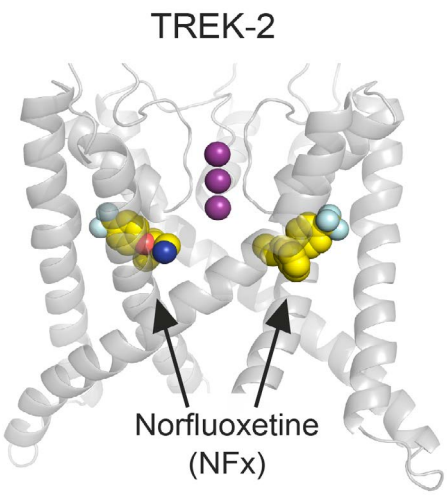
A TREK INHIBITOR TAKES MULTIPLE TRACKS

Single channel recordings reveal that norfluoxetine inhibits the two-pore domain K^+ channel TREK-2 by a complex array of mechanisms

The TREK subfamily of two-pore domain K^+ channels are expressed throughout the central and peripheral nervous systems and are involved in a diverse range of processes such as mechanosensation, thermosensation and nociception. Channel gating—which is thought to involve changes in the selectivity filter of TREKs—can, accordingly, be regulated by a wide variety of factors, including pressure, temperature, and multiple endogenous ligands.

Norfluoxetine binds exclusively to the "down" conformation of TREK-2 and prevents the channel's transmembrane domains from transitioning to the "up" configuration. But Proks et al. find that TREK-2 can be fully active in the down conformation and that norfluoxetine works via multiple mechanisms to inhibit both the open and closed states of the channel.

Stephen J. Tucker and colleagues at the University of Oxford previously helped solve the crystal structures of TREK-2 in the presence and absence of norfluoxetine, one of the known inhibitors of TREK activity. The channel can adopt two distinct conformations, named "up" or "down," depending on the orientation of its transmembrane helices, and norfluoxetine was found to bind within the inner cavity of TREK-2 in a gap that is only formed when the transmembrane helices are in the down configuration. Norfluoxetine can therefore block the transition from the down to up conformation, and it was originally suggested that this might inhibit channel activity by locking the selectivity filter in its closed state. But the mechanism of filter gating appears to be more complex. Tucker's group, for example, has shown using macroscopic recordings that TREK-2 can adopt several open states, some of which may occur in the down conformation.



To learn more about the mechanisms underlying filter gating and norfluoxetine inhibition, Tucker and colleagues, including first author Peter Proks, turned to single channel recordings of purified TREK-2 channels embedded in lipid bilayers. "We found that norfluoxetine affects both the open and closed states of the channel and is therefore a state-independent inhibitor of TREK-2," Tucker says. "That information is lost in macroscopic recordings."

Moreover, the fact that highly active channels were sensitive to norfluoxetine inhibition confirms that TREK channels can be fully open in the down conformation. It also indicates that, in addition to blocking changes in transmembrane conformation, norfluoxetine must inhibit TREK channels by other mechanisms as well.

"We found that there are several mechanisms involved, all of which converge on the selectivity filter gate," Tucker says. The researchers also observed a mild voltage dependence of norfluoxetine inhibition, suggesting that it can influence voltage-dependent gating as well.

"The complexity with which the drug works reflects the many different ways in which the selectivity filter can gate the channel," Tucker says. "This, in turn, reflects the polymodal regulation of TREK channels and their ability to integrate a wide variety of signals."

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ORIGINAL PAPER

Proks, P., M. Schewe, L.J. Conrad, S. Rao, K. Rathje, K.E.J. Rödström, E.P. Carpenter, T. Baukrowitz, and S.J. Tucker. 2021. Norfluoxetine inhibits TREK-2 K2P channels by multiple mechanisms including state-independent effects on the selectivity filter gate. *J. Gen. Physiol.* 153 (8): e202012812.

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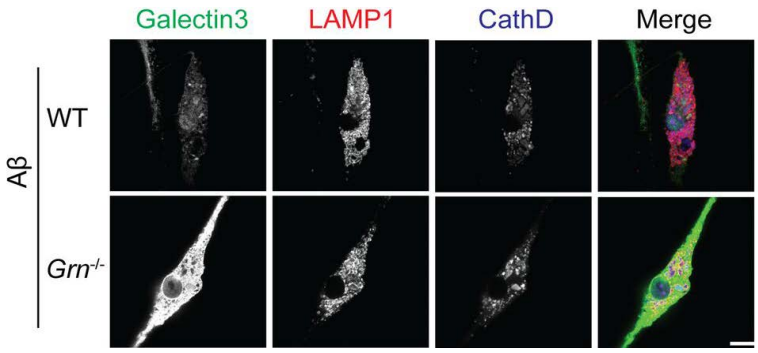
PROGRANULIN REGULATES AMYLOID-BETA DYNAMICS VIA LYSOSOMES

Progranulin is associated with neurodegeneration, and a new study shows the protein regulates lysosome function and microglia-mediated inflammation

Mutations in the *granulin* (*GRN*) gene are linked to a host of neurodegenerative diseases, including frontotemporal lobar degeneration and Alzheimer's disease. The secreted protein progranulin (PGRN) is encoded by the *GRN* gene, and lower levels of PGRN are associated with elevated Alzheimer's risk. In the brain, PGRN is mainly expressed by neurons and microglia, brain-resident immune cells that secrete especially high levels of PGRN. Past studies showed that PGRN regulates microglia immune functions, and that PGRN is highly expressed in the microglia surrounding Aβ plaques, which are a hallmark of Alzheimer's. Additionally, inflammation triggered by microglia, as well as lysosomal dysfunction are commonly observed in many other neurodegenerative diseases.

PGRN is known to be critical for proper lysosomal function. However, "how PGRN regulates lysosomal function and microglia-mediated inflammation was unclear, and whether lysosome abnormalities caused by PGRN deficiency contributes to microglial phenotypes remained to be tested," explains Fenghua Hu, an associate professor at Cornell University. Hu and colleagues, including first author Huan Du examined the role of PGRN in regulating lysosomes and inflammation in microglia associated with Aβ plaques.

In the 5XFAD mouse model of Alzheimer's disease, the team found that PGRN was mainly expressed in microglia around Aβ plaques and localized with lysosomes. When they crossed 5XFAD mice with *Grn* knockout mice, they observed a reduction in the area, number and intensity of Aβ plaques in young male, but not female, animals. The researchers also determined that PGRN deficiency in 5XFAD mice is associated with an up-regulation of proteins linked to microglial activation and neurodegeneration in microglia surrounding Aβ plaques in both male and female mice.

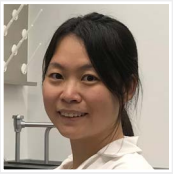


PGRN-deficient microglia (bottom row) are more susceptible to lysosome abnormalities in response to pathological Aβ fragments (Aβ fibrils), as indicated by an increase in Galectin-3, which is recruited to lysosomes upon lysosome membrane permeabilization, and Cathepsin D, a lysosomal enzyme. © 2021 Du et al.

Furthermore, PGRN deficiency in 5XFAD mice led to an up-regulation of lysosomal proteins, also in microglia near Aβ plaques, which may be due to increased activation of TFEB/TFE3, transcription factors involved in lysosome biogenesis that are activated by starvation or lysosome stress. Lysosome membrane integrity also seemed to be impaired in the absence of PGRN. Finally, the team found that treatment with Aβ fibrils, pathological Aβ fragments, led to enhanced inflammation and lysosomal responses in PGRN-deficient microglia.

Hu says, "Our data support that PGRN regulates microglia-mediated inflammation through modulating lysosomal functions and that PGRN deficiency leads to enhanced lysosome abnormalities and inflammatory responses in response to Aβ."

RESEARCHER DETAILS



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ORIGINAL PAPER

Du, H., M.Y. Wong, T. Zhang, M.N. Santos, C. Hsu, J. Zhang, H. Yu, W. Luo, and F. Hu. 2021. A multifaceted role of progranulin in regulating amyloid-beta dynamics and responses. *Life Science Alliance*. 4 (7) e202000874;

http://doi.org/10.26508/lsa.202000874

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SYNAPTIC ACTIVITY REGULATES AUTOPHAGY IN DENDRITES

Study shows that synaptic activity controls the motility and degradative function of autophagic vacuoles in neuronal dendrites

The autophagy pathway degrades cellular components by sequestering them into double-membraned autophagosomes that subsequently fuse with lysosomes to form degradative autolysosomes. Defects in autophagy can cause numerous problems in neuronal development and function, including deficits in learning and memory, that suggest the degradative pathway plays crucial roles at neuronal synapses.

Sandra Maday and colleagues at the University of Pennsylvania have previously shown that autophagosomes show distinct dynamics in neuronal axons and dendrites. In axons, the organelles mostly undergo long-range transport from axon terminals to the cell body, where they mature into autolysosomes. Dendritic autophagosomes, on the other hand, exhibit bidirectional movements or stay in place.

"However, how the dynamics of autophagic vacuoles—i.e., autophagosomes and autolysosomes—are influenced by the activity state of the neuron and how alterations in these dynamics impact autophagic function are largely unknown," Maday explains.

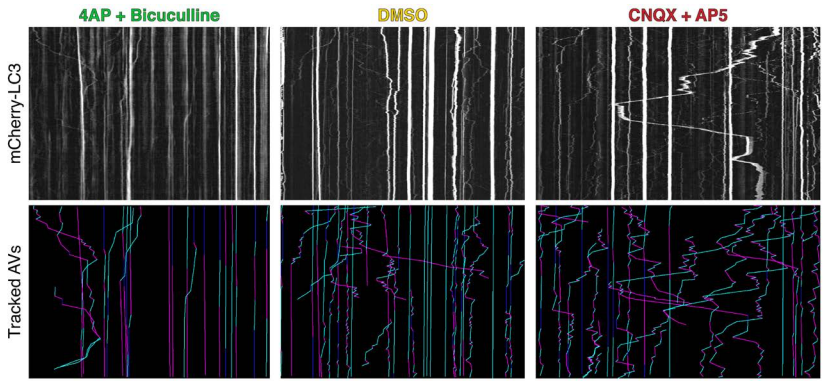
Maday and colleagues, including first author Vineet Kulkarni, therefore examined whether changes in the activity of primary hippocampal neurons alters the motility of autophagic vacuoles (AVs). The researchers found that increasing synaptic activity reduced the motility of AVs in dendrites, whereas dampening synaptic activity enhanced AV motility. "Importantly, we observed these effects specifically within dendrites and not within axons, indicating compartment-specific regulation of AV dynamics by synaptic activity," Kulkarni says.

Photouncaging experiments with the neurotransmitter glutamate revealed that synaptic activity can rapidly and locally regulate AV motility within dendrites. Moreover, Maday's team determined that this regulation is reversible: AVs immobilized by an increase in synaptic activity can recover their motility once synaptic activity is repressed.

Notably, increasing synaptic activity also increased the proportion of acidic, degradative autolysosomes in dendrites (but not axons), suggesting that changes in AV motility may be coupled to organelle maturation.

"We found that activity-dependent dampening of AV motility increases their residence time at or near post-synaptic compartments," Maday says. "This process may therefore facilitate local regulation of the synaptic proteome through degradation of post-synaptic components and/or through recycling of degradation products to fuel local protein synthesis." This could aid synaptic remodeling and explain why defects in autophagy impair learning and memory.

"We now want to delineate the mechanism by which AVs become more degradative in dendrites in response to synaptic stimulation," Maday says.



Kymographs of AVs labeled with mCherry-LC3 show that, compared with DMSO-treated controls (center), increasing synaptic activity (left) reduces AV motility in dendrites, whereas silencing synaptic activity (right) enhances AV dynamics. The retrograde movements of tracked AVs are colored magenta, anterograde movements are cyan, and stationary segments are dark blue.

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ORIGINAL PAPER

Kulkarni, V.V., A. Anand, J.B. Herr, C. Miranda, M.C. Vogel, and S. Maday. 2021. Synaptic activity controls autophagic vacuole motility and function in dendrites. *J. Cell Biol.* 220 (6): e202002084.

https://doi.org/10.1083/jcb.202002084

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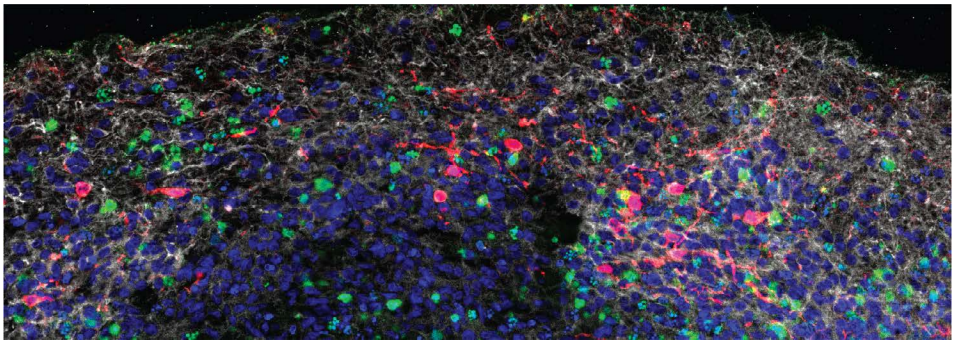
SARS-COV-2 CAN INFECT NEURONS AND DAMAGE BRAIN TISSUE

Study begins to unravel some of the virus's effects on brain cells, potentially explaining the various neurological symptoms associated with COVID-19



An image of a human brain organoid shows numerous dying cells (green) surrounding neurons (gray) that have been infected by SARS-CoV-2 (red). © 2021 Song et al.

Though COVID-19 is considered to primarily be a respiratory disease, SARS-CoV-2 can affect many other organs in the body, including, in some patients, the central nervous system, where infection is associated with a variety of symptoms ranging from headaches and loss of taste and smell to impaired consciousness, delirium, strokes, and cerebral hemorrhage.

Early in the pandemic, it was unclear whether SARS-CoV-2 could directly infect neurons or other types of brain cells. To address this question, Akiko Iwasaki and colleagues at Yale School of Medicine analyzed the ability of SARS-CoV-2 to invade human brain organoids. The researchers, including co-senior author Kaya Bilguvar and co-first authors Eric Song and Ce Zhang, found that the virus was able to infect neurons in these organoids and use the neuronal cell machinery to replicate. The virus appears to facilitate its replication by boosting the metabolism of infected cells, while neighboring, uninfected neurons die as their oxygen supply is reduced.

SARS-CoV-2 enters lung cells by binding to a protein called ACE2, but whether this protein is present on the surface of brain cells is unclear. Iwasaki and colleagues determined that the ACE2 protein is, in fact, produced by neurons and that blocking this protein prevents the virus from human brain organoids.

SARS-CoV-2 was also able to infect the brains of mice genetically engineered to produce human ACE2, causing dramatic alterations in the brain's blood vessels that could potentially disrupt the organ's oxygen supply. Central nervous system infection was much more lethal in mice than infections limited to the lungs, the researchers found.

Finally, Iwasaki and colleagues analyzed the brains of three patients who succumbed to COVID-19. SARS-CoV-2 was detected in the cortical neurons of one of these patients, and the infected brain regions were associated with ischemic infarcts in which decreased blood supply causes localized tissue damage and cell death. Microinfarcts were detected in the brain autopsy of all three patients.

"Our study clearly demonstrates that neurons can become a target of SARS-CoV-2 infection, with devastating consequences of localized ischemia in the brain and cell death," Bilguvar says. "Our results suggest that neurologic symptoms associated with COVID-19 may be related to these consequences, and may help guide rational approaches to the treatment of COVID-19 patients with neuronal disorders."

"Future studies will be needed to investigate what might predispose some patients to infections of the central nervous system and to determine the route of SARS-CoV-2 invasion into the brain and the sequence of infection in different cell types within the central nervous system that will help validate the temporal relationship between SARS-CoV-2 and ischemic infarcts in patients," Iwasaki adds.

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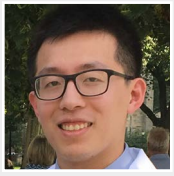

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https://doi.org/10.1084/jem.20202135

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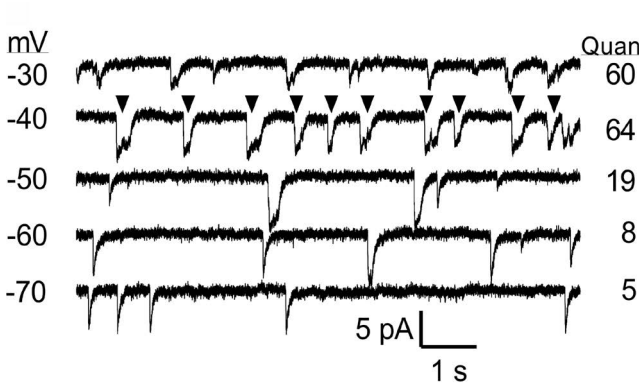
SYNAPTIC VESICLES BURST INTO SIGHT

Study shows that small voltage changes disrupt semi-regular bursts of vesicle release from rod photoreceptors, potentially facilitating low-light vision

The ability of vertebrates to see in low-light conditions depends on the extreme sensitivity of rod photoreceptors in the retina, which can detect single photons of light. But the membrane potential of rod cells only changes by a few millivolts in response to a single photon, and it is unclear how such small signals are reliably transmitted to the rest of the visual system.

In the dark, rod cells release a train of synaptic vesicles containing the neurotransmitter glutamate, which acts on downstream bipolar and horizontal cells. Upon stimulation, a slight hyperpolarization interrupts this release by reducing the activity of presynaptic Ca^{2+} channels. "But how does a downstream cell know if a change in glutamate release is due to the absorption of a photon, or just a random fluctuation?" asks Wallace Thoreson from the University of Nebraska Medical Center.

One possibility is that resting rod cells secrete vesicles at an extremely high rate—around 100 vesicles per second per synapse—thereby making it easier for downstream cells to detect a light-induced decrease in glutamate release. But this would be energetically expensive, and, when Thoreson and colleagues, including first author Cassandra Hays, measured vesicle release from voltage-clamped mouse rods, they found that individual cells secreted just ~12 vesicles per second under resting conditions.



At a resting membrane potential of −40 mV, rod cell vesicle release occurs in semi-regular bursts of 10–20 vesicles (black arrowheads). Small voltage changes eliminate these bursts and, instead, single vesicles are released at random intervals.

An alternative suggestion is that downstream cells could distinguish genuine from random signals if resting rod cells secrete glutamate at regular, predictable intervals. Hays et al. found that resting mouse rods released glutamate in coordinated bursts of 10–20 vesicles.

"These bursts occurred at fairly regular intervals and were quite sensitive to small changes in voltage," says Thoreson. Upon hyperpolarization, rod cells switched to secreting single vesicles at random intervals. Cone cells, in contrast, never showed bursts of vesicle release, suggesting that the ability of rod cells to change their release patterns in response to small voltage changes could be crucial for low-light vision.

The bursts of release by resting rod cells involved the readily releasable pool of vesicles, which are thought to be positioned near release sites by the synaptic ribbon, a large plate-like structure found at rod cell synapses. Indeed, the researchers determined that the bursts are triggered by the opening of ribbon-associated Ca^{2+} channels.

The bursts were dependent on the Ca^{2+} sensor synaptotagmin 1, which promotes exocytosis in response to Ca^{2+} influx. But it remains to be seen how rod cells coordinate the bursts such that, after an extended pause, multiple vesicles are released in quick succession.

"It seems like the regularity and voltage-sensitivity of the bursting might help downstream neurons detect single photos," Thoreson says. "We're now doing some modeling and additional experiments to determine whether this is actually true."

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ORIGINAL PAPER

Hays, C.L., A.L. Sladek, and W.B. Thoreson. 2020. Resting and stimulated mouse rod photoreceptors show distinct patterns of vesicle release at ribbon synapses. *J. Gen. Physiol.* 152 (12): e202012716.

https://doi.org/10.1085/jgp.202012716

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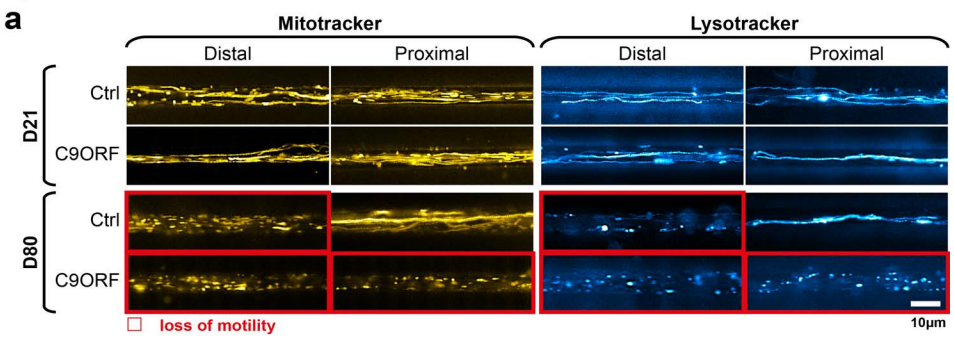
ALS-ASSOCIATED GENE CONTRIBUTES TO NEURODEGENERATION IN MULTIPLE WAYS

Study indicates that ALS-linked hexanucleotide repeat expansions in C9ORF72 can result in both loss and gain of function, contributing to different hallmarks of neurodegeneration

Amyotrophic lateral sclerosis (ALS), is a devastating, incurable disease caused by degeneration of spinal and cortical motor neurons, leading to progressive muscle paralysis and death. The most common genetic cause of ALS is intronic hexanucleotide repeat expansions (HREs) in the *C9ORF72* gene. However, precisely how HREs trigger ALS is still not fully understood.

HREs occur concomitantly with reduced expression of the exonic *C9ORF72* gene, and researchers have speculated that neurodegeneration could result from a subsequent loss of function in axonal trafficking. However, intronic HREs can undergo non-canonical translation to produce neurotoxic dipeptide repeat proteins (DPRs) that cause DNA damage—a gain-of-function contribution to neurodegeneration.

To dissect these different mechanisms, Arun Pal, Andreas Hermann, and colleagues at Technische Universität Dresden and University of Rostock in Germany generated iPSC-derived spinal motor neuron cell lines from ALS patients with HREs in *C9ORF72*, as well as lines with a gene-corrected (GC) variant with intronic HREs excised, a knockout (KO) of the exonic C9ORF72 with intronic HREs maintained, and a similar knockout of C9ORF72 in control cells with naturally no HREs.



Mitochondria (yellow) and lysosome (blue) motility is reduced at day 80 in distal and proximal axons from iPSC-derived spinal motor neurons with intronic HREs in the *C9ORF72* gene. Depicted are maximum projection images from videos, in which properly moving organelles appear as lines, while dots represent not moving organelles. © 2021 Pal et al.

Because C9ORF72 has been shown to have a role in trafficking, the team performed fast dual-color live imaging of mitochondria and lysosomes and found that while control cells had distal motility defects when aged to day 80, HRE *C9ORF72* showed both distal and proximal axonal organelle motility deficits, alongside augmented DNA double-strand breaks, non-canonical transcription (observed as RNA foci), DPRs, and neuronal apoptosis.

While GC cell lines showed distal trafficking defects only, additional KO of exonic *C9ORF72* aggravated all phenotypes, indicating that both the loss of function of C9ORF72 protein and gain of function of HRE/DPR mechanisms contribute to the overall C9ORF72 phenotype. Meanwhile, knockout of exonic *C9ORF72* in cells with naturally no HREs mimicked double-strand break accumulation in KO cells containing HREs, suggesting that loss of function of C9ORF72 is the driving factor for appearance of double-strand breaks, rather than augmented DPR production.

Hermann says their study "indicates that HREs in *C9ORF72* result in both gain- and loss-of-function mechanisms that cause trafficking defects along with accumulation of dipeptide repeat proteins, RNA foci, and DNA double-strand breaks."

RESEARCHER DETAILS

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ORIGINAL PAPER

Pal, A., B. Kretner, M. Abo-Rady, H. Glaß B.P. Dash, M. Naumann, J. Japtok, N. Kreiter, A. Dhingra, P. Heutink, T.M. Böckers, R. Günther, J. Sterneckert, and A. Hermann. 2021. Concomitant gain and loss of function pathomechanisms in C9ORF72 amyotrophic lateral sclerosis. Life Science Alliance. 4(4): e202000764.

http://doi.org/10.26508/lsa.202000764

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NEURONAL EXTRACELLULAR VESICLES PROMOTE NEURAL FATE

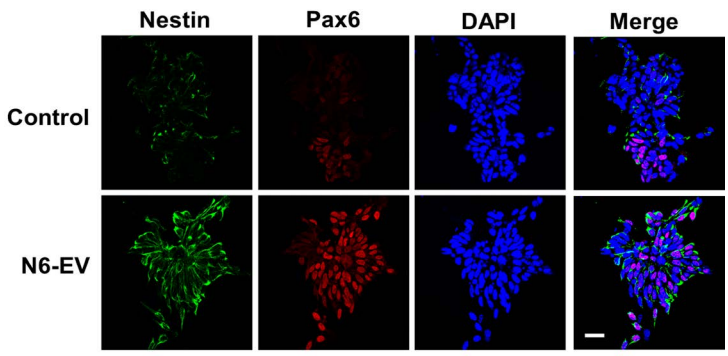
Differentiating neurons produce extracellular vesicles that induce stem cells to adopt a neural fate via the transfer of cyclin D1

Extracellular vesicles (EVs) are thought to enable intercellular communication—potentially over long distances—by transporting proteins and RNAs between cells. In the central nervous system, EVs are produced by neurons and glial cells, and, during development, neuronal EVs have been shown to promote the differentiation of neural progenitors into nerve cells.

"Although EVs have been suggested to facilitate these later neurogenesis events, little is known about the role of EVs during the earlier stage of neural induction, when pluripotent stem cells commit to a neural fate and convert into neural progenitors," explains Randy Schekman from the University of California, Berkeley.

To learn more about how EVs influence neural development, Schekman and colleagues, including first author Lu Song, used buoyant density centrifugation to purify the EVs secreted by cells undergoing neuronal differentiation in vitro. The researchers found that EV production increases during neurogenesis and that the physical properties and content of these EVs changes as the cells differentiate.

Schekman and colleagues then treated mouse embryonic stem cells (mESCs) with purified EVs to see if they had any effect on neural induction. EVs purified from differentiated neurons—but not EVs purified from undifferentiated neuronal precursors—promoted the conversion of mESCs to a neural fate, increasing the expression of several key neural markers.



Compared with control cells (top), mESCs treated with EVs purified from in differentiating PC12 cells (bottom) express increased amounts of the neural markers Nestin (green) and Pax6 (red). © 2021 Song et al.

One potential EV cargo that could promote neural induction is the cell cycle regulator cyclin D1, which drives the G1/S transition and is known to promote the expansion of the basal neural progenitor population in the mouse cortex and hippocampus. Schekman and colleagues found that cyclin D1 is specifically sorted into EVs secreted from differentiated neurons by the chaperone protein Hsc70, and that, when these EVs are taken up by mESCs, cyclin D1 is transferred to the nucleoplasm/cytoplasm of the cells.

Preventing cyclin D1's packaging into EVs—either by depleting the protein or by inhibiting Hsc70—reduced the ability of neuronal EVs to promote the neural induction of mESCs. In contrast, increasing cyclin D1's incorporation by overexpressing the protein enhanced the ability of neuronal EVs to induce a neural fate.

"Taken together, our results suggest that neuronal EVs contribute to neural fate determination through the sorting and transfer of cyclin D1," Schekman says. Though it remains to be seen how cyclin D1 moves from the lumen of EVs to the nucleus/cytoplasm of recipient stem cells, Schekman and colleagues have already identified some potential targets of cyclin D1 that could promote their commitment to the neural lineage.

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ORIGINAL PAPER

Song, L., X. Tian, and R. Schekman. 2021. Extracellular vesicles from neurons promote neural induction of stem cells through cyclin D1. *J. Cell Biol.* 220 (9): e202101075.

https://doi.org/10.1083/jcb.202101075

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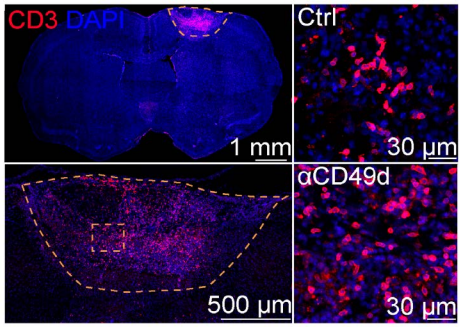
CHRONIC T CELL PROLIFERATION MAY CONFOUND STROKE IMMUNOTHERAPY

Local proliferation of T cells within brain lesions may limit the efficacy of therapeutic approaches that limit the invasion of lymphocytes across the blood–brain barrier after stroke

Stroke is one of the leading causes of death and disability worldwide, and, currently, the only way to limit stroke-induced brain damage is to quickly restore blood supply to the occluded region. In recent years, researchers have focused on the role of neuroinflammation in post-stroke injury and recovery. T cells in particular have been shown to invade the brain after stroke and drive secondary brain injury, and several therapeutic strategies that block this process have been investigated.

Natalizumab, for example, is a monoclonal antibody that targets CD49d (α4β1 integrin), a key cell adhesion molecule used by circulating lymphocytes to cross the blood–brain barrier. Originally developed to treat multiple sclerosis, Natalizumab was shown to reduce T cell brain invasion and improve acute outcome in experimental animal stroke models. But clinical trials in humans revealed no improvement in stroke patient recovery after 3 mo.

"The efficacy of Natalizumab was extraordinarily well characterized in preclinical models, and the clinical trials closely mimicked the treatment regimen and investigated outcome parameters, with the exception of analyzing different time points after stroke," explains Arthur Liesz from LMU Munich University Hospital. "The clinical trials analyzed the chronic phase after stroke as the primary endpoint, whereas the preclinical studies analyzed the acute phase."



T cells (red) accumulate in the brain lesions of mice 28 d after stroke. This accumulation is due to sustained, local proliferation and isn't reduced by anti-CD49d antibodies (bottom right) that inhibit lymphocyte invasion into the brain. © 2021 Heindl et al.

Liesz and colleagues, including first author Steffanie Heindl, therefore took a "reverse translational" approach, applying the design of the clinical trials to two different stroke models in mice. In both cases, anti-CD49d antibodies failed to improve the chronic outcomes of mice 3 mo after stroke, as assessed by lesion size, neuronal connectivity, and functional recovery.

Although the antibodies reduced lymphocyte invasion, T cells still accumulated within brain lesions in the weeks following a stroke. The clustering of these cells, along with DNA labeling experiments, indicated that the sustained, local proliferation of T cells drives this chronic accumulation. Liesz and colleagues were also able to observe the local proliferation and accumulation of T cells in autopsy samples taken from the brains of human stroke patients.

The ability of a small number of T cells to proliferate after they have entered the brain likely explains why reducing lymphocyte invasion is insufficient to promote long-term recovery from stroke. Liesz notes that over 40 clinical trials are currently investigating immunotherapies that seek to limit lymphocyte brain invasion in some way. "In the light of our findings, these trials need to be fundamentally reconsidered. Mechanisms of chronic neuroinflammation after stroke and the consequences for recovery need to be better understood for the rational design of efficient immunotherapies in stroke," Liesz says.

RESEARCHER DETAILS

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ORIGINAL PAPER

Heindl, S., A. Ricci, O. Carofiglio, Q. Zhou, T. Arzberger, N. Lenart, N. Franzmeier, T. Hortobagyi, P.T. Nelson, A.M. Stowe, A. Denes, D. Edbauer, and A. Liesz. 2021. Chronic T cell proliferation in brains after stroke could interfere with the efficacy of immunotherapies. *J. Exp. Med.* 218 (8): e20202411.

https://doi.org/10.1084/jem.20202411

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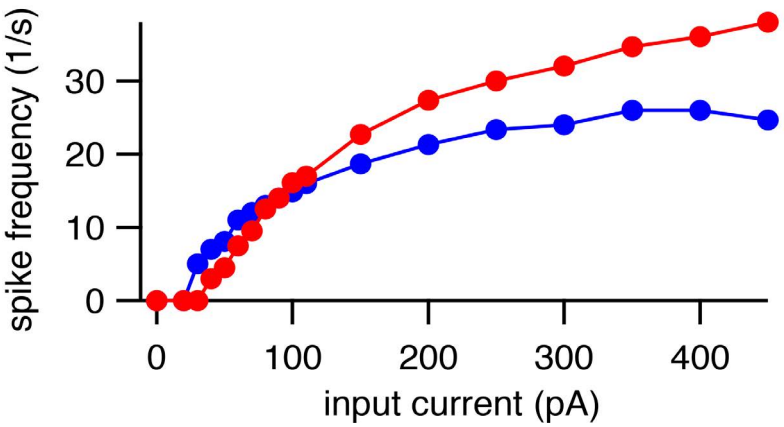
HOW ULTRASOUND MODULATES NEURONAL ACTIVITY

By activating K2P channels, high-frequency ultrasound can stimulate or inhibit neural activity, depending on the neuron's firing frequency



Compared with no ultrasound (blue line), ultrasound (red line) can inhibit or potentiate action potential firing, depending on input current. © 2020 Prieto et al.

High-frequency ultrasound can activate neurons and could therefore be a form of noninvasive brain stimulation. However, it's not fully understood how acoustic energy leads to changes in neuronal activity, and therefore it is not understood why some reports show excitation and other show inhibition of neural activity. To investigate how ultrasound impacts neurons, a research team led by Martin Loynaz Prieto and Merritt Maduke at Stanford University developed a technique to study the impact of high-frequency ultrasound on ion channels responsible for neurons' electrical activity. Their results support a hypothesis that ultrasound activates two-pore-domain potassium (K2P) channels, leading to a sustained potassium conductance, which can either excite or inhibit neurons, depending on the conditions.

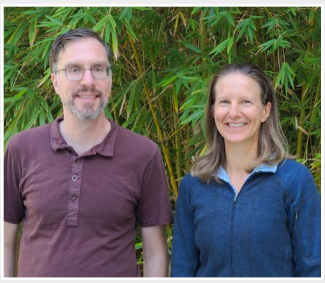
The research team used a patch-clamp technique to measure the activity of ion channels in hippocampal CA1 pyramidal cells within rodent brain slices. They found that when they stimulated the neurons at low input currents to induce low firing frequencies, ultrasound inhibited the neurons from firing, whereas at higher input currents that induce higher firing frequencies, ultrasound promoted neuron firing.

To learn more about how ultrasound physically impacts nerve cells, the team used computer modeling, which indicated that the effects of ultrasound on neuron firing frequency are caused by a small increase in temperature, with possible additional contributions from the mechanical effects of ultrasound waves.

The researchers suspected K2P channels are impacted by ultrasound because their data implicated channels that do not undergo extended voltage-dependent inactivation during sustained depolarizations. Moreover, K2P channels are both mechano– and thermosensitive. "We propose that ultrasound activates thermosensitive and mechanosensitive K2P channels through heating or mechanical effects of acoustic radiation force," Prieto explains.

"According to this hypothesis, the dual outcome occurs because activation of K2P channels can both oppose and potentiate action-potential firing. At high input currents, potentiation predominates because the K2P-induced hyperpolarization reduces sodium channel inactivation," says Maduke. The team believes this mechanistic insight will help future research by allowing scientists to use ultrasound to either excite or inhibit nerve signals, depending on the firing frequency.

RESEARCHER DETAILS



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Prieto, M.L., K. Firouzi, B.T. Khuri-Yakub, D.V. Madison, and M. Maduke. 2020. Spike frequency–dependent inhibition and excitation of neural activity by high-frequency ultrasound. *J. Gen. Physiol.* 152 (11): e202012672.

https://doi.org/10.1085/jgp.202012672

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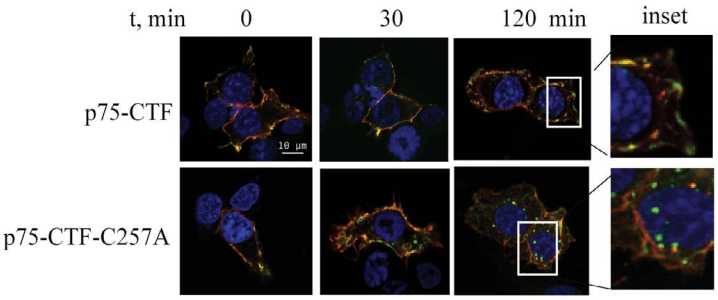
TRKA PROTECTS NEURONS FROM SIDE EFFECT OF γ-SECRETASE INHIBITION

γ-secretase inhibitors induce the accumulation of a p75NTR fragment that can promote neuronal death, potentially explaining why these drugs worsen cognitive symptoms in Alzheimer's patients



p75-CTF, which can form dimers that promote cell death signaling, is not internalized as much or as fast as a version of p75-CTF that cannot form dimers (p75-CTF-C257A). Surface p75-CTF is shown in red, and internalized p75-CTF is shown in green. © 2021 Franco et al.

Alzheimer's disease (AD) is one of the most commonly diagnosed types of dementia. A hallmark of the disease is amyloid plaques containing misfolded Aβ peptides generated by cleavage of the amyloid precursor protein (APP) by the β- and γ-secretases. γ-secretase inhibitors (GSIs) were developed to reduce the generation of Aβ peptides, but a clinical trial found that the drugs worsened the cognitive symptoms of AD patients, likely because GSI is required to cleave other type 1 transmembrane proteins. María Luisa Franco, Irmina García-Carpio, Marçal Vilar, and colleagues at the Institute of Biomedicine of València in Spain investigated the impact of GSI on the p75 neurotrophin receptor (p75NTR), which could explain the worsened cognitive symptoms.

p75NTR is expressed in cholinergic neurons, which are involved in complex cognitive tasks. p75NTR undergoes regulated intramembrane proteolysis (RIP) and is sequentially cleaved by α-secretase, generating a C-terminal membrane–anchored fragment (p75-CTF), followed by γ-secretase cleavage, which can occur within endosomes. RIP is required for p75NTR signaling, a pathway that regulates axonal growth and synaptic plasticity, as well as cell proliferation, migration, and neuronal death.

The team found that GSI treatment induced p75-CTF dimerization in a neuronal cell line, and that this promoted cell death. Dimerization also made the fragment more stable within the cell. These results "indicate that p75-CTF dimers are resistant to γ-secretase processing and this feature results in the increased accumulation of dimeric forms and concomitant exacerbated induction of cell death," Vilar says.

During aging, there is an increase in p75NTR expression that is accompanied by decreased levels of the receptor tyrosine kinase TrkA. The team found that activation of TrkA promotes p75-CTF endocytosis and rescues cells from p75-CTF–mediated cell death. They also showed that TRAF6, a p75 effector that promotes cell death, preferentially binds to p75-CTF dimers but that TrkA can compete with TRAF6 and reduce the effector's association with p75-CTF. Additionally, the team confirmed that in mature cholinergic neurons with increased p75 and decreased TrkA expression, GSI treatment promotes cell death.

Vilar says "our results reveal a novel mechanism underlying the regulated intramembrane proteolysis of p75, where the oligomerization of the receptor and its subcellular location protects it from γ-secretase–mediated processing and exacerbates its deadly function. We speculate that the worsening in cognition observed in a clinical trial of a GSI could be linked to the inhibition of p75-CTF turnover and its consequent accumulation in the cholinergic neurons of the treated Alzheimer's patients."

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Franco, M.L., I. García-Carpio, R. Comaposada-Baró, J.J Escribano-Saiz, L. Chávez-Gutiérrez, and M. Vilar. 2021. TrkA-mediated endocytosis of p75-CTF prevents cholinergic neuron death upon γ-secretase inhibition. *Life Science Alliance*. 4(4):e202000844.

http://doi.org/10.26508/lsa.202000844

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TDP-43 REGULATES OLIGODENDROCYTE MYELINATION

Study shows that loss of the neurodegenerative disease-associated protein disrupts the biosynthesis and uptake of cholesterol required to maintain the myelin sheath

Toxic TDP-43 aggregates are found in the brains of most amyotrophic lateral sclerosis (ALS) patients and ~45% of frontotemporal dementia (FTD) patients, and they are linked to several other neurodegenerative disorders, including some cases of Alzheimer's disease. The aggregates form not only in neurons but also in other brain cell types such as oligodendrocytes. These latter cells protect neurons and speed up the transmission of nerve impulses by wrapping neurons in a fatty, cholesterol-rich substance called myelin.

The formation of TDP-43 aggregates may prevent TDP-43 from performing its normal, vital functions within cells. Shuo-Chien Ling and colleagues at the Yong Loo Lin School of Medicine, National University of Singapore, previously found that oligodendrocytes need TDP-43 to survive and wrap neurons in myelin. "Specifically, we found that mice with oligodendrocytes lacking TDP-43 develop progressive neurological phenotypes leading to early lethality. These phenotypes were accompanied by the death of oligodendrocytes and progressive loss of myelin," Ling says.

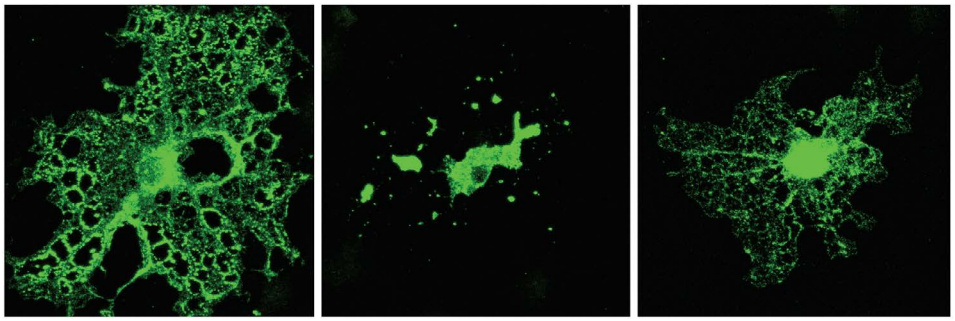
In a new study, Ling and colleagues, including co-first authors Wan Yun Ho, Jer-Cherng Chang, and Kenneth Lim, reveal that one reason oligodendrocytes are dysfunctional in the absence of TDP-43 is that they are unable to synthesize or take up the cholesterol they need to sustain myelin production.

Cholesterol is such a major component of myelin that 25% of the body's total cholesterol can be found in the central nervous system. Oligodendrocytes are known to synthesize large amounts of cholesterol for themselves, but they can also acquire it from other brain cells called astrocytes. Ling and colleagues determined that, in the absence of TDP-43, oligodendrocytes lack many of the enzymes required to synthesize cholesterol and also have reduced levels of the low density lipoprotein receptor that can take in cholesterol from outside the cell. Supplementing these TDP-43–deficient cells with cholesterol restored their ability to maintain the myelin sheath.

Similar defects in cholesterol metabolism may occur in patients, where the formation of aggregates might prevent TDP-43 from performing its normal functions. Ling and colleagues analyzed brain samples from FTD patients and found that their oligodendrocytes produced lower amounts of two key enzymes required for cholesterol synthesis, while the low density lipoprotein receptor was incorporated into TDP-43 aggregates.

"Our results indicate that simultaneous disruption of cholesterol synthesis and uptake is likely one of the causes of the demyelination phenotype observed in mice with TDP-43–deficient oligodendrocytes, and suggest that defects in cholesterol metabolism may contribute to ALS and FTD, as well as other neurodegenerative diseases characterized by TDP-43 aggregates," Ling says.

Drugs that modulate cholesterol metabolism might therefore be a novel therapeutic strategy to treat these diseases, the researchers suggest.



Compared with a normal cell (left), an oligodendrocyte lacking TDP-43 (center) produces less myelin (green) because it is unable to synthesize or take up sufficient amounts of cholesterol. Supplementing TDP-43–deficient cells with cholesterol (right) restores myelin production. © 2021 Ho et al.

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ORIGINAL PAPER

Ho, W.Y., J.-C. Chang, K. Lim, A. Cazenave-Gassiot, A.T. Nguyen, J.C. Foo, S. Muralidharan, A. Viera-Ortiz, S.J.M. Ong, J.H. Hor, I. Agrawal, S. Hoon, O.A. Arogundade, M.J. Rodriguez, S.M. Lim, S.H. Kim, J. Ravits, S.-Y. Ng, M.R. Wenk, E.B. Lee, G. Tucker-Kellogg, and S.-C. Ling. 2021. TDP-43 mediates SREBF2-regulated gene expression required for oligodendrocyte myelination. *J. Cell Biol.* 220 (9): e201910213.

https://doi.org/10.1083/jcb.201910213

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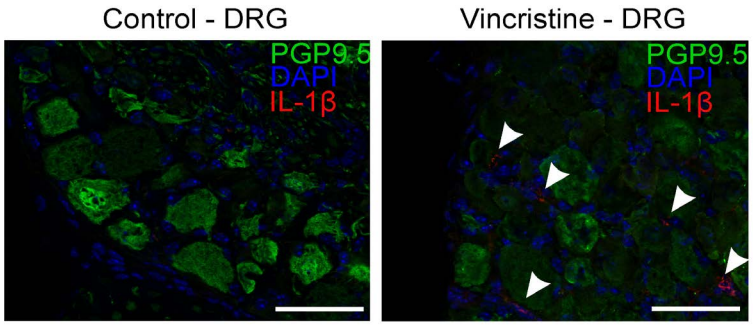
INFLAMMASOME SIGNALING DRIVES VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY

Study suggests that the IL-1 receptor antagonist anakinra may alleviate side effects of a commonly used chemotherapy drug



Vincristine treatment (right) increases the number of cells (arrowheads) expressing IL-1β (red) in close proximity to neurons (green) in the dorsal root ganglia of mice. © 2021 Starobova et al.

Vincristine is a microtubule-targeting chemotherapeutic agent used to treat a variety of adult and pediatric cancers, including childhood leukemias and medulloblastoma. Unfortunately, however, vincristine's side effects include the development of a peripheral neuropathy that can make it difficult to walk properly and causes pain in various parts of the body. This reduces the quality of life of cancer patients and makes it difficult for them to complete their treatment regimen.

Vincristine's effects on peripheral neurons have been attributed to its ability to disrupt microtubule-based transport but, in recent years, it has become clear that vincristine-induced neuropathy also involves neuroinflammatory processes, including the infiltration of peripheral macrophages and the release of proinflammatory cytokines. "As a major molecular complex mediating macrophage-induced inflammation, we wanted to investigate whether the NLRP3 inflammasome drives vincristine-induced neuropathy," explains Irina Vetter, a professor at the Institute for Molecular Bioscience at The University of Queensland.

In response to various proinflammatory signals, NLRP3 assembles into an inflammasome complex that recruits the protease caspase-1, enabling it to cleave and activate the proinflammatory cytokines IL-1β and IL-18. Vetter and colleagues, including first author Hana Starobova and co-senior author Kate Schroder, found that, unlike wild-type animals, mice lacking NLRP3 do not develop gait abnormalities and an increased sensitivity to touch in response to vincristine treatment.

Further experiments revealed that vincristine directly activates the NLRP3 inflammasome in macrophages, stimulating the release of IL-1β. This cytokine is known to sensitize sensory neurons to pain by modulating the activity of various ion channels, and Vetter and colleagues found that mice lacking IL-1β or its receptor, IL-1R, were protected from vincristine-induced touch sensitivity and gait abnormalities.

"We therefore sought to evaluate whether anakinra, an IL-1R antagonist used to treat rheumatoid and juvenile arthritis, could suppress the development of vincristine-induced mechanical allodynia and gait disturbances," Vetter says.

Sure enough, anakinra prevented the onset of these vincristine-induced symptoms in mice. Crucially, the cytokine blocker had no effect on tumor growth or the efficacy of vincristine treatment in a patient-derived xenograft model of medulloblastoma.

"Our results suggest that co-administering anakinra may reduce the suffering of cancer patients treated with vincristine and will enable these patients to carry through with chemotherapy, which, in turn, will lead to better outcomes," Vetter says.

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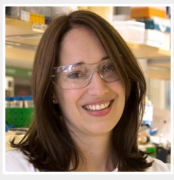

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ORIGINAL PAPER

Starobova, H., M. Monteleone, C. Adolphe, L. Batoon, C.J. Sandrock, B. Tay, J.R. Deuis, A.V. Smith, A. Mueller, E.I. Nadar, G.P. Lawrence, A. Mayor, E. Tolson, J.-P. Levesque, A.R. Pettit, B.J. Wainwright, K. Schroder, and I. Vetter. 2021. Vincristine-induced peripheral neuropathy is driven by canonical NLRP3 activation and IL-1β release. *J. Exp. Med.* 218 (5): e20201452.

https://doi.org/10.1084/jem.20201452

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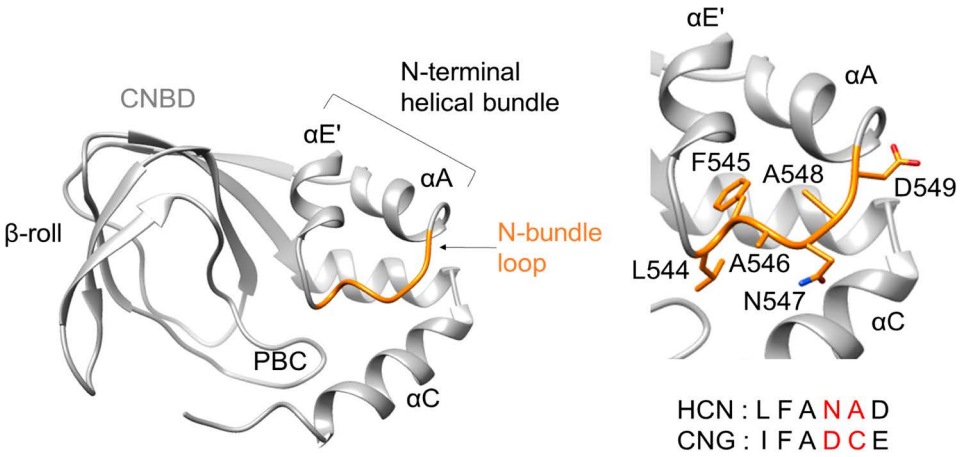
MUTATIONS TO BETTER UNDERSTAND HCN CHANNEL CAMP RESPONSE

Study identifies mutations to investigate the role of the brain protein TRIP8b in limiting the cAMP response of HCN channels in neurons

In the brain, hyperpolarization-activated and cyclic nucleotide–gated (HCN) channels are involved in neural activity, and evidence suggests that improper regulation of the channels is associated with the development of temporal lobe epilepsy. A protein called TRIP8b regulates both the function and localization of HCN channels, but it is hard for researchers to study these two effects of TRIP8b independently. However, Alessandro Porro and Andrea Saponaro and colleagues at the University of Milan, in Italy, developed a solution.

In cortical and hippocampal pyramidal neurons, HCN1 channel subunits are targeted to the distal regions of apical dendrites, where HCN channels control dendritic excitability. This is modulated by direct cAMP binding to HCN. TRIP8b interacts with HCN channels in two regions of HCN—one that ensures proper trafficking and another in HCN's cyclic nucleotide–binding domain (CNBD), where TRIP8b competes with cAMP binding and negatively shifts the voltage-dependent gating of HCN channels.



Structural representation of the CNBD domain of HCN2 (left). On the right is a close-up view showing the residues of the N-bundle loop, labeled in orange, where the two-point mutations (N547D/A548C) were introduced to reduce binding of TRIP8b and therefore limit TRIP8b's ability to modulate the cAMP response of HCN channels. © 2020 Porro et al.

Saponaro explained that "it would be useful to have an experimental tool able to dissect the two effects of TRIP8b on the channel in order to test the role of TRIP8b in modulating cAMP binding without altering expression and localization of the channel in the neuron."

Using a detailed atomic structure, the team identified possible mutations in HCN that would disrupt TRIP8b's interaction with the CNBD. They found that when combined, two-point mutations (N547D/A548C) in the loop connecting the CNBD to another part of the HCN structure, called the C-linker (N-bundle loop), strongly reduced the binding of TRIP8b to the CNBD. They confirmed that HCN's cAMP affinity was not changed by these two mutations, and, in primary cultured cortical neurons, the team showed that the double mutant inhibited the ability of TRIP8b to modulate HCN channel gating but did not perturb the regulation of HCN trafficking and expression.

Saponaro believes the HCN mutations will be useful in future research, "Given the fact that hippocampal and entorhinal neurons are both part of the network controlling spatial learning, the N547D/A548C genetic tool may become crucial in advancing the understanding of the hyperpolarization-activated cation current and cAMP signaling in the development of spatial memory and navigation."

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ORIGINAL PAPER

Porro, A., A. Binda, M. Pisoni, C. Donadoni, I. Rivolta, and A. Saponaro. 2020. Rational design of a mutation to investigate the role of the brain protein TRIP8b in limiting the cAMP response of HCN channels in neurons. *J. Gen. Physiol.* 152 (9): e202012596.

https://doi.org/10.1085/jgp.202012596

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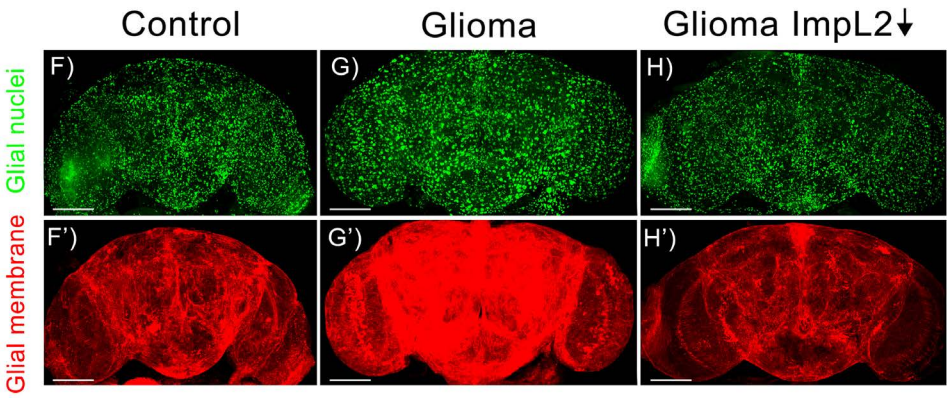
INSULIN SIGNALING IS INVOLVED IN GLIOBLASTOMA PROGRESSION

Study shows that insulin signaling is diminished in a* Drosophila *model of glioblastoma, and restoring insulin signaling halts tumor progression

Glioblastoma is one of the most aggressive kinds of cancers, and even with current treatment, it generally causes death within a year of diagnosis. A team led by Patricia Jarabo, Francisco Antonio Martín, and Sergio Casas-Tintó of the Cajal Institute in Madrid, Spain, revealed an important role for insulin signaling in a glioblastoma model, which they found could be targeted to halt tumor progression.

Past research hinted that there could be a link between insulin signaling and glioblastoma. In glioblastoma patients, studies showed that there is an increase in *insulin-like growth binding protein 7* (*IGFBP7*) gene expression. Additionally, short noncoding RNAs that control gene activity, called microRNAs, regulate insulin signaling genes—low levels of *miR-200*, which are associated with poor prognosis in glioblastoma, are linked to higher levels of IGFBP7. And research indicated that factors with a role in insulin signaling are involved in synaptic communication. Furthermore, there is loss of neurons and synapses in glioblastoma.

Glioblastoma originates in glial cells, and Jarabo and colleagues investigated whether tumoral glial cells are able to modify insulin signaling in surrounding neurons and whether microRNAs regulate insulin signaling genes during tumor progression. To do this, the team used a *Drosophila* glioblastoma model with glial expression of two of the most frequent mutations found in glioblastoma patients: constitutively active forms of the epidermal growth factor receptor (EGFR) and the phosphatidylinositol-3 kinase catalytic subunit p110α (PI3K), which recapitulates many of the features of the human disease.

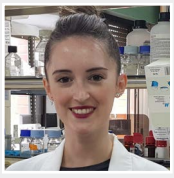


In a *Drosophila* model of glioblastoma, downregulating *ImpL2*, the homologue of insulin-like growth binding protein 7 (IGFBP7) (right), reduced the number of glial cells and tumor volume, indicating a role of insulin signaling in glioblastoma. © 2020 Jarabo et al.

The researchers observed an increase in expression of *ImpL2*, the *Drosophila* homologue of *IGFBP7*, which was associated with synapse loss. Glial cells secreted ImpL2 that truncates insulin signaling. Decreasing *ImpL2* expression counteracted the reduction in synapses and inhibited tumor progression. The team also found that the fly homologue of *miR-200*, called *miR-8*, regulated ImpL2, with increasing levels of *miR-8* inhibiting synapse loss. Furthermore, restoring insulin signaling by overexpressing a protein in the insulin signaling network, called Rheb, reduced tumor volume and extended the lifespan of the flies.

Together, the results "suggest that reducing insulin signaling, and the subsequent neurodegeneration, is critical for glioblastoma progression and invasion and ultimately for the lethality caused by glioblastoma," says Casas-Tintó.

RESEARCHER DETAILS



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ORIGINAL PAPER

Jarabo, P., C. de Pablo, H. Herranz, F.A. Martín, and S. Casas-Tintó. 2020. Insulin signaling mediates neurodegeneration in glioma. *Life Sci Alliance*. 4 (3): e202000693

http://doi.org/10.26508/lsa.202000693

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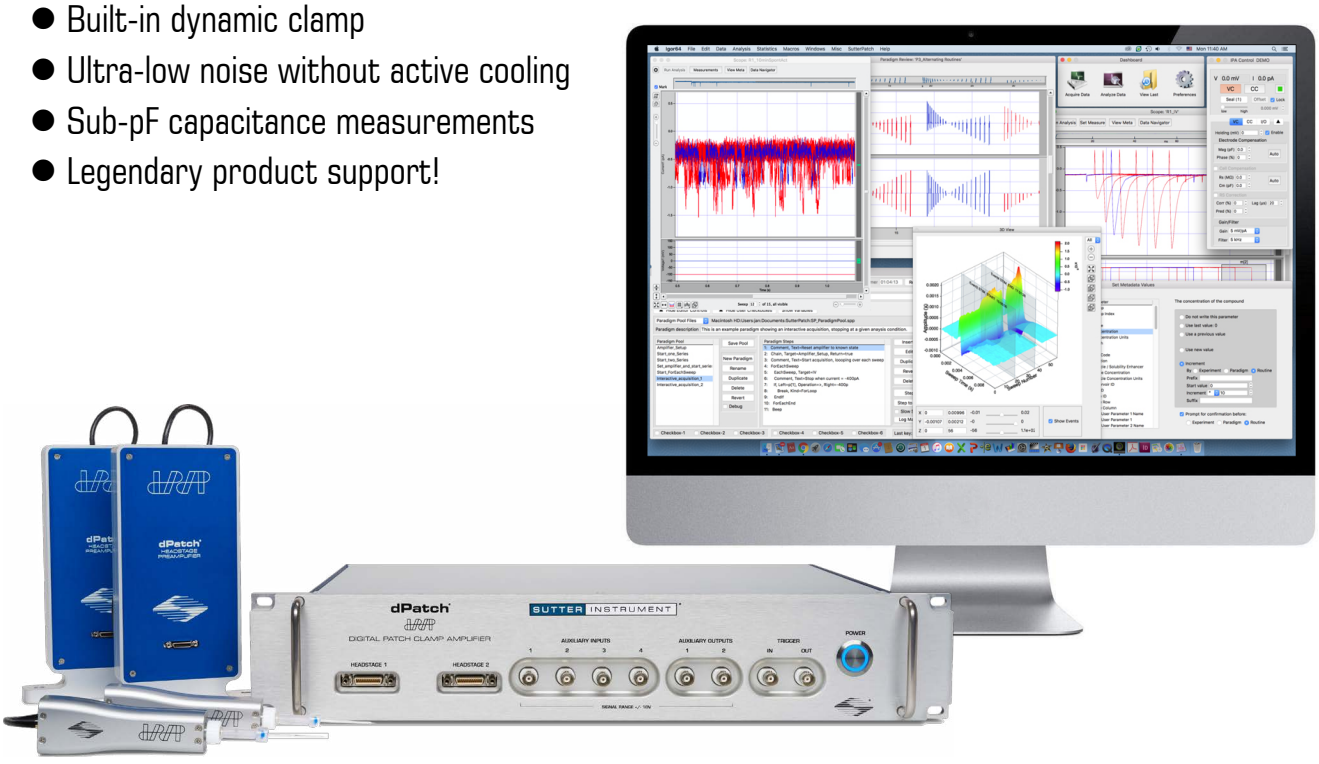

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