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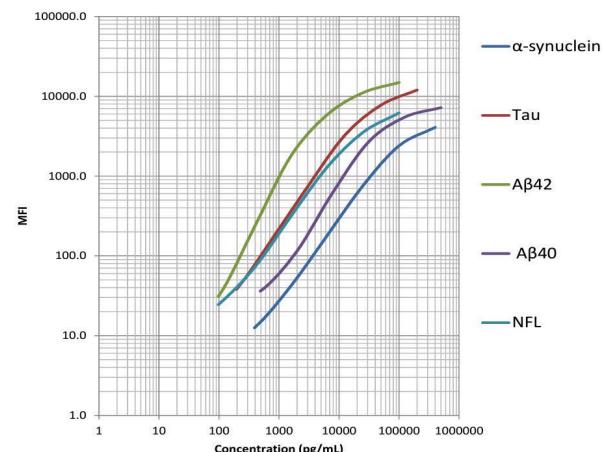
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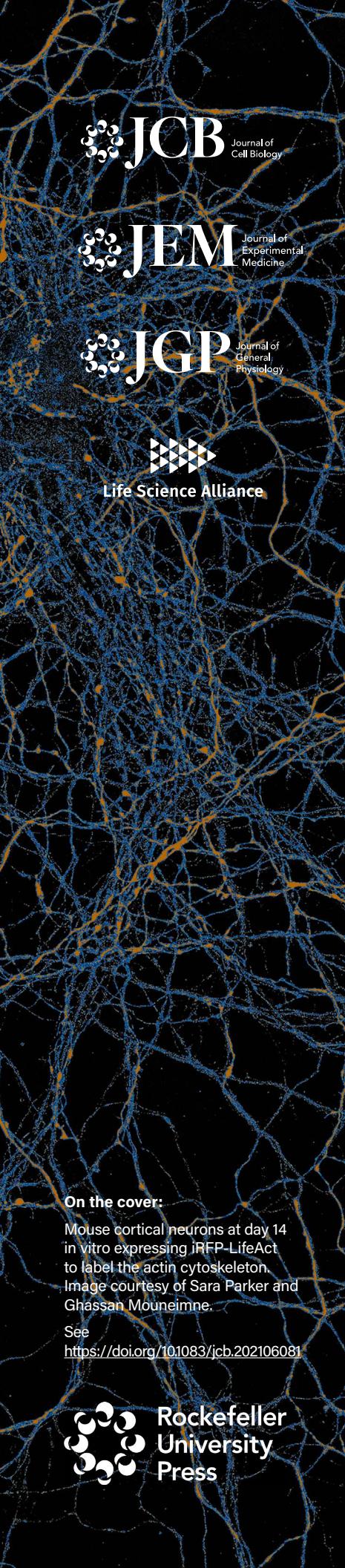
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The editors of the *Journal of Cell Biology* (JCB), *Journal of Experimental Medicine* (JEM), *Journal of General Physiology* (JGP), and *Life Science Alliance* (LSA) are pleased to present special collections of recently published articles that elucidate new advances within the field of neuroscience. If you enjoy the selected abstracts included in this magazine, we encourage you to scan the QR codes to view the full online collections and sign up for email alerts to receive the latest research.

Distinct actin nanostructures at presynapses	 6
Dominic Bingham... Marie-Jeanne Papandréou, and Christophe Leterrier	
Systematic analysis of mRNA/protein discordance in neural tissues	 6
Joshua Titlow, Maria Kiourlappou, Ana Palanca, Jeffrey Y. Lee... Ilan Davis	
LMX1B-autophagy crosstalk protects midbrain dopaminergic neurons	 7
Natalia Jiménez-Moreno... Jon D. Lane	
Microglia resolve aberrant myelination during development	 7
Minou Djannatian... Mikael Simons	
δ-Catenin controls astrocyte morphology	 8
Christabel Xin Tan... Cagla Eroglu	
New gene-editing technique reverses vision loss in mice	 9
Huan Qin, Wenliang Zhang, Shiyao Zhang, Yuan Feng... Kai Yao	
Modeling tauopathy in wild-derived mice	 9
Dominic J. Acri... Jungsu Kim	
Reexamining the function of arachnoid granulations	 10
Trishna Shah... Rupal I. Mehta	
TREM2 activation increases Aβ-induced tau pathology	 10
Nimansha Jain... David M. Holtzman	
CD8⁺ T cells drive demyelination in Krabbe's disease	 11
Pearl A. Sutter... Stephen J. Crocker	
Piezo1 regulates cholesterol biosynthesis in neural stem cells	 12
Jamison L. Nourse... Medha M. Pathak	
Five channel types underlie a BK channelopathy	 12
Yanyan Geng, Ping Li, Alice Butler... Lawrence Salkoff, Karl L. Magleby	
Temperature intensifies pain-linked sodium channel gating	 13
Sophia Kriegeskorte... Ralf Hausmann, Angelika Lampert	
D-type K⁺ current controls lateral excitation between coupled neurons	 13
Antonella Dapino... Sebastian Curti	
Epilepsy-associated SCN2A variants exhibit complex properties	 14
Christopher H. Thompson, Franck Potet... Alfred L. George, Jr.	
Parenchymal border macrophages regulate tau pathology	 15
Antoine Drieu... David M. Holtzman, Maud Gratuze	
Bats experience hearing loss in old age	 15
Yifat Chaya Tarnovsky... Yossi Yovel	
New botulinum constructs for pain relief	 16
Charlotte Leese, Claire Christmas... Bazbek Davletov	
Rational design of Parkin-activating mutations	 16
Michael U. Stevens, Nathalie Croteau, Mohamed A. Eldeeb... Edward A. Fon, Miratul M.K. Muqit, Jean-François Trempe	
ApoE4 intersects with neuronal Aβ	 17
Sabine C. Konings... Gunnar K. Gouras	

On the cover:

Mouse cortical neurons at day 14 in vitro expressing iRFP-LifeAct to label the actin cytoskeleton. Image courtesy of Sara Parker and Ghassan Mouneimne.

See

<https://doi.org/10.1083/jcb.202106081>

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DISTINCT ACTIN NANOSTRUCTURES AT PRESYNAPSES

The architecture of the actin cytoskeleton that concentrates at presynapses remains poorly known, hindering our understanding of its roles in synaptic physiology. In this work, we measure and visualize presynaptic actin by diffraction-limited and super-resolution microscopy, thanks to a validated model of bead-induced presynapses in cultured neurons.

We identify a major population of actin-enriched presynapses that concentrates more presynaptic components and shows higher synaptic vesicle cycling than their non-enriched counterparts. Pharmacological perturbations point to an optimal actin amount and

the presence of distinct actin structures within presynapses. We directly visualize these nanostructures using Single Molecule Localization Microscopy, defining three distinct types: an actin mesh at the active zone, actin rails between the active zone and deeper reserve pools, and actin corrals around the whole presynaptic compartment.

Finally, CRISPR tagging of endogenous actin allows us to validate our results in natural synapses between cultured neurons, confirming the role of actin enrichment and the presence of three types of presynaptic actin nanostructures.

ORIGINAL PAPER

Bingham, D., C.E. Jakobs, F. Wernert, F. Boroni-Rueda, N. Jullien, E.-M. Schentarra, K. Friedl, J. Da Costa Moura, D.M. van Bommel, G. Caillol, Y. Ogawa, M.-J. Papandréou, and C. Leterrier. 2023. Presynapses contain distinct actin nanostructures. *J. Cell Biol.* 222 (10): e202208110. <https://doi.org/10.1083/jcb.202208110>

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SYSTEMATIC ANALYSIS OF mRNA/PROTEIN DISCORDANCE IN NEURAL TISSUES

While post-transcriptional control is thought to be required at the periphery of neurons and glia, its extent is unclear. We investigate systematically the spatial distribution and expression of mRNA at single molecule sensitivity and their corresponding proteins of 200 YFP trap lines across the intact *Drosophila* nervous system.

97.5% of the genes studied showed discordance between the distribution of mRNA and the proteins they encode in at least one region of the nervous system. These data suggest

that post-transcriptional regulation is very common, helping to explain the complexity of the nervous system. We also discovered that 68.5% of these genes have transcripts present at the periphery of neurons, with 9.5% at the glial periphery. Peripheral transcripts include many potential new regulators of neurons, glia, and their interactions.

Our approach is applicable to most genes and tissues and includes powerful novel data annotation and visualization tools for post-transcriptional regulation.

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ORIGINAL PAPER

Titlow, J.S., M. Kiourlappou, A. Palanca, J.Y. Lee, D.S. Gala, D. Ennis, J.J.S. Yu, F.L. Young, D.M. Susano Pinto, S. Garforth, H.S. Francis, F. Strivens, H. Mulvey, A. Dallman-Porter, S. Thornton, D. Arman, M.J. Millard, A.I. Järvelin, M.K. Thompson, M. Sargent, I. Kounatidis, R.M. Parton, S. Taylor, and I. Davis. 2023. Systematic analysis of YFP traps reveals common mRNA/protein discordance in neural tissues. *J. Cell Biol.* 222 (6): e202205129. <https://doi.org/10.1083/jcb.202205129>

LMX1B-AUTOPHAGY CROSSTALK PROTECTS MIDBRAIN DOPAMINERGIC NEURONS

The LIM homeodomain transcription factors LMX1A and LMX1B are essential mediators of midbrain dopaminergic neuronal (mDAN) differentiation and survival. We show that LMX1A and LMX1B are autophagy transcription factors that provide cellular stress protection. Their suppression dampens the autophagy response, lowers mitochondrial respiration, and elevates mitochondrial ROS, and their inducible overexpression protects against rotenone toxicity in human iPSC-derived mDANs *in vitro*.

Significantly, we show that LMX1A and LMX1B stability is in part regulated by

autophagy and that these transcription factors bind to multiple ATG8 proteins. Binding is dependent on subcellular localization and nutrient status, with LMX1B interacting with LC3B in the nucleus under basal conditions and associating with both cytosolic and nuclear LC3B during nutrient starvation.

Crucially, ATG8 binding stimulates LMX1B-mediated transcription for efficient autophagy and cell stress protection, thereby establishing a novel LMX1B-autophagy regulatory axis that contributes to mDAN maintenance and survival in the adult brain.

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ORIGINAL PAPER

Jiménez-Moreno, N., M. Kollareddy, P. Stathakos, J.J. Moss, Z. Antón, D.K. Shoemark, R.B. Sessions, R. Witzgall, M. Caldwell, and J.D. Lane. 2023. ATG8-dependent LMX1B-autophagy crosstalk shapes human midbrain dopaminergic neuronal resilience. *J. Cell Biol.* 222 (5): e201910133. <https://doi.org/10.1083/jcb.201910133>

MICROGLIA RESOLVE ABERRANT MYELINATION DURING DEVELOPMENT

To enable rapid propagation of action potentials, axons are ensheathed by myelin, a multilayered insulating membrane formed by oligodendrocytes. Most of the myelin is generated early in development, resulting in the generation of long-lasting stable membrane structures. We explored structural and dynamic changes in central nervous system myelin during development.

To achieve this, we performed an ultrastructural analysis of mouse optic nerves by serial block face scanning electron microscopy and confocal time-lapse imaging in the zebrafish spinal cord. We found that myelin

undergoes extensive ultrastructural changes during early postnatal development. Myelin degeneration profiles were engulfed and phagocytosed by microglia using exposed phosphatidylserine as one “eat me” signal. In contrast, retractions of entire myelin sheaths occurred independently of microglia and involved uptake of myelin by the oligodendrocyte itself.

Our findings show that the generation of myelin early in development is an inaccurate process associated with aberrant ultrastructural features that require substantial refinement.

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ORIGINAL PAPER

Djannatian, M., S. Radha, U. Weikert, S. Safaiyan, C. Wrede, C. Deichsel, G. Kislinger, A. Rhomberg, T. Ruhwedel, D.S. Campbell, T. van Ham, B. Schmid, J. Hegermann, W. Möbius, M. Schifferer, and M. Simons. 2023. Myelination generates aberrant ultrastructure that is resolved by microglia. *J. Cell Biol.* 222 (3): e202204010. <https://doi.org/10.1083/jcb.202204010>

δ-CATENIN CONTROLS ASTROCYTE MORPHOLOGY

Astrocytes control the formation of specific synaptic circuits via cell adhesion and secreted molecules. Astrocyte synaptogenic functions are dependent on the establishment of their complex morphology. However, it is unknown if distinct neuronal cues differentially regulate astrocyte morphogenesis.

δ-Catenin was previously thought to be a neuron-specific protein that regulates dendrite morphology. We found δ-catenin is also highly expressed by astrocytes and required both in astrocytes and neurons for astrocyte morphogenesis. δ-Catenin is hypothesized to mediate transcellular interactions through the cadherin family of cell adhesion proteins. We used structural

modeling and biochemical analyses to reveal that δ-catenin interacts with the N-cadherin juxtamembrane domain to promote N-cadherin surface expression. An autism-linked δ-catenin point mutation impaired N-cadherin cell surface expression and reduced astrocyte complexity.

In the developing mouse cortex, only lower-layer cortical neurons express N-cadherin. Remarkably, when we silenced astrocytic N-cadherin throughout the cortex, only lower-layer astrocyte morphology was disrupted. These findings show that δ-catenin controls astrocyte–neuron cadherin interactions that regulate layer-specific astrocyte morphogenesis.

ORIGINAL PAPER

Tan, C.X., D.S. Bindu, E.J. Hardin, K. Sakers, R. Baumert, J.J. Ramirez, J.T. Savage, and C. Eroglu. 2023. δ-Catenin controls astrocyte morphogenesis via layer-specific astrocyte–neuron cadherin interactions. *J. Cell Biol.* 222 (11): e202303138. <https://doi.org/10.1083/jcb.202303138>

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NEW GENE-EDITING TECHNIQUE REVERSES VISION LOSS IN MICE

Retinitis pigmentosa (RP) is an inherited retinal dystrophy causing progressive and irreversible loss of retinal photoreceptors. We developed a genome-editing tool characterized by the versatility of prime editors (PEs) and unconstrained protospacer adjacent motif (PAM) requirement of a SpCas9 variant (SpRY), referred to as PE^{SpRY}.

The diseased retinas of a *Pde6b*-associated RP mouse model were transduced via a dual adeno-associated virus system packaging PE^{SpRY} for in vivo genome editing through a non-NGG PAM (GTG). The progressing cell loss was reversed once the mutation was corrected, leading to substantial

rescue of photoreceptors and production of functional PDE6 β . The treated mice exhibited significant responses in electroretinogram and displayed good performance in both passive and active avoidance tests. Moreover, they presented an apparent improvement in visual stimuli-driven optomotor responses and efficiently completed visually guided water-maze tasks.

Together, our study provides convincing evidence for the prevention of vision loss caused by RP-associated gene mutations via unconstrained in vivo prime editing in the degenerating retinas.

ORIGINAL PAPER

Qin, H., W. Zhang, S. Zhang, Y. Feng, W. Xu, J. Qi, Q. Zhang, C. Xu, S. Liu, J. Zhang, Y. Lei, W. Liu, S. Feng, J. Wang, X. Fu, Z. Xu, P. Li, and K. Yao. 2023. Vision rescue via unconstrained in vivo prime editing in degenerating neural retinas. *J. Exp. Med.* 220 (5): e20220776. <https://doi.org/10.1084/jem.20220776>

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MODELING TAUOPATHY IN WILD-DERIVED MICE

Previous research demonstrated that genetic heterogeneity is a critical factor in modeling amyloid accumulation and other Alzheimer's disease phenotypes. However, it is unknown what mechanisms underlie these effects of genetic background on modeling tau aggregate-driven pathogenicity.

In this study, we induced tau aggregation in wild-derived mice by expressing *MAPT*. To investigate the effect of genetic background on the action of tau aggregates, we performed RNA sequencing with brains of C57BL/6J, CAST/EiJ, PWK/PhJ, and WSB/EiJ mice ($n = 64$) and determined core

transcriptional signature conserved in all genetic backgrounds and signature unique to wild-derived backgrounds. By measuring tau seeding activity using the cortex, we identified 19 key genes associated with tau seeding and amyloid response. Interestingly, microglial pathways were strongly associated with tau seeding activity in CAST/EiJ and PWK/PhJ backgrounds. Collectively, our study demonstrates that mouse genetic context affects tau-mediated alteration of transcriptome and tau seeding. The gene modules associated with tau seeding provide an important resource to better model tauopathy.

ORIGINAL PAPER

Acri, D.J., Y. You, M.D. Tate, H. Karahan, P. Martinez, B. McCord, A.D. Sharify, S. John, B. Kim, L.C. Dabin, S. Philtjens, H.R.S. Wijeratne, T.J. McCray, D.C. Smith, S.J. Bissel, B.T. Lamb, C.A. Lasagna-Reeves, and J. Kim. 2023. Network analysis identifies strain-dependent response to tau and tau seeding-associated genes. *J. Exp. Med.* 220 (11): e20230180. <https://doi.org/10.1084/jem.20230180>

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REEXAMINING THE FUNCTION OF ARACHNOID GRANULATIONS

Arachnoid granulations (AG) are poorly investigated structures that uniquely localize to the mammalian brain–dura interface. Historical reports suggest that they regulate brain volume by passively transporting cerebrospinal fluid (CSF) into dural venous sinuses. We studied the microstructure of cerebral AG in humans with the aim of understanding their roles in physiology.

We discovered marked variations in AG size, lobation, location, content, and degree of surface encapsulation. High-resolution microscopy shows that AG consist of outer capsule and inner stromal core regions. The fine and porous framework suggests uncharac-

terized functions of AG in mechanical CSF filtration. Moreover, internal cytokine and immune cell enrichment imply unexplored neuroimmune properties of these structures that localize to the brain–meningeal lymphatic interface. Dramatic age-associated changes in AG structure are additionally identified.

This study depicts for the first time microscopic networks of internal channels that communicate with perisinus spaces, suggesting that AG subserve important functions as transsarachnoidal flow passageways. These data raise new theories regarding glymphatic–lymphatic coupling and mechanisms of CSF antigen clearance, homeostasis, and diseases.

ORIGINAL PAPER

Shah, T., S.E. Leurgans, R.I. Mehta, J. Yang, C.A. Galloway, K.L. de Mesy Bentley, J.A. Schneider, and R.I. Mehta. 2023. Arachnoid granulations are lymphatic conduits that communicate with bone marrow and dura-arachnoid stroma. *J. Exp. Med.* 220 (2): e20220618. <https://doi.org/10.1084/jem.20220618>

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TREM2 ACTIVATION INCREASES A β -INDUCED TAU PATHOLOGY

Variants in the triggering receptor expressed on myeloid cells 2 (*TREM2*) gene are associated with increased risk for late-onset Alzheimer's disease (AD). Genetic loss of or decreased *TREM2* function impairs the microglial response to amyloid- β (A β) plaques, resulting in more diffuse A β plaques and increased peri-plaque neuritic dystrophy and AD-tau seeding. Thus, microglia and *TREM2* are at a critical intersection of A β and tau pathologies in AD.

Since genetically decreasing *TREM2* function increases A β -induced tau seeding, we hypothesized that chronically increasing *TREM2* signaling would decrease amyloid-induced tau

seeding and spreading. Using a mouse model of amyloidosis in which AD-tau is injected into the brain to induce A β -dependent tau seeding/spreading, we found that chronic administration of an activating *TREM2* antibody increases peri-plaque microglial activation but surprisingly increases peri-plaque neuritic plaque-tau pathology and neuritic dystrophy without altering A β plaque burden.

Our data suggest that sustained microglial activation through *TREM2* that does not result in strong amyloid removal may exacerbate A β -induced tau pathology, which may have important clinical implications.

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ORIGINAL PAPER

Jain, N., C.A. Lewis, J.D. Ulrich, and D.M. Holtzman. 2023. Chronic *TREM2* activation exacerbates A β -associated tau seeding and spreading. *J. Exp. Med.* 220 (1): e20220654. <https://doi.org/10.1084/jem.20220654>

CD8⁺ T CELLS DRIVE DEMYELINATION IN KRABBE'S DISEASE

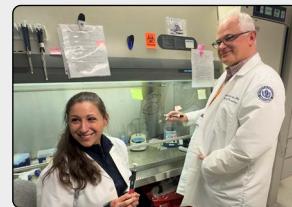
Globoid cell leukodystrophy (GLD), or Krabbe's disease, is a fatal genetic demyelinating disease of the central nervous system (CNS) caused by loss-of-function mutations in the galactosylceramidase (galc) gene. While the metabolic basis for disease is known, the understanding of how this results in neuropathology is not well understood.

We report that the rapid and protracted elevation of CD8⁺ cytotoxic T lymphocytes occurs coincident with clinical disease in a mouse model of GLD. Administration of a function-blocking antibody against CD8α effectively pre-

vented disease onset, reduced morbidity and mortality, and prevented CNS demyelination in mice.

These data indicate that subsequent to the genetic cause of disease, neuropathology is driven by pathogenic CD8⁺ T cells, thus offering novel therapeutic potential for treatment of GLD.

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ORIGINAL PAPER

Sutter, P.A., A. Ménoret, E.R. Jellison, A.M. Nicaise, A.M. Bradbury, A.T. Vella, E.R. Bongarzone, and S.J. Crocker. 2023. CD8⁺ T cell depletion prevents neuropathology in a mouse model of globoid cell leukodystrophy. *J. Exp. Med.* 220 (9): e20221862. <https://doi.org/10.1084/jem.20221862>

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PIEZ01 REGULATES CHOLESTEROL BIOSYNTHESIS IN NEURAL STEM CELLS

Mechanical forces and tissue mechanics influence the morphology of the developing brain, but the underlying molecular mechanisms have been elusive. We examine the role of mechanotransduction in brain development by focusing on *Piezo1*, a mechanically activated ion channel.

We find that *Piezo1* deletion results in a thinner neuroepithelial layer, disrupts pseudostratification, and reduces neurogenesis in E10.5 mouse embryos. Proliferation and differentiation of *Piezo1* knockout (KO) mouse neural stem cells (NSCs) isolated from E10.5 embryos are reduced in vitro compared to littermate WT NSCs. Transcriptome analysis of E10.5 *Piezo1* KO brains reveals downregulation of the cholesterol biosynthesis superpathway,

in which 16 genes, including *Hmgcr*, the gene encoding the rate-limiting enzyme of the cholesterol biosynthesis pathway, are downregulated by 1.5-fold or more. Consistent with this finding, membrane lipid composition is altered, and the cholesterol levels are reduced in *Piezo1* KO NSCs. Cholesterol supplementation of *Piezo1* KO NSCs partially rescues the phenotype in vitro.

These findings demonstrate a role for *Piezo1* in the neurodevelopmental process that modulates the quantity, quality, and organization of cells by influencing cellular cholesterol metabolism. Our study establishes a direct link in NSCs between PIEZO1, intracellular cholesterol levels, and neural development.

ORIGINAL PAPER

Nourse, J.L., V.M. Leung, H. Abuwarda, E.L. Evans, E. Izquierdo-Ortiz, A.T. Ly, N. Truong, S. Smith, H. Bhavsar, G. Bertaccini, E.S. Monuki, M.M. Panicker, and M.M. Pathak. 2022. *Piezo1* regulates cholesterol biosynthesis to influence neural stem cell fate during brain development. *J. Gen. Physiol.* 154 (10): e202213084. <https://doi.org/10.1085/jgp.202213084>

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FIVE CHANNEL TYPES UNDERLIE A BK CHANNELOPATHY

The molecular basis of Liang-Wang Syndrome, a severe developmental and neurological disorder associated with a de novo G375R variant of the tetrameric BK channel, is unknown. We address this question by recording from single BK channels expressed to mimic a G375R mutation heterozygous with a WT allele.

Five different types of functional BK channels were expressed: 3% were consistent with WT, 12% with homotetrameric mutant, and 85% with three different types of hybrid (heterotetrameric) channels assembled from both mutant and WT subunits. All channel types except WT showed a marked gain of function in voltage activation and a smaller decrease of function in single-channel conductance, with both changes in function becoming more pronounced as the number of mutant subunits per tetrameric channel increased. The net cellular response

from the five different types of channels comprising the molecular phenotype was a shift of -120 mV in the voltage required to activate half of the maximal current through BK channels, giving a net gain of function.

The WT and homotetrameric mutant channels in the molecular phenotype were consistent with genetic codominance as each displayed properties of a channel arising from only one of the two alleles. The three types of hybrid channels in the molecular phenotype were consistent with partial dominance as their properties were intermediate between those of mutant and WT channels. A model in which BK channels randomly assemble from mutant and WT subunits, with each subunit contributing increments of activation and conductance, approximated the molecular phenotype of the heterozygous G375R mutation.

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Geng, Y., P. Li, A. Butler, B. Wang, L. Salkoff, and K.L. Magleby. 2023. BK channels of five different subunit combinations underlie the de novo KCNMA1 G375R channelopathy. *J. Gen. Physiol.* 155 (5): e202213302. <https://doi.org/10.1085/jgp.202213302>

TEMPERATURE INTENSIFIES PAIN-LINKED SODIUM CHANNEL GATING

Voltage-gated sodium channels (Na_v) are key players in excitable tissues with the capability to generate and propagate action potentials. Mutations in the genes encoding Na_v s can lead to severe inherited diseases, and some of these so-called channelopathies show temperature-sensitive phenotypes, for example, paramyotonia congenita, Brugada syndrome, febrile seizure syndromes, and inherited pain syndromes like erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD). Nevertheless, most investigations of mutation-induced gating effects have been conducted at room temperature, and thus the role of cooling or warming in channelopathies remains poorly understood.

We investigated the temperature sensitivity of four Na_v subtypes: $Na_v1.3$, $Na_v1.5$, $Na_v1.6$, and $Na_v1.7$, and two mutations in $Na_v1.7$ causing IEM ($Na_v1.7/L823R$) and PEPD

($Na_v1.7/I1461T$) expressed in cells of the human embryonic kidney cell line using an automated patch clamp system. Our experiments at 15°C, 25°C, and 35°C revealed a shift of the voltage dependence of activation to more hyperpolarized potentials with increasing temperature for all investigated subtypes. $Na_v1.3$ exhibited strongly slowed inactivation kinetics compared with the other subtypes that resulted in enhanced persistent current, especially at 15°C, indicating a possible role in cold-induced hyperexcitability. Impaired fast inactivation of $Na_v1.7/I1461T$ was significantly enhanced by a cooling temperature of 15°C.

The subtype-specific modulation as well as the intensified mutation-induced gating changes stress the importance to consider temperature as a regulator for channel gating and its impact on cellular excitability as well as disease phenotypes.

ORIGINAL PAPER

Kriegeskorte, S., R. Bott, M. Hampl, A. Korngreen, R. Hausmann, and A. Lampert. 2023. Cold and warmth intensify pain-linked sodium channel gating effects and persistent currents. *J. Gen. Physiol.* 155 (9): e202213312. <https://doi.org/10.1085/jgp.202213312>

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D-TYPE K⁺ CURRENT CONTROLS LATERAL EXCITATION BETWEEN COUPLED NEURONS

Electrical synapses supported by gap junctions are known to form networks of electrically coupled neurons in many regions of the mammalian brain, where they play relevant functional roles. Yet, how electrical coupling supports sophisticated network operations and the contribution of the intrinsic electrophysiological properties of neurons to these operations remain incompletely understood.

Through a comparative analysis of electrically coupled mesencephalic trigeminal (MesV) neurons, we uncovered remarkable differences in the operation of these networks in highly related species. While spiking of MesV neurons might support the recruitment of coupled cells in rats, this rarely occurs in mice. Using whole-cell recordings, we determined that the higher efficacy in postsynaptic recruitment in rat MesV neurons does not result from

coupling strength of larger magnitude, but instead from the higher excitability of coupled neurons. Consistently, MesV neurons from rats present a lower rheobase, more hyperpolarized threshold, as well as a higher ability to generate repetitive discharges, in comparison to their counterparts from mice.

This difference in neuronal excitability results from a significantly higher magnitude of the D-type K⁺ current in MesV neurons from mice, indicating that the magnitude of this current gates the recruitment of postsynaptic-coupled neurons. Since MesV neurons are primary afferents critically involved in the organization of orofacial behaviors, activation of a coupled partner could support lateral excitation, which by amplifying sensory inputs may significantly contribute to information processing and the organization of motor outputs.

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ORIGINAL PAPER

Dapino, A., F. Davoine, and S. Curti. 2023. D-type K⁺ current rules the function of electrically coupled neurons in a species-specific fashion. *J. Gen. Physiol.* 155 (9): e202313353. <https://doi.org/10.1085/jgp.202313353>

EPILEPSY-ASSOCIATED SCN2A VARIANTS EXHIBIT COMPLEX PROPERTIES

Pathogenic variants in voltage-gated sodium (Na_v) channel genes including *SCN2A*, encoding $Na_v1.2$, are discovered frequently in neurodevelopmental disorders with or without epilepsy. *SCN2A* is also a high-confidence risk gene for autism spectrum disorder (ASD) and nonsyndromic intellectual disability (ID). Previous work to determine the functional consequences of *SCN2A* variants yielded a paradigm in which predominantly gain-of-function variants cause neonatal-onset epilepsy, whereas loss-of-function variants are associated with ASD and ID. However, this framework was derived from a limited number of studies conducted under heterogeneous experimental conditions, whereas most disease-associated *SCN2A* variants have not been functionally annotated.

We determined the functional properties of *SCN2A* variants using automated patch-clamp recording to demonstrate the validity of this method and to examine whether a binary classification of variant dysfunction

is evident in a larger cohort studied under uniform conditions. We studied 28 disease-associated variants and 4 common variants using two alternatively spliced isoforms of $Na_v1.2$ expressed in HEK293T cells. Automated patch-clamp recording provided a valid high throughput method to ascertain detailed functional properties of $Na_v1.2$ variants with concordant findings for variants that were previously studied using manual patch clamp. Many epilepsy-associated variants in our study exhibited complex patterns of gain- and loss-of-functions that are difficult to classify by a simple binary scheme.

The higher throughput achievable with automated patch clamp enables study of variants with greater standardization of recording conditions, freedom from operator bias, and enhanced experimental rigor. This approach offers an enhanced ability to discern relationships between channel dysfunction and neurodevelopmental disorders.

ORIGINAL PAPER

Thompson, C.H., F. Potet, T.V. Abramova, J.-M. DeKeyser, N.F. Ghabra, C.G. Vanoye, J.J. Millichap, and A.L. George. 2023. Epilepsy-associated *SCN2A* ($Na_v1.2$) variants exhibit diverse and complex functional properties. *J. Gen. Physiol.* 155 (10): e202313375. <https://doi.org/10.1085/jgp.202313375>

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PARENCHYMAL BORDER MACROPHAGES REGULATE TAU PATHOLOGY

Parenchymal border macrophages (PBMs) reside close to the central nervous system parenchyma and regulate cerebral spinal fluid (CSF) flow dynamics. We recently demonstrated that PBMs provide a clearance pathway for amyloid- β peptide, which accumulates in the brain in Alzheimer's disease (AD).

Given the emerging role for PBMs in AD, we explored how tau pathology affects the CSF flow and the PBM populations in the PS19 mouse model of tau pathology. We demonstrated a reduction of CSF flow, and an increase in an MHCII $^+$ PBM subpopulation in

PS19 mice compared with WT littermates. Consequently, we asked whether PBM dysfunction could exacerbate tau pathology and tau-mediated neurodegeneration. Pharmacological depletion of PBMs in PS19 mice led to an increase in tau pathology and tau-dependent neurodegeneration, which was independent of gliosis or aquaporin-4 depolarization, essential for the CSF-interstitial fluid exchange. Together, our results identify PBMs as novel cellular regulators of tau pathology and tau-mediated neurodegeneration.

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Drieu, A., S. Du, M. Kipnis, M.E. Bosch, J. Herz, C. Lee, H. Jiang, M. Manis, J.D. Ulrich, J. Kipnis, D.M. Holtzman, and M. Gratuze. 2023. Parenchymal border macrophages regulate tau pathology and tau-mediated neurodegeneration. *Life Science Alliance*. 6 (11) e202302087. <https://doi.org/10.26508/lsa.202302087>

BATS EXPERIENCE HEARING LOSS IN OLD AGE

Hearing loss is a hallmark of aging, typically initially affecting the higher frequencies. In echolocating bats, the ability to discern high frequencies is essential. However, nothing is known about age-related hearing loss in bats, and they are often assumed to be immune to it.

We tested the hearing of 47 wild Egyptian fruit bats by recording their auditory brainstem response and cochlear microphonics, and we also assessed the cochlear histology in four of these bats. We used the bats' DNA methylation profile to evaluate their age and found that bats exhibit age-related

hearing loss, with more prominent deterioration at the higher frequencies. The rate of the deterioration was \sim 1 dB per year, comparable to the hearing loss observed in humans.

Assessing the noise in the fruit bat roost revealed that these bats are exposed to continuous immense noise—mostly of social vocalizations—supporting the assumption that bats might be partially resistant to loud noise. Thus, in contrast to previous assumptions, our results suggest that bats constitute a model animal for the study of age-related hearing loss.

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Tarnovsky, Y.C., S. Taiber, Y. Nissan, A. Boonman, Y. Assaf, G.S. Wilkinson, K.B. Avraham, and Y. Yovel. 2023. Bats experience age-related hearing loss (presbycusis). *Life Science Alliance*. 6 (6) e202201847. <https://doi.org/10.26508/lsa.202201847>



NEW BOTULINUM CONSTRUCTS FOR PAIN RELIEF

Chronic pain affects one in five people across human societies, with few therapeutic options available. Botulinum neurotoxin (BoNT) can provide long-lasting pain relief by inhibiting local release of neuropeptides and neurotransmitters, but its highly paralytic nature has limited its analgesic potential. Recent advances in protein engineering have raised the possibility of synthesizing non-paralyzing botulinum molecules for translation to pain sufferers. However, the synthesis of these molecules, via several synthetic steps, has been challenging.

We describe a simple platform for safe production of botulinum molecules

for treating nerve injury-induced pain. We produced two versions of iso-peptide-bonded BoNT from separate botulinum parts using an iso-peptide bonding system. Although both molecules cleaved their natural substrate, SNAP25, in sensory neurons, the structurally elongated iBoNT did not cause motor deficit in rats. We show that the non-paralytic elongated iBoNT targets specific cutaneous nerve fibers and provides sustained pain relief in a rat nerve injury model.

Our results demonstrate that novel botulinum molecules can be produced in a simple and safe manner and be useful for treating neuropathic pain.

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Leese, C., C. Christmas, J. Mészáros, S. Ward, M. Maiaru, S.P. Hunt, and B. Davletov. 2023. New botulinum neurotoxin constructs for treatment of chronic pain. *Life Science Alliance*. 6 (6) e202201631.
<https://doi.org/10.26508/lsa.202201631>

RATIONAL DESIGN OF PARKIN-ACTIVATING MUTATIONS

Autosomal recessive mutations in the Parkin gene cause Parkinson's disease. Parkin encodes an ubiquitin E3 ligase that functions together with the kinase PINK1 in a mitochondrial quality control pathway. Parkin exists in an inactive conformation mediated by autoinhibitory domain interfaces. Thus, Parkin has become a target for the development of therapeutics that activate its ligase activity. Yet, the extent to which different regions of Parkin can be targeted for activation remained unknown.

We have used a rational structure-based approach to design new activating mutations in both human and rat Parkin across interdomain

interfaces. Out of 31 mutations tested, we identified 11 activating mutations that all cluster near the RING0:RING2 or REP:RING1 interfaces. The activity of these mutants correlates with reduced thermal stability. Furthermore, three mutations V393D, A401D, and W403A rescue a Parkin S65A mutant, defective in mitophagy, in cell-based studies.

Overall, our data extend previous analysis of Parkin activation mutants and suggests that small molecules that would mimic RING0:RING2 or REP:RING1 destabilization offer therapeutic potential for Parkinson's disease patients harboring select Parkin mutations.

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APOE4 INTERSECTS WITH NEURONAL A_β

Apolipoprotein E4 (ApoE4) is the most important genetic risk factor for Alzheimer's disease (AD). Among the earliest changes in AD is endosomal enlargement in neurons, which was reported as enhanced in ApoE4 carriers. ApoE is thought to be internalized into endosomes of neurons, whereas β -amyloid (A β) accumulates within neuronal endosomes early in AD. However, it remains unknown whether ApoE and A β intersect intracellularly.

We show that internalized astrocytic ApoE localizes mostly to lysosomes in neuroblastoma cells and astrocytes,

whereas in neurons, it preferentially localizes to endosomes-autophagosomes of neurites. In AD transgenic neurons, astrocyte-derived ApoE intersects intracellularly with amyloid precursor protein/A β . Moreover, ApoE increases the levels of endogenous and internalized A β_{42} in neurons.

Taken together, we demonstrate differential localization of ApoE in neurons, astrocytes, and neuron-like cells, and show that internalized ApoE intersects with amyloid precursor protein/A β in neurons, which may be of considerable relevance to AD.

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ORIGINAL PAPER

Konings, S.C., E. Nyberg, I. Martinsson, L. Torres-Garcia, O. Klementieva, C. Guimas Almeida, and G.K. Gouras. 2023. Apolipoprotein E intersects with amyloid- β within neurons. *Life Science Alliance*. 6 (8) e202201887.
<https://doi.org/10.26508/lsa.202201887>

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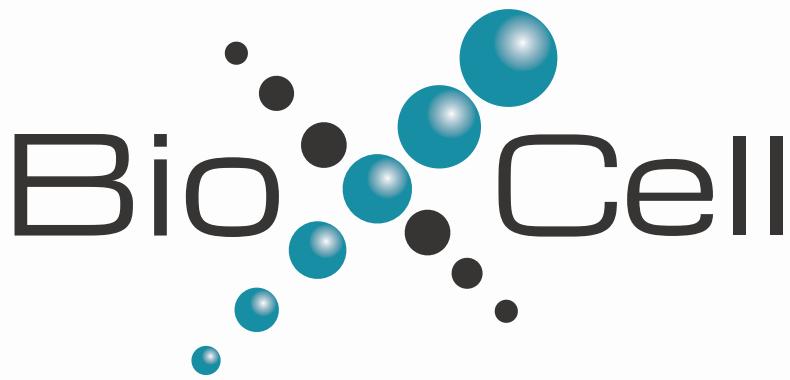
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