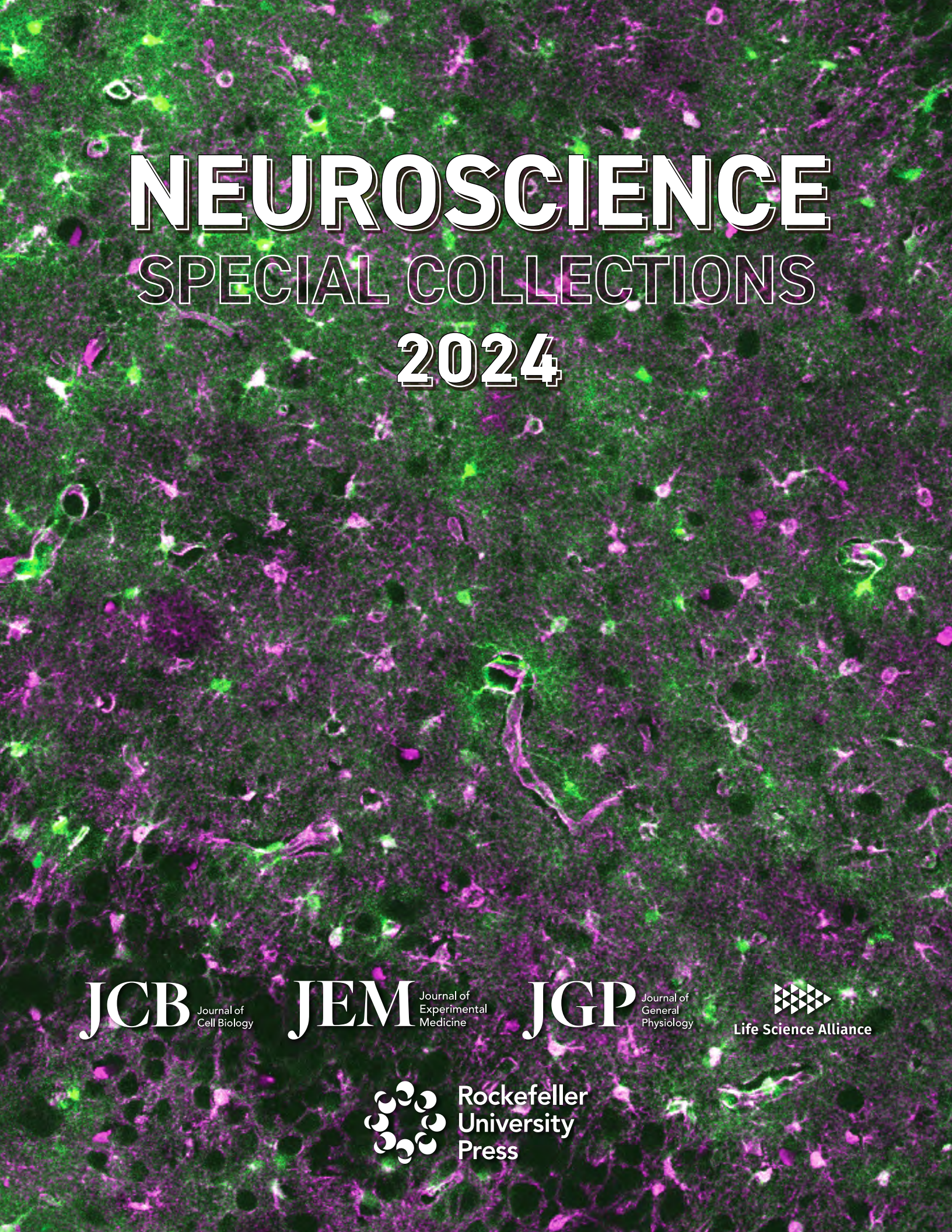
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Design by Yuko Tonohira

On the cover:
Astrocytes in the hippocampal CA3 region of a mouse brain labeled for S100β (green) and the tdTomato fluorescent reporter (magenta). Image © 2023 Altas et al.
See "Nedd4-2 regulates neuronal network activity by ubiquitinating astrocytic ion channels" on page 5.

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NEUROSCIENCE SPECIAL COLLECTIONS 2024

The editors of the *Journal of Cell Biology* (*JCB*), *Journal of Experimental Medicine* (*JEM*), *Journal of General Physiology* (*JGP*), and *Life Science Alliance* (*LSA*) 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.

IκB KINASE INDUCES TDP-43 DEGRADATION

Cytoplasmic aggregation of TDP-43 in neurons is a pathological feature common to amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). We demonstrate that the IκB kinase (IKK) complex promotes the degradation of cytoplasmic TDP-43 through proteasomes. While IKKβ is a major factor in TDP-43 degradation, IKKα acts as a cofactor, and NEMO functions as a scaffold for the recruitment of TDP-43 to the IKK complex.

Furthermore, we identified IKKβ-induced phosphorylation sites of TDP-43 and found that phosphorylation at Thr8 and Ser92 is important for the reduction of TDP-43 by IKK. TDP-43 phosphorylation at Ser92 was detected in a pattern different from that of C-terminal phosphorylation in the pathological inclusion of ALS. IKKβ was also found to significantly reduce the expression level and toxicity of the disease-causing TDP-43 mutation.

Finally, the favorable effect of IKKβ on TDP-43 aggregation was confirmed in the hippocampus of mice. IKK and the N-terminal phosphorylation of TDP-43 are potential therapeutic targets for ALS and FTLD.

ORIGINAL PAPER

Iguchi, Y., Y. Takahashi, J. Li, K. Araki, Y. Amakusa, Y. Kawakami, K. Kobayashi, S. Yokoi, and M. Katsuno. 2024. IκB kinase phosphorylates cytoplasmic TDP-43 and promotes its proteasome degradation. *J. Cell Biol.* 223 (2): e202302048. https://doi.org/10.1083/jcb.202302048

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HYPERACTIVE LRRK2 IMPAIRS AXONAL TRANSPORT OF SYNAPTIC VESICLE PRECURSORS

Gain-of-function mutations in the *LRRK2* gene cause Parkinson's disease (PD), characterized by debilitating motor and non-motor symptoms. Increased phosphorylation of a subset of RAB GTPases by LRRK2 is implicated in PD pathogenesis.

We find that increased phosphorylation of RAB3A, a cardinal synaptic vesicle precursor (SVP) protein, disrupts anterograde axonal transport of SVPs in iPSC-derived human neurons (iNeurons) expressing hyperactive *LRRK2*-p.R1441H. Knockout of the opposing protein phosphatase 1H (*PPM1H*) in iNeurons phenocopies this effect. In these models, the compartmental distribution of synaptic proteins is altered; synaptophysin and synaptobrevin-2 become sequestered in the neuronal soma with decreased delivery to presynaptic sites along the axon.

We find that RAB3A phosphorylation disrupts binding to the motor adaptor MADD, potentially preventing the formation of the RAB3A–MADD-KIF1A/1Bβ complex driving anterograde SVP transport. RAB3A hyperphosphorylation also disrupts interactions with RAB3GAP and RAB-GDI1.

Our results reveal a mechanism by which pathogenic hyperactive LRRK2 may contribute to the altered synaptic homeostasis associated with characteristic non-motor and cognitive manifestations of PD.

ORIGINAL PAPER

Dou, D., J. Aiken, and E.L.F. Holzbaur. 2024. RAB3 phosphorylation by pathogenic LRRK2 impairs trafficking of synaptic vesicle precursors. *J. Cell Biol.* 223 (6): e202307092. https://doi.org/10.1083/jcb.202307092

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JCB Journal of Cell Biology

NEDD4-2 REGULATES NEURONAL NETWORK ACTIVITY BY UBIQUITINATING ASTROCYTIC ION CHANNELS

Nedd4-2 is an E3 ubiquitin ligase in which missense mutations are related to familial epilepsy, indicating its critical role in regulating neuronal network activity. However, Nedd4-2 substrates involved in neuronal network function have yet to be identified.

Using mouse lines lacking Nedd4-1 and Nedd4-2, we identified astrocytic channel proteins inwardly rectifying K^+ channel 4.1 (Kir4.1) and Connexin43 as Nedd4-2 substrates. We found that the expression of Kir4.1 and Connexin43 is increased upon conditional deletion of Nedd4-2 in astrocytes, leading to an elevation of astrocytic membrane ion permeability and gap junction activity, with a consequent reduction of γ-oscillatory neuronal network activity.

Interestingly, our biochemical data demonstrate that missense mutations found in familial epileptic patients produce gain-of-function of the *Nedd4-2* gene product. Our data reveal a process of coordinated astrocytic ion channel proteostasis that controls astrocyte function and astrocyte-dependent neuronal network activity and elucidate a potential mechanism by which aberrant Nedd4-2 function leads to epilepsy.

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Altas, B., H.-J. Rhee, A. Ju, H.C. Solís, S. Karaca, J. Winchenbach, O. Kaplan-Arabaci, M. Schwark, M.C. Ambrozkiewicz, C. Lee, L. Spieth, G.L. Wieser, V.K. Chaugule, I. Majoul, M.A. Hassan, R. Goel, S.M. Wojcik, N. Koganezawa, K. Hanamura, D. Rotin, A. Pichler, M. Mitkovski, L. de Hoz, A. Poulopoulos, H. Urlaub, O. Jahn, G. Saher, N. Brose, J. Rhee, and H. Kawabe. 2024. Nedd4-2-dependent regulation of astrocytic Kir4.1 and Connexin43 controls neuronal network activity. *J. Cell Biol.* 223 (1): e201902050. https://doi.org/10.1083/jcb.201902050

INVESTIGATING THE FUNCTION OF SYNAPTIC EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) are released by many cell types, including neurons, carrying cargoes involved in signaling and disease. It is unclear whether EVs promote intercellular signaling or serve primarily to dispose of unwanted materials.

We show that loss of multivesicular endosome-generating endosomal sorting complex required for transport (ESCRT) machinery disrupts release of EV cargoes from *Drosophila* motor neurons. Surprisingly, ESCRT depletion does not affect the signaling activities of the EV cargo Synaptotagmin-4 (Syt4) and disrupts only some signaling activities of the EV cargo evenness interrupted (Evi). Thus, these cargoes may not require intercellular transfer via EVs and instead may be conventionally secreted or function cell-autonomously in the neuron.

We find that EVs are phagocytosed by glia and muscles and that ESCRT disruption causes compensatory autophagy in presynaptic neurons, suggesting that EVs are one of several redundant mechanisms to remove cargoes from synapses. Our results suggest that synaptic EV release serves primarily as a proteostatic mechanism for certain cargoes.

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ORIGINAL PAPER

Dresselhaus, E.C., K.P. Harris, C.R. Blanchette, K. Koles, S.J. Del Signore, M.F. Pescosolido, B. Ermanoska, M. Rozencwaig, R.C. Soslowsky, M.J. Parisi, B.A. Stewart, T.J. Mosca, and A.A. Rodal. 2024. ESCRT disruption provides evidence against trans-synaptic signaling via extracellular vesicles. *J. Cell Biol.* 223 (9): e202405025. https://doi.org/10.1083/jcb.202405025

POLARIZED KINESIN-1 AND RIC-7 LEAD ANTEROGRADE MITOCHONDRIA

Mitochondria transport is crucial for axonal mitochondria distribution and is mediated by kinesin-1-based anterograde and dynein-based retrograde motor complexes. While Miro and Milton/TRAK were identified as key adaptors between mitochondria and kinesin-1, recent studies suggest the presence of additional mechanisms.

In *C. elegans, ric-7* is the only single gene described so far, other than kinesin-1, that is absolutely required for axonal mitochondria localization. Using CRISPR engineering in *C. elegans*, we find that Miro is important but is not essential for anterograde traffic, whereas it is required for retrograde traffic. Both the endogenous RIC-7 and kinesin-1 act at the leading end to transport mitochondria anterogradely. RIC-7 binding to mitochondria requires its N-terminal domain and partially relies on MIRO-1, whereas RIC-7 accumulation at the leading end depends on its disordered region, kinesin-1, and metaxin2.

We conclude that transport complexes containing kinesin-1 and RIC-7 polarize at the leading edge of mitochondria and are required for anterograde axonal transport in *C. elegans*.

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ORIGINAL PAPER
Wu, Y., C. Ding, B. Sharif, A. Weinreb, G. Swaim, H. Hao, S. Yogev, S. Watanabe, and M. Hammarlund. 2024. Polarized localization of kinesin-1 and RIC-7 drives axonal mitochondria anterograde transport. *J. Cell Biol.* 223 (5): e202305105. https://doi.org/10.1083/jcb.202305105

JEM Journal of Experimental Medicine

PROPHYLACTIC VEGF-C TREATMENT PROVIDES NEUROLOGICAL PROTECTION FROM ISCHEMIC STROKE

Meningeal lymphatic vessels (MLVs) promote tissue clearance and immune surveillance in the central nervous system (CNS). Vascular endothelial growth factor-C (VEGF-C) regulates MLV development and maintenance and has therapeutic potential for treating neurological disorders.

We investigated the effects of VEGF-C overexpression on brain fluid drainage and ischemic stroke outcomes in mice. Intracerebrospinal administration of an adeno-associated virus expressing mouse full-length VEGF-C (AAV-mVEGF-C) increased cerebrospinal fluid drainage to the deep cervical lymph nodes (dCLNs) by enhancing lymphatic growth and upregulated neuroprotective signaling pathways identified by single nuclei RNA sequencing of brain cells.

In a mouse model of ischemic stroke, AAV-mVEGF-C pretreatment reduced stroke injury and ameliorated motor performances in the subacute stage, associated with mitigated microglia-mediated inflammation and increased brain-derived neurotrophic factor signaling in brain cells. Neuroprotective effects of VEGF-C were lost upon cauterization of the dCLN afferent lymphatics and not mimicked by acute post-stroke VEGF-C injection.

We conclude that VEGF-C prophylaxis promotes multiple vascular, immune, and neural responses that culminate in protection against neurological damage in acute ischemic stroke.

ORIGINAL PAPER

Boisserand, L.S.B., L.H. Geraldo, J. Bouchart, M.-R. El Kamouh, S. Lee, B.G. Sanganahalli, M. Spajer, S. Zhang, S. Lee, M. Parent, Y. Xue, M. Skarica, X. Yin, J. Guegan, K. Boyé, F.S. Leser, L. Jacob, M. Poulet, M. Li, X. Liu, S.E. Velazquez, R. Singhababu, M.E. Robinson, M.H. Askenase, A. Osherov, N. Sestan, J. Zhou, K. Alitalo, E. Song, A. Eichmann, L.H. Sansing, H. Benveniste, F. Hyder, and J.-L. Thomas. 2024. VEGF-C prophylaxis favors lymphatic drainage and modulates neuroinflammation in a stroke model. *J. Exp. Med.* 221 (4): e20221983. https://doi.org/10.1084/jem.20221983

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CHOLESTEROL 25-HYDROXYLASE PROMOTES NEUROINFLAMMATION AND TAU-MEDIATED NEURODEGENERATION

Alzheimer's disease (AD) is characterized by amyloid plaques and neurofibrillary tangles, in addition to neuroinflammation and changes in brain lipid metabolism. 25-Hydroxycholesterol (25-HC), a known modulator of both inflammation and lipid metabolism, is produced by cholesterol 25-hydroxylase encoded by *Ch25h* expressed as a "disease-associated microglia" signature gene. However, whether Ch25h influences tau-mediated neuroinflammation and neurodegeneration is unknown.

We show that in the absence of Ch25h and the resultant reduction in 25-HC, there is strikingly reduced age-dependent neurodegeneration and neuroinflammation in the hippocampus and entorhinal/piriform cortex of PS19 mice, which express the P301S mutant human tau transgene. Transcriptomic analyses of bulk hippocampal tissue and single nuclei revealed that Ch25h deficiency in PS19 mice strongly suppressed proinflammatory signaling in microglia.

Our results suggest a key role for Ch25h/25-HC in potentiating proinflammatory signaling to promote tau-mediated neurodegeneration. Ch25h may represent a novel therapeutic target for primary tauopathies, AD, and other neuroinflammatory diseases.

ORIGINAL PAPER

Toral-Rios, D., J.M. Long, J.D. Ulrich, J. Yu, M.R. Strickland, X. Han, D.M. Holtzman, A.G. Cashikar, and S.M. Paul. 2024. Cholesterol 25-hydroxylase mediates neuroinflammation and neurodegeneration in a mouse model of tauopathy. *J. Exp. Med.* 221 (4): e20232000. https://doi.org/10.1084/jem.20232000

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ADUCANUMAB INDUCES MICROGLIAL AND IMMUNE ALTERATIONS

Aducanumab, an anti-amyloid immunotherapy for Alzheimer's disease, efficiently reduces Aβ, though its plaque clearance mechanisms, long-term effects, and effects of discontinuation are not fully understood.

We assessed the effect of aducanumab treatment and withdrawal on Aβ, neuritic dystrophy, astrocytes, and microglia in the APP/PS1 amyloid mouse model. We found that reductions in amyloid and neuritic dystrophy during acute treatment were accompanied by microglial and astrocytic activation, and microglial recruitment to plaques and adoption of an aducanumab-specific pro-phagocytic and pro-degradation transcriptomic signature, indicating a role for microglia in aducanumab-mediated Aβ clearance.

Reductions in Aβ and dystrophy were sustained 15 but not 30 wk after discontinuation, and reaccumulation of plaques coincided with loss of the microglial aducanumab signature and failure of microglia to reactivate. This suggests that despite the initial benefit from treatment, microglia are unable to respond later to restrain plaque reaccumulation, making further studies on the effect of amyloid-directed immunotherapy withdrawal crucial for assessing long-term safety and efficacy.

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ORIGINAL PAPER
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ARL6IP1 IS A POTENTIAL TARGET FOR HEREDITARY SPASTIC PARAPLEGIA GENE THERAPY

ARL6IP1 is implicated in hereditary spastic paraplegia (HSP), but the specific pathogenic mechanism leading to neurodegeneration has not been elucidated. We clarified the molecular mechanism of ARL6IP1 in HSP using in vitro and in vivo models.

The *Arl6ip1* knockout (KO) mouse model was generated to represent the clinically involved frameshift mutations and mimicked the HSP phenotypes. Notably, in vivo brain histopathological analysis revealed demyelination of the axon and neuroinflammation in the white matter, including the corticospinal tract. In in vitro experiments, *ARL6IP1* silencing caused cell death during neuronal differentiation and mitochondrial dysfunction by dysregulated autophagy. ARL6IP1 localized on mitochondria-associated membranes (MAMs) to maintain endoplasmic reticulum and mitochondrial homeostasis via direct interaction with LC3B and BCl2L13. ARL6IP1 played a crucial role in connecting the endoplasmic reticulum and mitochondria as a member of MAMs.

ARL6IP1 gene therapy reduced HSP phenotypes and restored pathophysiological changes in the *Arl6ip1* KO model. Our results established that *ARL6IP1* could be a potential target for HSP gene therapy.

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Lim, J.H., H.M. Kang, D.H. Kim, B. Jeong, D.Y. Lee, J.-R. Lee, J.Y. Baek, H.-S. Cho, M.-Y. Son, D.S. Kim, N.-S. Kim, and C.-R. Jung. 2024. *ARL6IP1* gene delivery reduces neuroinflammation and neurodegenerative pathology in hereditary spastic paraplegia model. *J. Exp. Med.* 221 (1): e20230367. https://doi.org/10.1084/jem.20230367

JEM Journal of Experimental Medicine

A TBK1 MUTANT CAUSES AUTOPHAGOLYSOSOMAL DEFECTS AND NEURODEGENERATION

Heterozygous mutations in the *TBK1* gene can cause amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The majority of TBK1-ALS/FTD patients carry deleterious loss-of-expression mutations, and it is still unclear which TBK1 function leads to neurodegeneration.

We investigated the impact of the pathogenic *TBK1* missense variant p.E696K, which does not abolish protein expression, but leads to a selective loss of TBK1 binding to the autophagy adaptor protein and TBK1 substrate optineurin. Using organelle-specific proteomics, we found that in a knock-in mouse model and human iPSC–derived motor neurons, the p.E696K mutation causes presymptomatic onset of autophagolysosomal dysfunction in neurons precipitating the accumulation of damaged lysosomes. This is followed by a progressive, age-dependent motor neuron disease.

Contrary to the phenotype of mice with full *Tbk1* knock-out, RIPK/TNF-α–dependent hepatic, neuronal necroptosis, and overt autoinflammation were not detected. Our in vivo results indicate autophagolysosomal dysfunction as a trigger for neurodegeneration and a promising therapeutic target in TBK1-ALS/FTD.

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ORIGINAL PAPER
Brenner, D., K. Sieverding, J. Srinidhi, S. Zellner, C. Secker, R. Yilmaz, J. Dyckow, S. Amr, A. Ponomarenko, E. Tunaboylu, Y. Douahem, J.S. Schlag, L. Rodríguez Martínez, G. Kislinger, C. Niemann, K. Nalbach, W.P. Ruf, J. Uhl, J. Hollenbeck, L. Schirmer, A. Catanese, C.S. Lobsiger, K.M. Danzer, D. Yilmazer-Hanke, C. Münch, P. Koch, A. Freischmidt, M. Fetting, C. Behrends, R. Parlato, and J.H. Weishaupt. 2024. A TBK1 variant causes autophagolysosomal and motoneuron pathology without neuroinflammation in mice. *J. Exp. Med.* 221 (5): e20221190. https://doi.org/10.1084/jem.20221190

MODULATION OF K_V7.2 CHANNEL DEACTIVATION BY pH

Potassium-selective, voltage-gated channels of the K_V7 family are critical regulators of electrical excitability in many cell types. Removing the outermost putative sensing charge (R198) of human K_V7.2 shifts its activation voltage dependence toward more negative potentials. This suggests that removing a charge "at the top" of the fourth (S4) segment of the voltage-sensing domain facilitates activation.

We hypothesized that restoring that charge would bring back the activation to its normal voltage range. We introduced the mutation R198H in K_V7.2 with the idea that titrating the introduced histidine with protons would reinstate the sensing charge. As predicted, the mutant's activation voltage dependence changed as a function of the external pH (pH_{EXT}) while modest changes in the activation voltage dependence were observed with the wild-type (WT) channel. On the other hand, the deactivation kinetics of the R198H mutant were remarkably sensitive to pH_{EXT} changes, readily deactivating at pH_{EXT} 6, while becoming slower to deactivate at pH_{EXT} 8. In contrast, K_V7.2 WT displayed modest changes in deactivation kinetics as a function of pH_{EXT}. This indicated that the mutation had an asymmetrical impact on activity, with a remarkable effect on channel closing, suggesting that the charge of residue 198 was critical for deactivation.

However, in a surprising turn, the mutant R198Q—a non-titratable mutation—also displayed a high pH_{EXT} sensitivity activity. We thus concluded that rather than the charge at position 198, the protonation status of the channel's extracellular face modulates the open channel stabilization and that the charge of residue 198 is required for the voltage sensor to effectively deactivate the channel, overcoming the stabilizing effect of high pH_{EXT}.

ORIGINAL PAPER

Mehrdel, B., and C.A. Villalba-Galea. 2024. Effect of a sensing charge mutation on the deactivation of K_V7.2 channels. *J. Gen. Physiol.* 156 (3): e202213284. https://doi.org/10.1085/jgp.202213284

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HOW MUTATIONS IN A SODIUM CHANNEL CAUSE SEVERE INHERITED PAIN SYNDROME

Voltage-gated sodium channels in peripheral nerves conduct nociceptive signals from nerve endings to the spinal cord. Mutations in voltage-gated sodium channel Na_V1.7 are responsible for a number of severe inherited pain syndromes, including inherited erythromelalgia (IEM).

We describe the negative shifts in the voltage dependence of activation in the bacterial sodium channel Na_VAb as a result of the incorporation of four different IEM mutations in the voltage sensor, which recapitulate the gain-of-function effects observed with these mutations in human Na_V1.7. Crystal structures of Na_VAb with these IEM mutations revealed that a mutation in the S1 segment of the voltage sensor facilitated the outward movement of S4 gating charges by widening the pathway for gating charge translocation. In contrast, mutations in the S4 segments modified hydrophobic interactions with surrounding amino acid side chains or membrane phospholipids that would enhance the outward movement of the gating charges.

These results provide key structural insights into the mechanisms by which these IEM mutations in the voltage sensors can facilitate outward movements of the gating charges in the S4 segment and cause hyperexcitability and severe pain in IEM. Our work gives new insights into IEM pathogenesis at the near-atomic level and provides a molecular model for mutation-specific therapy of this debilitating disease.

ORIGINAL PAPER

Wisedchaisri, G., T.M. Gamal El-Din, N.M. Powell, N. Zheng, and W.A. Catterall. 2023. Structural basis for severe pain caused by mutations in the voltage sensors of sodium channel Na_V1.7. 2023. *J. Gen. Physiol.* 155 (12): e202313450. https://doi.org/10.1085/jgp.202313450

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JGP Journal of General Physiology

KCNMA1 CHANNELOPATHY MUTANTS REDUCE CIRCADIAN CLOCK ROBUSTNESS

KCNMA1 encodes the voltage- and calcium-activated K^+ (BK) channel, which regulates suprachiasmatic nucleus (SCN) neuronal firing and circadian behavioral rhythms. Gain-of-function (GOF) and loss-of-function (LOF) alterations in BK channel activity disrupt circadian behavior, but the effect of human disease-associated *KCNMA1* channelopathy variants has not been studied on clock function.

We assess circadian behavior in two GOF and one LOF mouse lines. Heterozygous *Kcnma1*$^{N999S/WT}$ and homozygous *Kcnma1*$^{D434G/D434G}$ mice are validated as GOF models of paroxysmal dyskinesia (PNKD3), but whether circadian rhythm is affected in this hypokinetic locomotor disorder is unknown. Conversely, homozygous LOF *Kcnma1*$^{H444Q/H444Q}$ mice do not demonstrate PNKD3. We assessed circadian behavior by locomotor wheel running activity. All three mouse models were rhythmic, but *Kcnma1*$^{N999S/WT}$ and *Kcnma1*$^{D434G/D434G}$ showed reduced circadian amplitude and decreased wheel activity, corroborating prior studies focused on acute motor coordination. In addition, *Kcnma1*$^{D434G/D434G}$ mice had a small decrease in period.

However, the phase-shifting sensitivity for both GOF mouse lines was abnormal. Both *Kcnma1*$^{N999S/WT}$ and *Kcnma1*$^{D434G/D434G}$ mice displayed increased responses to light pulses and took fewer days to re-entrain to a new light:dark cycle. In contrast, the LOF *Kcnma1*$^{H444Q/H444Q}$ mice showed no difference in any of the circadian parameters tested. The enhanced sensitivity to phase-shifting stimuli in *Kcnma1*$^{N999S/WT}$ and *Kcnma1*$^{D434G/D434G}$ mice was similar to other *Kcnma1* GOF mice. Together with previous studies, these results suggest that increasing BK channel activity decreases circadian clock robustness without rhythm ablation.

ORIGINAL PAPER

Dinsdale, R.L., C.E. Roache, and A.L. Meredith. 2023. Disease-associated *KCNMA1* variants decrease circadian clock robustness in channelopathy mouse models. *J. Gen. Physiol.* 155 (11): e202313357. https://doi.org/10.1085/jgp.202313357

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DISTINCT CHOLINERGIC PATHWAYS IN RETINAL AMACRINE CELLS

Cholinergic signaling in the retina is mediated by acetylcholine (ACh) released from starburst amacrine cells (SACs), which are key neurons for motion detection. SACs comprise ON and OFF subtypes, which morphologically show mirror symmetry to each other. Although many physiological studies on SACs have targeted ON cells only, the synaptic computation of ON and OFF SACs is assumed to be similar. Recent studies demonstrated that gene expression patterns and receptor types differed between ON and OFF SACs, suggesting differences in their functions.

We compared cholinergic signaling pathways between ON and OFF SACs in the mouse retina using the patch clamp technique. The application of ACh increased GABAergic feedback, observed as post-synaptic currents to SACs, in both ON and OFF SACs; however, the mode of GABAergic feedback differed. Nicotinic receptors mediated GABAergic feedback in both ON and OFF SACs, while muscarinic receptors mediated GABAergic feedback in ON SACs only in adults. Neither tetrodotoxin, which blocked action potentials, nor LY354740, which blocked neurotransmitter release from SACs, eliminated ACh-induced GABAergic feedback in SACs.

These results suggest that ACh-induced GABAergic feedback in ON and OFF SACs is regulated by different feedback mechanisms in adults and mediated by non-spiking amacrine cells other than SACs.

ORIGINAL PAPER

Gangi, M., T. Maruyama, T. Ishii, and M. Kaneda. 2024. ON and OFF starburst amacrine cells are controlled by distinct cholinergic pathways. *J. Gen. Physiol.* 156 (8): e202413550. https://doi.org/10.1085/jgp.202413550

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LIGHT SHAPES THE MATURATION OF PHOTORECEPTOR SIGNALING PATHWAYS

The complex nature of rod and cone photoreceptors and the light-evoked responsivity of bipolar cells in the mature rodent retina have been well characterized. However, little is known about the emergent light-evoked response properties of the mouse retina and the role light plays in shaping these emergent responses.

We have previously demonstrated that the outer retina is responsive to green light as early as postnatal day 8 (P8). Here, we characterize the progression of both photoreceptors (rods and cones) and bipolar cell responses during development and into adulthood using ex vivo electroretinogram recordings. Our data show that the majority of photoreceptor response at P8 originates from cones and that these outputs drive second-order bipolar cell responses as early as P9.

We find that the magnitude of the photoresponse increases concurrently with each passing day of postnatal development and that many functional properties of these responses, as well as the relative rod/cone contributions to the total light-evoked response, are age dependent.

We compared these responses at eye opening and maturity to age-matched animals raised in darkness and found that the absence of light diminishes emergent and mature cone-to-bipolar cell signaling. Furthermore, we found cone-evoked responses to be significantly slower in dark-reared retinas. Together, this work characterizes the developmental photoresponsivity of the mouse retina while highlighting the importance of properly timed sensory input for the maturation of the first visual system synapse.

ORIGINAL PAPER

Bonezzi, P.J., M.J. Tarchick, B.D. Moore, and J.M. Renna. 2023. Light drives the developmental progression of outer retinal function. *J. Gen. Physiol.* 155 (9): e202213262. https://doi.org/10.1085/jgp.202213262

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COMPARING THE LIPOTYPES OF DEVELOPING NEURONS IN VIVO AND IN VITRO

During development, different tissues acquire distinct lipotypes that are coupled to tissue function and homeostasis. In the brain, where complex membrane trafficking systems are required for neural function, specific glycerophospholipids, sphingolipids, and cholesterol are highly abundant, and defective lipid metabolism is associated with abnormal neural development and neurodegenerative disease. Notably, the production of specific lipotypes requires appropriate programming of the underlying lipid metabolic machinery during development, but when and how this occurs is unclear.

To address this, we used high-resolution MS^{ALL} lipidomics to generate an extensive time-resolved resource of mouse brain development covering early embryonic and postnatal stages. This revealed a distinct bifurcation in the establishment of the neural lipotype, whereby the canonical lipid biomarkers 22:6-glycerophospholipids and 18:0-sphingolipids begin to be produced in utero, whereas cholesterol attains its characteristic high levels after birth. Using the resource as a reference, we next examined to which extent this can be recapitulated by commonly used protocols for in vitro neuronal differentiation of stem cells. Here, we found that the programming of the lipid metabolic machinery is incomplete and that stem cell–derived cells can only partially acquire a neural lipotype when the cell culture media is supplemented with brain-specific lipid precursors.

Altogether, our work provides an extensive lipidomic resource for early mouse brain development and highlights a potential caveat when using stem cell–derived neuronal progenitors for mechanistic studies of lipid biochemistry, membrane biology and biophysics, which nonetheless can be mitigated by further optimizing in vitro differentiation protocols.

ORIGINAL PAPER

Gopalan, A.B., L. van Uden, R.R. Sprenger, N. Fernandez-Novel Marx, H. Bogetofte, P.A. Neveu, M. Meyer, K.-M. Noh, A. Diz-Muñoz, and C.S. Ejsing. 2024. Lipotype acquisition during neural development is not recapitulated in stem cell–derived neurons. *Life Science Alliance*. 7 (5): e202402622. https://doi.org/10.26508/lsa.202402622

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GLYCOLIC ACID AND D-LACTIC ACID RESTORE NEURODEGENERATION IN ALS

Amyotrophic lateral sclerosis (ALS) leads to death within 2–5 yr. Currently, available drugs only slightly prolong survival. We present novel insights into the pathophysiology of *Superoxide Dismutase 1* (SOD1)- and, in particular, *Fused In Sarcoma* (FUS)-ALS by revealing a supposedly central role of glycolic acid (GA) and D-lactic acid (DL)—both putative products of the Parkinson's disease–associated glyoxylase DJ-1.

Combined, not single, treatment with GA/DL restored axonal organelle phenotypes of mitochondria and lysosomes in FUS- and SOD1-ALS patient-derived motoneurons (MNs). This was not only accompanied by restoration of mitochondrial membrane potential but even dependent on it. Despite presenting an axonal transport deficiency as well, TDP43 patient-derived MNs did not share mitochondrial depolarization and did not respond to GA/DL treatment. GA and DL also restored cytoplasmic mislocalization of FUS and FUS recruitment to DNA damage sites, recently reported as being upstream of the mitochondrial phenotypes in FUS-ALS.

Whereas these data point toward the necessity of individualized (gene-) specific therapy stratification, it also suggests common therapeutic targets across different neurodegenerative diseases characterized by mitochondrial depolarization.

ORIGINAL PAPER

Pal, A., D. Grossmann, H. Glaß, V. Zimyanin, R. Günther, M. Catinozzi, T.M. Boeckers, J. Sterneckert, E. Storkebaum, S. Petri, F. Wegner, S.W. Grill, F. Pan-Montojo, and A. Hermann. 2024. Glycolic acid and D-lactate—putative products of DJ-1—restore neurodegeneration in FUS- and SOD1-ALS. *Life Science Alliance*. 7 (8): e202302535. https://doi.org/10.26508/lsa.202302535

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LITHIUM REGULATES PIP_2 AND CALCIUM SIGNALING IN NEURONS

Lithium (Li) is widely used as a mood stabilizer to treat bipolar affective disorder. However, the molecular targets of Li that underpin its therapeutic effect remain unresolved. Inositol monophosphatase (IMPA1) is an enzyme involved in phosphatidylinositol 4,5-bisphosphate (PIP_2) resynthesis after PLC signaling. In vitro, Li inhibits IMPA1, but the relevance of this inhibition within neural cells remains unknown.

We report that treatment with therapeutic concentrations of Li reduces receptor-activated calcium release from intracellular stores and delays PIP_2 resynthesis. These effects of Li are abrogated in *IMPA1* deleted cells. We also observed that in human forebrain cortical neurons, treatment with Li reduced neuronal excitability and calcium signals. After Li treatment of human cortical neurons, transcriptome analyses revealed down-regulation of signaling by glutamate, a key excitatory neurotransmitter in the human brain.

Collectively, our findings suggest that inhibition of IMPA1 by Li reduces receptor-activated PLC signaling and neuronal excitability.

ORIGINAL PAPER

Saha, S., H. Krishnan, and P. Raghu. 2023. IMPA1 dependent regulation of phosphatidylinositol 4,5-bisphosphate and calcium signalling by lithium. *Life Science Alliance*. 7 (2): e202302425. https://doi.org/10.26508/lsa.202302425

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TARGETING OXIDATIVE STRESS AS A THERAPEUTIC STRATEGY FOR *C9orf72*-rALS/FTD

Mitochondrial dysfunction is a common feature of *C9orf72* amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD); however, it remains unclear whether this is a cause or consequence of the pathogenic process.

Analyzing multiple aspects of mitochondrial biology across several *Drosophila* models of *C9orf72*-ALS/FTD, we found morphology, oxidative stress, and mitophagy are commonly affected, which correlated with progressive loss of locomotor performance. Notably, only genetic manipulations that reversed the oxidative stress levels were also able to rescue *C9orf72* locomotor deficits, supporting a causative link between mitochondrial dysfunction, oxidative stress, and behavioral phenotypes. Targeting the key antioxidant Keap1/Nrf2 pathway, we found that genetic reduction of Keap1 or pharmacological inhibition by dimethyl fumarate significantly rescued the *C9orf72*-related oxidative stress and motor deficits. Finally, mitochondrial ROS levels were also elevated in *C9orf72* patient-derived iNeurons and were effectively suppressed by dimethyl fumarate treatment.

These results indicate that mitochondrial oxidative stress is an important mechanistic contributor to *C9orf72* pathogenesis, affecting multiple aspects of mitochondrial function and turnover. Targeting the Keap1/Nrf2 signaling pathway to combat oxidative stress represents a therapeutic strategy for *C9orf72*-related ALS/FTD.

ORIGINAL PAPER

Au, W.H., L. Miller-Fleming, A. Sanchez-Martinez, J.A.K. Lee, M.J. Twyning, H.A. Prag, L. Raik, S.P. Allen, P.J. Shaw, L. Ferraiuolo, H. Mortiboys, and A.J. Whitworth. 2024. Activation of the Keap1/Nrf2 pathway suppresses mitochondrial dysfunction, oxidative stress, and motor phenotypes in *C9orf72* ALS/FTD models. *Life Science Alliance*. 7 (9): e202402853. https://doi.org/10.26508/lsa.202402853

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Life Science Alliance

REVIEW

REGULATION OF ALTERNATIVE SPLICING AND POLYADENYLATION IN NEURONS

Cell type–specific gene expression is a fundamental feature of multicellular organisms and is achieved by combinations of regulatory strategies. Although cell-restricted transcription is perhaps the most widely studied mechanism, co-transcriptional and post-transcriptional processes are also central to the spatiotemporal control of gene functions.

One general category of expression control involves the generation of multiple transcript isoforms from an individual gene, whose balance and cell specificity are frequently tightly regulated via diverse strategies. The nervous system makes particularly extensive use of cell-specific isoforms, specializing the neural function of genes that are expressed more broadly. We review regulatory strategies and RNA-binding proteins that direct neural-specific isoform processing. These include various classes of alternative splicing and alternative polyadenylation events, both of which broadly diversify the neural transcriptome.

Importantly, global alterations of splicing and alternative polyadenylation are characteristic of many neural pathologies, and recent genetic studies demonstrate how misregulation of individual neural isoforms can directly cause mutant phenotypes.

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ORIGINAL PAPER
Lee, S., J.I. Aubee, and E.C. Lai. 2023. Regulation of alternative splicing and polyadenylation in neurons. *Life Science Alliance*. 6 (12): e202302000. https://doi.org/10.26508/lsa.202302000

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