

This Week in The Journal

● Cellular/Molecular

Hap1 Helps Tsc1 Inhibit mTORC1

Luis A. Mejia, Nadia Litterman,
Yoshiho Ikeuchi, Luis de la Torre-Ubieta,
Eric J. Bennett, et al.

(see pages 18015–18021)

The neurodevelopmental disorder tuberous sclerosis complex (TSC) is characterized not only by the presence of abnormal tuber-like growths in the brain, but also by intellectual disability, autism spectrum disorder, and epilepsy. The disease is caused by mutations in the proteins Tsc1 and Tsc2, which normally inhibit the mTORC1 signaling pathway and thus prevent excessive protein synthesis in response to extracellular signals. Using interaction proteomics analyses, Mejia et al. discovered that huntingtin-associated protein 1 (Hap1) directly binds to Tsc1 in neurons and participates in the inhibition of mTORC1 signaling. Knocking down Hap1 or Tsc1 *in vitro* caused hippocampal neurons to form multiple axons. Knockdown *in vivo* also disrupted migration of pyramidal neurons to the proper layer in the hippocampus. Inhibiting mTORC1 with the exogenous inhibitor rapamycin rescued the effects of Hap1 knockdown *in vitro*.

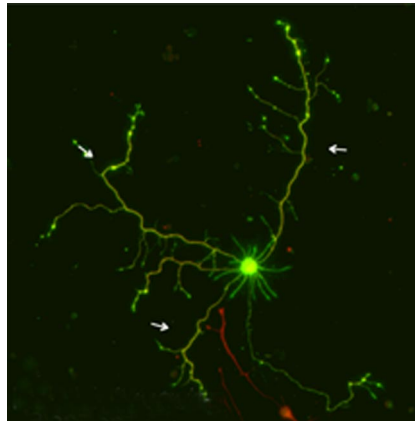
● Development/Plasticity/Repair

Gpr126 Is Necessary for Axon Myelination

Amit Mogha, Andrew E. Benesh,
Chinmoy Patra, Felix B. Engel,
Torsten Schöneberg, et al.

(see pages 17976–17985)

During development of the peripheral nervous system, immature Schwann cells migrate along bundles of growing axons and extend processes into the bundles to separate individual axons. Such interactions with axons are required to trigger Schwann cell maturation, which enables them to form myelin. Mice lacking the adhesion G-protein-coupled receptor Gpr126 lack myelin, suggesting that this protein is required at some stage of myelination. But because Gpr126-null mice die before birth (likely as a result of abnormal heart development), the role of Gpr126



Knocking down Hap1 in hippocampal cultures caused formation of multiple axons (arrows). See the article by Mejia et al. for details.

in myelination has not been fully explored. Therefore, Mogha et al. restricted knockout of Gpr126 to Schwann cells. Although more Schwann cells were present in mutant nerves than in controls, axon sorting was delayed in mutants, and myelination did not occur. These phenotypes were partially rescued by activating adenylate cyclase in Schwann cells or treating nerves with a cAMP analog. Further experiments in cell lines indicated that Gpr126 increases cAMP levels, but couples to G_i as well as G_s proteins.

● Systems/Circuits

The Amygdala Encodes Neutral Cues

Rotem Genud-Gabai, Oded Klavir,
and Rony Paz

(see pages 17986–17994)

To function in the world while avoiding injury, animals must learn not only to identify cues that predict danger, but also to discriminate between danger cues and similar, innocuous cues. The amygdala has a central role in learning about cues that predict reward or danger, but Genud-Gabai et al. now show that neurons in this structure also respond to cues that have not been linked with any unconditioned stimulus. Similar numbers of neurons in macaque basolateral amygdala (BLA) acquired responses (increased or decreased activity) to cues paired

with an aversive stimulus, cues of the same modality (auditory or visual) that were not paired with an unconditioned stimulus, and unpaired cues of a different modality. The magnitude of the acquired response was also similar for danger and neutral cues. Most neurons that responded to neutral cues stopped responding after the cues were paired with the aversive stimulus, suggesting separate populations of BLA neurons encode neutral and aversive cues.

● Neurobiology of Disease

LGI1 Autoantibodies Are Tightly Linked to Limbic Encephalitis

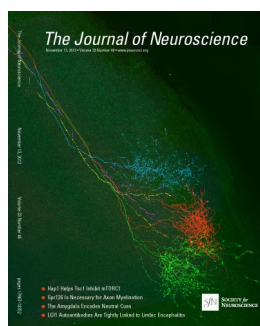
Toshika Ohkawa, Yuko Fukata,
Miwako Yamasaki, Taisuke Miyazaki,
Norihiko Yokoi, et al.

(see pages 18161–18174)

Mutations in the secreted protein LGI1 cause lateral temporal lobe epilepsy, and autoantibodies against LGI1 are present in people with limbic encephalitis (LE), characterized partly by seizures. LGI1 interacts with multiple proteins and influences several neuronal processes; perhaps most relevant to seizure susceptibility, it prevents inactivation of voltage-gated potassium channels, and through interactions with ADAM22 and ADAM23, it promotes synapse formation and glutamate receptor expression. Having screened serum from patients with various immune-mediated neurological disorders, Ohkawa et al. found that ~90% of people with LE had autoantibodies only against LGI1. Serum from these patients reduced interactions between LGI1 and ADAM22 and ADAM23, whereas LGI1 autoantibodies from patients without LE did not. Furthermore, serum from LE patients reduced synaptic AMPA receptor clusters in rat hippocampal neurons, as did incubating neurons with an ADAM22 fragment that disrupts LGI1–ADAM22 interactions. Moreover, expression of AMPARs, ADAM22, and ADAM23 were lower in the dentate gyrus of LGI1-null mice than in controls. Together, the data suggest that LGI1 autoantibodies lead to seizures by disrupting interactions between LGI1 and ADAM22 and ADAM23, and thus reducing synaptic expression of AMPARs.

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Cover legend: Composition of three mouse retinal ganglion cells arbors in the superior colliculus. Individual retinal ganglion cells were labeled by injection and electroporation of plasmids encoding reporter proteins into the embryonic retina. Arbors in the superior colliculus of nine-day-old mice were imaged individually, superimposed in a single image, and artificially colored. Blue, red, and green arbors correspond to control retinal ganglion cells, while magenta and yellow axons come from retinal ganglion cells that ectopically express the rectifying potassium channel Kir2.1. Although all the axons reach similar topographic points in the superior colliculus, the Kir2.1-expressing axons do not arborize properly. For more information, see Benjumea et al. (18208–18218).

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A Novel Hap1–Tsc1 Interaction Regulates Neuronal mTORC1 Signaling and Morphogenesis in the Brain

Luis A. Mejia,^{1,2} Nadia Litterman,^{1,2*} Yoshiho Ikeuchi,^{1,3*} Luis de la Torre-Ubieta,^{1,2} Eric J. Bennett,⁴ Chi Zhang,¹ J. Wade Harper,⁴ and Azad Bonni^{1,2,3}

¹Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, ²Program in Neuroscience, Harvard Medical School, Boston, Massachusetts 02115, ³Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110, and ⁴Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115

Tuberous sclerosis complex (TSC) is a leading genetic cause of autism. The TSC proteins Tsc1 and Tsc2 control the mTORC1 signaling pathway in diverse cells, but how the mTORC1 pathway is specifically regulated in neurons remains to be elucidated. Here, using an interaction proteomics approach in neural cells including neurons, we uncover the brain-enriched protein huntingtin-associated protein 1 (Hap1) as a novel functional partner of Tsc1. Knockdown of Hap1 promotes specification of supernumerary axons in primary hippocampal neurons and profoundly impairs the positioning of pyramidal neurons in the mouse hippocampus *in vivo*. The Hap1 knockdown-induced phenotypes in primary neurons and *in vivo* recapitulate the phenotypes induced by Tsc1 knockdown. We also find that Hap1 knockdown in hippocampal neurons induces the downregulation of Tsc1 and stimulates the activity of mTORC1, as reflected by phosphorylation of the ribosomal protein S6. Inhibition of mTORC1 activity suppresses the Hap1 knockdown-induced polarity phenotype in hippocampal neurons. Collectively, these findings define a novel link between Hap1 and Tsc1 that regulates neuronal mTORC1 signaling and neuronal morphogenesis, with implications for our understanding of developmental disorders of cognition.

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The σ -1 Receptor Interacts Directly with GluN1 But Not GluN2A in the GluN1/GluN2A NMDA Receptor

Dilshan Balasuriya, Andrew P. Stewart, and J. Michael Edwardson

Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, United Kingdom

The σ -1 receptor (Sig1R) is widely expressed in the CNS, where it has a neuroprotective role in ischemia and stroke and an involvement in schizophrenia. The Sig1R interacts functionally with a variety of ion channels, including the NMDA receptor (NMDAR). Here, we used atomic force microscopy (AFM) imaging to investigate the interaction between the Sig1R and the NMDAR. The Sig1R bound directly to GluN1/GluN2A NMDAR heterotetramers. Furthermore, the mean angle between pairs of bound Sig1Rs was 72°. This result suggested that the Sig1R interacts with either GluN1 or GluN2A, but not both, and supports our recent demonstration that the NMDAR subunits adopt an adjacent (i.e., 1/1/2/2) arrangement. The Sig1R could be coisolated with GluN1 but not with GluN2A, indicating that GluN1 is its specific target within the NMDAR. Consistent with this conclusion, AFM imaging of coisolated Sig1R and GluN1 revealed GluN1 dimers decorated with Sig1Rs. *In situ* proximity ligation assays demonstrated that the Sig1R interacts with GluN1 (but not with GluN2A) within intact cells and also that its C terminus is extracellular. We conclude that the Sig1R binds to the GluN1/GluN2A NMDAR specifically via the GluN1 subunit. This interaction likely accounts for at least some of the modulatory effects of Sig1R ligands on the NMDAR.

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Early Auditory Processing in Area V5/MT+ of the Congenitally Blind Brain

Kate E. Watkins,^{1,2} Timothy J. Shakespeare,¹ M. Clare O'Donoghue,^{1,2} Iona Alexander,¹ Nicola Ragge,³ Alan Cowey,^{1†} and Holly Bridge²

¹Department of Experimental Psychology, University of Oxford, Oxford, OX1 3UD, United Kingdom, ²Functional MRI of the Brain Centre, Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom, and ³Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, OX3 0BP, United Kingdom

Previous imaging studies of congenital blindness have studied individuals with heterogeneous causes of blindness, which may influence the nature and extent of cross-modal plasticity. Here, we scanned a homogeneous group of blind people with bilateral congenital anophthalmia, a condition in which both eyes fail to develop, and, as a result, the visual pathway is not stimulated by either light or retinal waves. This model of congenital blindness presents an opportunity to investigate the effects of very early visual deafferentation on the functional organization of the brain. In anophthalmic animals, the occipital cortex receives direct subcortical auditory input. We hypothesized that this pattern of subcortical reorganization ought to result in a topographic mapping of auditory frequency information in the occipital cortex of anophthalmic people. Using functional MRI, we examined auditory-evoked activity to pure tones of high, medium, and low frequencies. Activity in the superior temporal cortex was significantly reduced in anophthalmic compared with sighted participants. In the occipital cortex, a region corresponding to the cytoarchitectural area V5/MT+ was activated in the anophthalmic participants but not in sighted controls. Whereas previous studies in the blind indicate that this cortical area is activated to auditory motion, our data show it is also active for trains of pure tone stimuli and in some anophthalmic participants shows a topographic mapping (tonotopy). Therefore, this region appears to be performing early sensory processing, possibly served by direct subcortical input from the pulvinar to V5/MT+.

The Journal of Neuroscience, November 13, 2013 • 33(46):18242–18246

Microglial CD33-Related Siglec-E Inhibits Neurotoxicity by Preventing the Phagocytosis-Associated Oxidative Burst

Janine Claude,¹ Bettina Linnartz-Gerlach,¹ Alexei P. Kudin,² Wolfram S. Kunz,² and Harald Neumann¹

¹Neural Regeneration Group, Institute of Reconstructive Neurobiology and ²Division of Neurochemistry, Department of Epileptology, University of Bonn Medical Center, 53127 Bonn, Germany

Sialic acid-binding Ig-like lectins (Siglecs) are members of the Ig superfamily that recognize sialic acid residues of glycoproteins. Siglec-E is a mouse CD33-related Siglec that preferentially binds to sialic acid residues of the cellular glycocalyx. Here, we demonstrate gene transcription and protein expression of Siglec-E by cultured mouse microglia. Siglec-E on microglia inhibited phagocytosis of neural debris and prevented the production of superoxide radicals induced by challenge with neural debris. Soluble extracellular Siglec-E receptor protein bound to the neural glycocalyx. Coculture of mouse microglia and neurons demonstrated a neuroprotective effect of microglial Siglec-E that was dependent on neuronal sialic acid residues. Increased neurotoxicity of microglia after knockdown of *Siglece* mRNA was neutralized by the reactive oxygen species scavenger Trolox. Data suggest that Siglec-E recognizes the intact neuronal glycocalyx and has neuroprotective function by preventing phagocytosis and the associated oxidative burst.

The Journal of Neuroscience, November 13, 2013 • 33(46):18270–18276

Articles

CELLULAR/MOLECULAR

$\alpha 6\beta 1$ and $\alpha 7\beta 1$ Integrins Are Required in Schwann Cells to Sort Axons

Marta Pellegatta,^{1,2} Adèle De Arcangelis,³ Alessandra D'Urso,¹ Alessandro Nodari,¹ Desirée Zambroni,¹ Monica Ghidinelli,^{1,2} Vittoria Matafora,¹ Courtney Williamson,² Elisabeth Georges-Labouesse,^{3†} Jordan Kreidberg,⁴ Ulrike Mayer,⁵ Karen K. McKee,⁶ Peter D. Yurchenco,⁶ Angelo Quattrini,¹ Lawrence Wrabetz,^{1,2} and Maria Laura Feltri^{1,2}

¹San Raffaele Scientific Institute, Milano 20132, Italy, ²Hunter James Kelly Research Institute, University at Buffalo, State University of New York, New York 14203, ³Development and Stem Cells Program, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7104, Institut National de la Santé et de la Recherche Médicale U964, Université de Strasbourg, Illkirch 67404, France, ⁴Department of Medicine, Children's Hospital Boston and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, ⁵Biomedical Research Centre, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom, and ⁶Robert Wood Johnson Medical School, Piscataway, New Jersey, New Jersey 08854

During development, Schwann cells extend lamellipodia-like processes to segregate large- and small-caliber axons during the process of radial sorting. Radial sorting is a prerequisite for myelination and is arrested in human neuropathies because of laminin deficiency. Experiments in mice using targeted mutagenesis have confirmed that laminins 211, 411, and receptors containing the $\beta 1$ integrin subunit are required for radial sorting; however, which of the 11 α integrins that can pair with $\beta 1$ forms the functional receptor is unknown. Here we conditionally deleted all the α subunits that form predominant laminin-binding $\beta 1$ integrins in Schwann cells and show that only $\alpha 6\beta 1$ and $\alpha 7\beta 1$ integrins are required and that $\alpha 7\beta 1$ compensates for the absence of $\alpha 6\beta 1$ during development. The absence of either $\alpha 7\beta 1$ or $\alpha 6\beta 1$ integrin impairs the ability of Schwann cells to spread and to bind laminin 211 or 411, potentially explaining the failure to extend cytoplasmic processes around axons to sort them. However, double $\alpha 6/\alpha 7$ integrin mutants show only a subset of the abnormalities found in mutants lacking all $\beta 1$ integrins, and a milder phenotype. Double-mutant Schwann cells can properly activate all the major signaling pathways associated with radial sorting and show normal Schwann cell proliferation and survival. Thus, $\alpha 6\beta 1$ and $\alpha 7\beta 1$ are the laminin-binding integrins required for axonal sorting, but other Schwann cell $\beta 1$ integrins, possibly those that do not bind laminins, may also contribute to radial sorting during peripheral nerve development.

The Journal of Neuroscience, November 13, 2013 • 33(46):17995–18007

Tonic Activation of Bax Primes Neural Progenitors for Rapid Apoptosis through a Mechanism Preserved in Medulloblastoma

Andrew J. Crowther,^{1*} Vivian Gama,^{6*} Ariana Bevilacqua,⁴ Sha X. Chang,^{2,5} Hong Yuan,^{3,5} Mohanish Deshmukh,^{4,5,6} and Timothy R. Gershon^{1,4,5}

Departments of ¹Neurology, ²Radiation Oncology, and ³Radiology, ⁴Neuroscience Center, ⁵Lineberger Comprehensive Cancer Center, and ⁶Department of Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599

Commitment to survival or apoptosis within expanding progenitor populations poses distinct risks and benefits to the organism. We investigated whether specialized mechanisms regulate apoptosis in mouse neural progenitors and in the progenitor-derived brain tumor medulloblastoma. Here, we identified constitutive activation of proapoptotic Bax, maintained in check by Bcl-xL, as a mechanism for rapid cell death, common to postnatal neural progenitors and medulloblastoma. We found that tonic activation of Bax in cerebellar progenitors, along with sensitivity to DNA damage, was linked to differentiation state. In cerebellar progenitors, active Bax localized to mitochondria, where it was bound to Bcl-xL. Disruption of Bax:Bcl-xL binding by BH3-mimetic ABT 737 caused rapid apoptosis of cerebellar progenitors and primary

murine medulloblastoma cells. Conditional deletion of Mcl-1, in contrast, did not cause death of cerebellar progenitors. Our findings identify a mechanism for the sensitivity of brain progenitors to typical anticancer therapies and reveal that this mechanism persists in medulloblastoma, a malignant brain tumor markedly sensitive to radiation and chemotherapy.

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D₁ Dopamine Receptor Coupling to PLC β Regulates Forward Locomotion in Mice

Ivan O. Medvedev,^{1*} Amy J. Ramsey,^{2*} Shababa T. Masoud,² Marie Kristel Bermejo,² Nikhil Urs,¹ Tatyana D. Sotnikova,³ Jean-Martin Beaulieu,⁶ Raul R. Gainetdinov,^{3,4,5} and Ali Salahpour²

¹Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710, ²Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A8, Canada, ³Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, 16163 Genova, Italy, ⁴Skolkovo Institute of Science and Technology, Skolkovo, Moscow Region, 143025, Russia, ⁵Faculty of Biology and Soil Science, St. Petersburg State University, St. Petersburg 199034, Russia, and ⁶Department of Psychiatry and Neuroscience, Faculty of Medicine, Laval University and Institut Universitaire en Santé Mentale de Québec, Québec City, Québec G1J 2G3, Canada

Several studies have reported the coupling of dopamine signaling to phospholipase C β (PLC β) both *in vitro* and *in vivo*. However, the precise physiological relevance of this signaling pathway in mediating dopamine behaviors is still unclear. Here we report that stimulation of dopamine receptor signaling *in vivo* with systemic administration of apomorphine, amphetamine, and cocaine leads to increased production of inositol triphosphate (IP₃) in the mouse striatum. Using selective antagonists and dopamine D₁ and D₂ receptor knock-out animals, we show that the production of IP₃ is mediated by the D₁ receptor, but not the D₂ receptor. A selective blocker of PLC β , U73122, was used to assess the physiological relevance of D₁-mediated IP₃ production. We show that U73122 inhibits the locomotor-stimulating effects of apomorphine, amphetamine, cocaine, and SKF81297. Furthermore, U73122 also suppresses the spontaneous hyperactivity exhibited by dopamine transporter knock-out mice. Importantly, the effects of U73122 are selective to dopamine-mediated hyperactivity, as this compound does not affect hyperactivity induced by the glutamate NMDA receptor antagonist MK801. Finally, we present evidence showing that an imbalance of D₁- and D₂-mediated signaling following U73122 treatment modifies the locomotor output of animals from horizontal locomotor activity to vertical activity, further highlighting the importance of the PLC β pathway in the regulation of forward locomotion via dopamine receptors.

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The Fragile X Mental Retardation Protein Regulates Matrix Metalloproteinase 9 mRNA at Synapses

Aleksandra Janusz,¹ Jacek Milek,^{1,2} Małgorzata Perycz,³ Laura Pacini,⁴ Claudia Bagni,^{4,5,6} Leszek Kaczmarek,¹ and Magdalena Dziembowska¹

¹Laboratory of Neurobiology, The Nencki Institute, 02-093 Warsaw, Poland, ²Institute of Biochemistry and Biophysics, 02-106 Warsaw, Poland,

³Laboratory of Molecular and Cellular Neurobiology, The International Institute of Molecular and Cell Biology, 02-109 Warsaw, Poland, ⁴Department of Biomedicine and Prevention, University “Tor Vergata,” 00133 Rome, Italy, ⁵VIB Center for the Biology of Disease, 3000 Leuven, Belgium, and ⁶Center for Human Genetics, KU Leuven, 3000 Leuven, Belgium

Activity-dependent protein synthesis at synapses is dysregulated in the Fragile X syndrome (FXS). This process contributes to dendritic spine dysmorphogenesis and synaptic dysfunction in FXS. Matrix Metalloproteinase 9 (MMP-9) is an enzyme involved in activity-dependent reorganization of dendritic spine architecture and was shown to regulate spine morphology in a mouse model of FXS, the Fmr1 knock-out mice. Here we show that MMP-9 mRNA is part of the FMRP complex and colocalizes in dendrites. In the absence of FMRP MMP-9 mRNA translation is increased at synapses, suggesting that this mechanism contributes to the increased metalloproteinase level at synapses of Fmr1 knock-out mice. We propose that such a local effect can contribute to the aberrant dendritic spine morphology observed in the Fmr1 knock-out mice and in patients with FXS.

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CRMP2 Tethers Kainate Receptor Activity to Cytoskeleton Dynamics during Neuronal Maturation

Joana M. Marques,^{1*} Ricardo J. Rodrigues,^{1*} Sergio Valbuena,¹ Jose L. Rozas,¹ Sanja Selak,¹ Philippe Marin,² Maria I. Aller,¹ and Juan Lerma¹

¹Instituto de Neurociencias, Centro mixto de la Universidad Miguel Hernández de Elche y el Consejo Superior de Investigaciones Científicas, 03550 San Juan de Alicante, Spain; and ²Institute of Functional Genomics, Centre National de la Recherche Scientifique–Unité Mixte de Recherche 5203, INSERM U661, F34094 Montpellier Cedex 5, France

The CRMP2 and CRMP4 proteins are strongly expressed in the developing nervous system, mediating neurite outgrowth, neuronal polarity, and axon guidance. In the present study, we demonstrate the interaction of the CRMP2 and CRMP4 proteins with the GluK5 subunit of the kainate (KA) receptor (KAR) and investigated the role of KARs in modulating the development of cultured mouse DRG neurons. We found that KARs modulate neuronal maturation and neurite outgrowth in a bidirectional manner. Accordingly, low concentrations of KA delayed maturation and enhanced neurite outgrowth, whereas maturation was promoted by higher concentrations of KA that attenuated neuritic elongation. The effects of weak KAR activation were prevented by blocking their noncanonical signaling and involved a differential regulation of CRMP2. Whereas the delay in maturation involves PKC-mediated phosphorylation of CRMP2 at T555 leading to a downregulation of membrane Cav2.2, the promotion of

neurite outgrowth is achieved by dephosphorylation at T514 at the growth cones, the latter reflecting PKC-driven enhancement of GSK3 β phosphorylation at S9. Together, these findings indicate that noncanonical KAR signaling influences neuronal development by modulating CRMP2 activity.
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Glial Control of Endocannabinoid Heterosynaptic Modulation in Hypothalamic Magnocellular Neuroendocrine Cells

Shi Di,¹ Ion R. Popescu,¹ and Jeffrey G. Tasker^{1,2}

¹Department of Cell and Molecular Biology, and ²Neuroscience Program, Tulane University, New Orleans, Louisiana 70118

Cannabinoid receptors are functionally operant at both glutamate and GABA synapses on hypothalamic magnocellular neuroendocrine cells; however, retrograde endocannabinoid actions are evoked at only glutamate synapses. We tested whether the functional targeting of evoked retrograde endocannabinoid actions to glutamate, and not GABA, synapses on magnocellular neurons is the result of the spatial restriction of extracellular endocannabinoids by astrocytes. Whole-cell GABA synaptic currents were recorded in magnocellular neurons in rat hypothalamic slices following manipulations to reduce glial buffering of extracellular signals. Depolarization- and glucocorticoid-evoked retrograde endocannabinoid suppression of synaptic GABA release was not detected under normal conditions, but occurred in both oxytocin and vasopressin neurons under conditions of attenuated glial coverage and depressed glial metabolic function, suggesting an emergent endocannabinoid modulation of GABA synapses with the loss of astrocyte function. Tonic endocannabinoid suppression of GABA release was insensitive to glial manipulation. Blocking cannabinoid transport mimicked, and increasing the extracellular viscosity reversed, the effect of suppressed glial buffering on the endocannabinoid modulation of GABA release. Evoked, but not tonic, endocannabinoid modulation of GABA synapses was mediated by 2-arachidonoylglycerol. Therefore, depolarization- and glucocorticoid-evoked 2-arachidonoylglycerol release from magnocellular neurons is spatially restricted to glutamate synapses by astrocytes, but spills over onto GABA synapses under conditions of reduced astrocyte buffering; tonic endocannabinoid modulation of GABA release, in contrast, is likely mediated by anandamide and is insensitive to astrocytic buffering. Astrocytes, therefore, provide dynamic control of stimulus-evoked 2-arachidonoylglycerol, but not tonic anandamide, regulation of GABA synaptic inputs to magnocellular neuroendocrine cells under different physiological conditions.

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DEVELOPMENT/PLASTICITY/REPAIR

The Protein Kinase A Regulatory Subunit R1A (Prkar1a) Plays Critical Roles in Peripheral Nerve Development

Li Guo,¹ Audrey A. Lee,² Tilat A. Rizvi, Nancy Ratner,¹ and Lawrence S. Kirschner^{2,3}

¹Division of Experimental Hematology and Cancer Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229, ²Department of Molecular, Virology, Immunology, and Medical Genetics, and ³Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine The Ohio State University, Columbus, Ohio 43210

Signaling through cAMP has been implicated in Schwann cell (SC) proliferation and myelination, but the signaling pathway components downstream of cAMP required for SC function remain unknown. Protein kinase A (PKA) is a potential downstream effector of cAMP. Here, we induced loss of *Prkar1a*, the gene encoding the type 1A regulatory subunit of PKA, in SC to study its role in nerve development; loss of *Prkar1a* is predicted to elevate PKA activity. Conditional *Prkar1a* knock-out in mouse SC (*Prkar1a*-SCKO) resulted in a dramatic and persistent axonal sorting defect, and unexpectedly decreased SC proliferation in *Prkar1a*-SCKO nerves *in vivo*. Effects were cell autonomous as they were recapitulated *in vitro* in *Prkar1a*-SCKO SC, which showed elevated PKA activity. In the few SCs sorted into 1:1 relationships with axons *in vivo*, SC myelination was premature in *Prkar1a*-SCKO nerves, correlating with global increase in the cAMP-regulated transcription factor Oct-6 and expression of myelin basic protein. These data reveal a previously unknown role of PKA in axon sorting, an unexpected inhibitory role of PKA on SC cell proliferation *in vivo* and define the importance of *Prkar1a* in peripheral nerve development.

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Gpr126 Functions in Schwann Cells to Control Differentiation and Myelination via G-Protein Activation

Amit Mogha,¹ Andrew E. Benesh,¹ Chinmoy Patra,³ Felix B. Engel,^{3,4} Torsten Schöneberg,⁵ Ines Liebscher,⁵ and Kelly R. Monk^{1,2}

¹Department of Developmental Biology, ²Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, Missouri 63110

³Department of Cardiac Development and Remodeling, Max Planck Institute for Heart and Lung Research, 61231 Bad Nauheim, Germany, ⁴Experimental Renal and Cardiovascular Research, Department of Nephropathology, Institute of Pathology, University of Erlangen-Nürnberg, 91054 Erlangen, Germany, and ⁵Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, University of Leipzig, 04103 Leipzig, Germany

The myelin sheath surrounding axons ensures that nerve impulses travel quickly and efficiently, allowing for the proper function of the vertebrate nervous system. We previously showed that the adhesion G-protein-coupled receptor (aGPCR) Gpr126 is essential for peripheral nervous system myelination, although the molecular mechanisms by which Gpr126 functions were incompletely understood. aGPCRs are a significantly understudied protein class, and it was unknown whether Gpr126 couples to G-proteins. Here, we analyze *Dhh^{Cre};Gpr126^{fl/fl}* conditional mutants, and show that Gpr126 functions in Schwann cells (SCs) for radial sorting of axons and myelination.

Furthermore, we demonstrate that elevation of cAMP levels or protein kinase A activation suppresses myelin defects in *Gpr126* mouse mutants and that cAMP levels are reduced in conditional *Gpr126* mutant peripheral nerve. Finally, we show that GPR126 directly increases cAMP by coupling to heterotrimeric G-proteins. Together, these data support a model in which Gpr126 functions in SCs for proper development and myelination and provide evidence that these functions are mediated via G-protein-signaling pathways.

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Cortical Reorganization after Long-Term Adaptation to Retinal Lesions in Humans

Susana T. L. Chung

School of Optometry, University of California, Berkeley, Berkeley, California 94720-2020

Single-unit recordings demonstrated that the adult mammalian visual cortex is capable of reorganizing after induced retinal lesions. In humans, whether the adult cortex is capable of reorganizing has only been studied using functional magnetic resonance imaging, with equivocal results. Here, we exploited the phenomenon of visual crowding, a major limitation on object recognition, to show that, in humans with long-standing retinal (macular) lesions that afflict the fovea and thus use their peripheral vision exclusively, the signature properties of crowding are distinctly different from those of the normal periphery. Crowding refers to the inability to recognize objects when the object spacing is smaller than the critical spacing. Critical spacing depends only on the retinal location of the object, scales linearly with its distance from the fovea, and is approximately two times larger in the radial than the tangential direction with respect to the fovea, thus demonstrating the signature radial–tangential anisotropy of the crowding zone. Using retinal imaging combined with behavioral measurements, we mapped out the crowding zone at the precise peripheral retinal locations adopted by individuals with macular lesions as the new visual reference loci. At these loci, the critical spacings are substantially smaller along the radial direction than expected based on the normal periphery, resulting in a lower scaling of critical spacing with the eccentricity of the peripheral locus and a loss in the signature radial–tangential anisotropy of the crowding zone. These results imply a fundamental difference in the substrate of cortical processing in object recognition following long-term adaptation to macular lesions.

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N-Cadherin Sustains Motility and Polarity of Future Cortical Interneurons during Tangential Migration

Camilla Luccardini,^{1,2} Laetitia Hennekinne,^{1,2} Lucie Viou,^{1,2} Mitsutoshi Yanagida,³ Fujio Murakami,³ Nicoletta Kessaris,⁴ Xufei Ma,⁵ Robert S. Adelstein,⁵ René-Marc Mège,^{1,2} and Christine Métin^{1,2}

¹Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 839, Institut du Fer à Moulin, 75005 Paris, France, ²Université Pierre et Marie Curie–Paris 6, 75005 Paris, France, ³Graduate School of Frontier Biosciences, Osaka University, Osaka 565-0871, Japan, ⁴Wolfson Institute for Biomedical Research and Department of Cell and Developmental Biology, University College London, London, WC1E 6BT, United Kingdom, and

⁵Laboratory of Molecular Cardiology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

In the developing brain, cortical GABAergic interneurons migrate long distances from the medial ganglionic eminence (MGE) in which they are generated, to the cortex in which they settle. MGE cells express the cell adhesion molecule N-cadherin, a homophilic cell–cell adhesion molecule that regulates numerous steps of brain development, from neuroepithelium morphogenesis to synapse formation. N-cadherin is also expressed in embryonic territories crossed by MGE cells during their migration. In this study, we demonstrate that N-cadherin is a key player in the long-distance migration of future cortical interneurons. Using N-cadherin-coated substrate, we show that N-cadherin-dependent adhesion promotes the migration of mouse MGE cells *in vitro*. Conversely, mouse MGE cells electroporated with a construct interfering with cadherin function show reduced cell motility, leading process instability, and impaired polarization associated with abnormal myosin IIB dynamics. *In vivo*, the capability of electroporated MGE cells to invade the developing cortical plate is altered. Using genetic ablation of *N-cadherin* in mouse embryos, we show that *N-cadherin*-depleted MGEs are severely disorganized. MGE cells hardly exit the disorganized proliferative area. *N-cadherin* ablation at the postmitotic stage, which does not affect MGE morphogenesis, alters MGE cell motility and directionality. The tangential migration to the cortex of *N-cadherin* ablated MGE cells is delayed, and their radial migration within the cortical plate is perturbed. Altogether, these results identify N-cadherin as a pivotal adhesion substrate that activates cell motility in future cortical interneurons and maintains cell polarity over their long-distance migration to the developing cortex.

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Uncoupling of EphA/ephrinA Signaling and Spontaneous Activity in Neural Circuit Wiring

Isabel Benjumeda,^{1*} Augusto Escalante,^{1*} Chris Law,^{2*} Daniel Morales,^{2,3} Geraud Chauvin,¹ Gerald Muça,¹ Yaiza Coca,¹ Joaquín Márquez,¹ Guillermina López-Bendito,¹ Artur Kania,^{2,3,4,5} Luis Martínez,¹ and Eloísa Herrera¹

¹Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas and Universidad Miguel Hernández, Campus San Juan de Alicante, 03550 Alicante, Spain, ²Institut de Recherches Cliniques de Montréal, Montréal, Québec H2W 1R7, Canada, ³McGill University Integrated Program in Neuroscience, Montréal, Québec H3A 2B4, ⁴Departments of Anatomy and Cell Biology, and Biology, Division of Experimental Medicine, McGill University, Montréal, Québec H3A 2B2, and ⁵Faculté de Médecine, Université de Montréal, Montréal, Québec H3C 3J7, Canada

Classic studies have proposed that genetically encoded programs and spontaneous activity play complementary but independent roles in the development of neural circuits. Recent evidence, however, suggests that these two mechanisms could interact extensively, with spontaneous activity affecting the expression and function of guidance molecules at early developmental stages. Here, using the developing chick spinal cord and the mouse visual system to ectopically express the inwardly rectifying potassium channel Kir2.1 in individual embryonic neurons, we demonstrate that cell-intrinsic blockade of spontaneous activity *in vivo* does not affect neuronal identity

specification, axon pathfinding, or EphA/ephrinA signaling during the development of topographic maps. However, intrinsic spontaneous activity is critical for axon branching and pruning once axonal growth cones reach their correct topographic position in the target tissues. Our experiments argue for the dissociation of spontaneous activity from hard-wired developmental programs in early phases of neural circuit formation.

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SYSTEMS/CIRCUITS

Safety Signals in the Primate Amygdala

Rotem Genud-Gabai, Oded Klavir, and Rony Paz

Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel 76100

The ability to distinguish danger from safety is crucial for survival. On the other hand, anxiety disorders can result from failures to dissociate safe cues from those that predict dangerous outcomes. The amygdala plays a major role in learning and signaling danger, and recently, evidence accumulates that it also acquires information to signal safety. Traditionally, safety is explored by paradigms that change the value of a previously dangerous cue, such as extinction or reversal; or by paradigms showing that a safe cue can inhibit responses to another danger-predicting cue, as in conditioned-inhibition. In real-life scenarios, many cues are never paired or tested with danger and remain neutral all along. A detailed study of neural responses to unpaired conditioned-stimulus (CS–) can therefore indicate whether information on safety-by-comparison is also acquired in the amygdala. We designed a multiple-CS study, with CS– from both visual and auditory modalities. Using discriminative aversive-conditioning, we find that responses in the primate amygdala develop for CS– of the same modality and of a different modality from that of the aversive CS+. Moreover, we find that responses are comparable in proportion, sign (increase/decrease), onset, and magnitude. These results indicate that the primate amygdala actively acquires signals about safety, and strengthen the hypothesis that failure in amygdala processing can result in failure to distinguish dangerous cues from safe ones and lead to maladaptive behaviors.

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Medial Habenula Output Circuit Mediated by $\alpha 5$ Nicotinic Receptor-Expressing GABAergic Neurons in the Interpeduncular Nucleus

Yun-Wei A. Hsu,¹ Lynne Tempest,¹ Lely A. Quina,¹ Aguan D. Wei,^{1,4} Hongkui Zeng,⁵ and Eric E. Turner^{1,2,3}

¹Center for Integrative Brain Research, Seattle Children's Research Institute, and ²Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington 98101, ³Center on Human Development and Disability and ⁴Department of Neurological Surgery, University of Washington, Seattle, Washington 98195, and ⁵Allen Institute for Brain Science, Seattle, Washington 98103

The *Chrna5* gene encodes the $\alpha 5$ nicotinic acetylcholine receptor subunit, an “accessory” subunit of pentameric nicotinic receptors, that has been shown to play a role in nicotine-related behaviors in rodents and is genetically linked to smoking behavior in humans. Here we have used a BAC transgenic mouse line, $\alpha 5^{GFP}$, to examine the cellular phenotype, connectivity, and function of $\alpha 5$ -expressing neurons. Although the medial habenula (MHb) has been proposed as a site of $\alpha 5$ function, $\alpha 5^{GFP}$ is not detectable in the MHb, and $\alpha 5$ mRNA is expressed there only at very low levels. However, $\alpha 5^{GFP}$ is strongly expressed in a subset of neurons in the interpeduncular nucleus (IP), median raphe/paramedian raphe (MnR/PMnR), and dorsal tegmental area (DTg). Double-label fluorescence *in situ* hybridization reveals that these neurons are exclusively GABAergic. Transgenic and conventional tract tracing show that $\alpha 5^{GFP}$ neurons in the IP project principally to the MnR/PMnR and DTg/interfascicular dorsal raphe, both areas rich in serotonergic neurons. The $\alpha 5^{GFP}$ neurons in the IP are located in a region that receives cholinergic fiber inputs from the ventral MHb, and optogenetically assisted circuit mapping demonstrates a monosynaptic connection between these cholinergic neurons and $\alpha 5^{GFP}$ IP neurons. Selective inhibitors of both $\alpha 4\beta 2$ - and $\alpha 3\beta 4$ -containing nicotinic receptors were able to reduce nicotine-evoked inward currents in $\alpha 5^{GFP}$ neurons in the IP, suggesting a mixed nicotinic receptor profile in these cells. Together, these findings show that the $\alpha 5$ -GABAergic interneurons form a link from the MHb to serotonergic brain centers, which is likely to mediate some of the behavioral effects of nicotine.

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An Updated Midline Rule: Visual Callosal Connections Anticipate Shape and Motion in Ongoing Activity across the Hemispheres

Christiane Peiker,¹ Thomas Wunderle,¹ David Eriksson,¹ Anne Schmidt,¹ and Kerstin E. Schmidt^{1,2}

¹Max Planck Institute for Brain Research, 60528 Frankfurt am Main, Germany, and ²Brain Institute, Federal University of Rio Grande do Norte, 59056-450 Natal, Brazil

It is generally thought that callosal connections (CCs) in primary visual cortices serve to unify the visual scenery parted in two at the vertical midline (VM). Here, we present evidence that this applies also to visual features that do not cross yet but might cross the VM in the future. During reversible deactivation of the contralateral visual cortex in cats, we observed that ipsilaterally recorded neurons close to the border between areas 17 and 18 receive selective excitatory callosal input on both ongoing and evoked activity. In detail, neurons responding well to a vertical Gabor patch moving away from the deactivated hemifield decreased prestimulus and stimulus-driven activity much more than those preferring motion toward the cooled hemifield. Further, activity of neurons responding to horizontal lines decreased more than the response to vertical lines. Embedding a single Gabor into a collinear line context selectively stabilized responses, especially when the context was limited to the intact hemifield. These findings indicate that CCs interconnect not only neurons coding for similar orientations but also for similar directions of motion. We conclude that CCs anticipate stimulus features that are potentially relevant for both hemifields (i.e., coherent motion but also collinear shape) because already prestimulus activity and activity to stimuli not crossing the VM revealed feature specificity. Finally, we hypothesize that intrinsic and callosal networks processing different orientations and directions are anisotropic close to the VM facilitating perceptual grouping along likely future motion or (shape) trajectories before the visual stimulus arrives.

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Convergent Rhythm Generation from Divergent Cellular Mechanisms

Jason C. Rodriguez, Dawn M. Blitz, and Michael P. Nusbaum

Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Different modulatory inputs commonly elicit distinct rhythmic motor patterns from a central pattern generator (CPG), but they can instead elicit the same pattern. We are determining the rhythm-generating mechanisms in this latter situation, using the gastric mill (chewing) CPG in the crab (*Cancer borealis*) stomatogastric ganglion, where stimulating the projection neuron MCN1 (modulatory commissural neuron 1) or bath applying CabPK (*C. borealis* pyrokinin) peptide elicits the same gastric mill motor pattern, despite configuring different gastric mill circuits. In both cases, the core rhythm generator includes the same reciprocally inhibitory neurons LG (lateral gastric) and Int1 (interneuron 1), but the pyloric (food-filtering) circuit pacemaker neuron AB (anterior burster) is additionally necessary only for CabPK rhythm generation. MCN1 drives this rhythm generator by activating in the LG neuron the modulator-activated inward current (I_{MI}), which waxes and wanes periodically due to phasic feedback inhibition of MCN1 transmitter release. Each buildup of I_{MI} enables the LG neuron to generate a self-terminating burst and thereby alternate with Int1 activity. Here we establish that CabPK drives gastric mill rhythm generation by activating in the LG neuron I_{MI} plus a slowly activating transient, low-threshold inward current ($I_{Trans-LTS}$) that is voltage, time, and Ca^{2+} dependent. Unlike MCN1, CabPK maintains a steady I_{MI} activation, causing a subthreshold depolarization in LG that facilitates a periodic postinhibitory rebound burst caused by the regular buildup and decay of the availability of $I_{Trans-LTS}$. Thus, different modulatory inputs can use different rhythm-generating mechanisms to drive the same neuronal rhythm. Additionally, the same ionic current (I_{MI}) can play different roles under these different conditions, while different currents (I_{MI} , $I_{Trans-LTS}$) can play the same role.

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Frontal Cortex Activation Causes Rapid Plasticity of Auditory Cortical Processing

Daniel E. Winkowski,^{1*} Sharba Bandyopadhyay,^{1,2*} Shihab A. Shamma,^{1,3} and Patrick O. Kanold^{1,2}

¹Institute for Systems Research, ²Department of Biology, and ³Department for Electrical and Computer Engineering, University of Maryland, College Park, Maryland 20742

Neurons in the primary auditory cortex (A1) can show rapid changes in receptive fields when animals are engaged in sound detection and discrimination tasks. The source of a signal to A1 that triggers these changes is suspected to be in frontal cortical areas. How or whether activity in frontal areas can influence activity and sensory processing in A1 and the detailed changes occurring in A1 on the level of single neurons and in neuronal populations remain uncertain. Using electrophysiological techniques in mice, we found that pairing orbitofrontal cortex (OFC) stimulation with sound stimuli caused rapid changes in the sound-driven activity within A1 that are largely mediated by noncholinergic mechanisms. By integrating *in vivo* two-photon Ca^{2+} imaging of A1 with OFC stimulation, we found that pairing OFC activity with sounds caused dynamic and selective changes in sensory responses of neural populations in A1. Further, analysis of changes in signal and noise correlation after OFC pairing revealed improvement in neural population-based discrimination performance within A1. This improvement was frequency specific and dependent on correlation changes. These OFC-induced influences on auditory responses resemble behavior-induced influences on auditory responses and demonstrate that OFC activity could underlie the coordination of rapid, dynamic changes in A1 to dynamic sensory environments.

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Ventromedial Prefrontal Cortex Pyramidal Cells Have a Temporal Dynamic Role in Recall and Extinction of Cocaine-Associated Memory

Michel C. Van den Oever,¹ Diana C. Rotaru,² Jasper A. Heinsbroek,¹ Yvonne Gouwenberg,¹ Karl Deisseroth,³ Garret D. Stuber,^{4,5} Huibert D. Mansvelder,² and August B. Smit¹

Departments of ¹Molecular and Cellular Neurobiology and ²Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University Amsterdam, 1081 HV, The Netherlands, ³Department Bioengineering, Stanford University School of Medicine, Stanford, California 94305, Departments of ⁴Psychiatry and ⁵Cell Biology and Physiology, UNC Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

In addicts, associative memories related to the rewarding effects of drugs of abuse can evoke powerful craving and drug seeking urges, but effective treatment to suppress these memories is not available. Detailed insight into the neural circuitry that mediates expression of drug-associated memory is therefore of crucial importance. Substantial evidence from rodent models of addictive behavior points to the involvement of the ventromedial prefrontal cortex (vmPFC) in conditioned drug seeking, but specific knowledge of the temporal role of vmPFC pyramidal cells is lacking. To this end, we used an optogenetics approach to probe the involvement of vmPFC pyramidal cells in expression of a recent and remote conditioned cocaine memory. In mice, we expressed Channelrhodopsin-2 (ChR2) or Halorhodopsin (eNpHR3.0) in pyramidal cells of the vmPFC and studied the effect of activation or inhibition of these cells during expression of a cocaine-contextual memory on days 1–2 (recent) and ~3 weeks (remote) after conditioning. Whereas optical activation of pyramidal cells facilitated extinction of remote memory, without affecting recent memory, inhibition of pyramidal cells acutely impaired recall of recent cocaine memory, without affecting recall of remote memory. In addition, we found that silencing pyramidal cells blocked extinction learning at the remote memory time-point. We provide causal evidence of a critical time-dependent switch in the contribution of vmPFC pyramidal cells to recall and extinction of cocaine-associated memory, indicating that the circuitry that controls expression of cocaine memories reorganizes over time.

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Compression and Suppression of Shifting Receptive Field Activity in Frontal Eye Field Neurons

Wilsaan M. Joiner,^{1,2*} James Cavanaugh,^{1*} and Robert H. Wurtz¹

¹Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892-2510 and ²George Mason University, Department of Bioengineering, Fairfax, Virginia 22030

Before each saccade, neurons in frontal eye field anticipate the impending eye movement by showing sensitivity to stimuli appearing where the neuron's receptive field will be at the end of the saccade, referred to as the future field (FF) of the neuron. We explored the time course of this anticipatory activity in monkeys by briefly flashing stimuli in the FF at different times before saccades. Different neurons showed substantial variation in FF time course, but two salient observations emerged. First, when we compared the time span of stimulus probes before the saccade to the time span of FF activity, we found a striking temporal compression of FF activity, similar to compression seen for perisaccadic stimuli in human psychophysics. Second, neurons with distinct FF activity also showed suppression at the time of the saccade. The increase in FF activity and the decrease with suppression were temporally independent, making the patterns of activity difficult to separate. We resolved this by constructing a simple model with values for the start, peak, and duration of FF activity and suppression for each neuron. The model revealed the different time courses of FF sensitivity and suppression, suggesting that information about the impending saccade triggering suppression reaches the frontal eye field through a different pathway, or a different mechanism, than that triggering FF activity. Recognition of the variations in the time course of anticipatory FF activity provides critical information on its function and its relation to human visual perception at the time of the saccade.

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Neural Codes Formed by Small and Temporally Precise Populations in Auditory Cortex

Robin A. A. Ince,^{1,2} Stefano Panzeri,^{2,3} and Christoph Kayser^{1,2,4}

¹Max Planck Institute for Biological Cybernetics, 72076 Tübingen, Germany, ²Institute of Neuroscience and Psychology, University of Glasgow, Glasgow G12 8QB, United Kingdom, ³Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, 38068 Rovereto, Italy, and ⁴Bernstein Centre for Computational Neuroscience, 72076 Tübingen, Germany

The encoding of sensory information by populations of cortical neurons forms the basis for perception but remains poorly understood. To understand the constraints of cortical population coding we analyzed neural responses to natural sounds recorded in auditory cortex of primates (*Macaca mulatta*). We estimated stimulus information while varying the composition and size of the considered population. Consistent with previous reports we found that when choosing subpopulations randomly from the recorded ensemble, the average population information increases steadily with population size. This scaling was explained by a model assuming that each neuron carried equal amounts of information, and that any overlap between the information carried by each neuron arises purely from random sampling within the stimulus space. However, when studying subpopulations selected to optimize information for each given population size, the scaling of information was strikingly different: a small fraction of temporally precise cells carried the vast majority of information. This scaling could be explained by an extended model, assuming that the amount of information carried by individual neurons was highly nonuniform, with few neurons carrying large amounts of information. Importantly, these optimal populations can be determined by a single biophysical marker—the neuron's encoding time scale—allowing their detection and readout within biologically realistic circuits. These results show that extrapolations of population information based on random ensembles may overestimate the population size required for stimulus encoding, and that sensory cortical circuits may process information using small but highly informative ensembles.

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Fast Hemodynamic Responses in the Visual Cortex of the Awake Mouse

M. Andrea Pisauero, Neel T. Dhruv, Matteo Carandini, and Andrea Benucci

Institute of Ophthalmology, University College London, London EC1V 9EL, United Kingdom

Hemodynamic responses in mice and other species are typically measured under anesthesia. However, anesthesia could influence their relationship to neural activity. To investigate this relationship, we used optical imaging in mouse primary visual cortex (V1). Hemodynamic responses yielded clear maps of retinotopy in both anesthetized and awake mice. However, during wakefulness, responses were four times larger and twice as fast. These differences held whether we induced anesthesia with urethane or isoflurane and whether awake mice were stationary or running on a treadmill. With electrode recordings, we established that the effects of wakefulness reflect changes in neurovascular coupling, not in neural activity. By activating V1 directly via optogenetics, we replicated the effects of wakefulness in terms of timing but not of amplitude. We conclude that neurovascular coupling depends critically on anesthesia and wakefulness: during wakefulness, neural activity is followed by much stronger and quicker hemodynamic responses.

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Preparing for Selective Inhibition within Frontostriatal Loops

Peter Smittenaar, Marc Guitart-Masip, Antoine Lutti, and Raymond J. Dolan

Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, 12 Queen Square, WC1N 3BG, London, United Kingdom

Action inhibition can globally prevent all motor output or selectively cancel specific actions during concurrent motor output. Here we examine the behavioral and neural basis of selective inhibition focusing on the role of preparation. In 18 healthy human participants we manipulated the extent to which they could prepare for selective inhibition by providing or withholding information on what actions might need to be stopped. We show that, on average, information improves both speed and selectivity of inhibition. Functional magnetic resonance imaging data show that preparation for selective inhibition engages the inferior frontal gyrus, supplementary motor area, and striatum. Examining interindividual differences, we find the benefit of proactive control to speed and selectivity of inhibition trade off against each other, such that an improvement in stopping speed leads to a deterioration of selectivity of inhibition, and vice versa. This trade-off is implemented through engagement of the dorsolateral prefrontal cortex and putamen. Our results suggest proactive selective inhibition is implemented within frontostriatal structures, and we provide evidence that a speed-selectivity trade-off might underlie a range of findings reported previously.

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Longitudinal Growth Curves of Brain Function Underlying Inhibitory Control through Adolescence

Sarah J. Ordaz,¹ William Foran,² Katerina Velanova,^{1,2} and Beatriz Luna^{1,2}

¹Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, and ²Department of Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213

Neuroimaging studies suggest that developmental improvements in inhibitory control are primarily supported by changes in prefrontal executive function. However, studies are contradictory with respect to how activation in prefrontal regions changes with age, and they have yet to analyze longitudinal data using growth curve modeling, which allows characterization of dynamic processes of developmental change, individual differences in growth trajectories, and variables that predict any interindividual variability in trajectories. In this study, we present growth curves modeled from longitudinal fMRI data collected over 302 visits (across ages 9 to 26 years) from 123 human participants. Brain regions within circuits known to support motor response control, executive control, and error processing (i.e., aspects of inhibitory control) were investigated. Findings revealed distinct developmental trajectories for regions within each circuit and indicated that a hierarchical pattern of maturation of brain activation supports the gradual emergence of adult-like inhibitory control. Mean growth curves of activation in motor response control regions revealed no changes with age, although interindividual variability decreased with development, indicating equifinality with maturity. Activation in certain executive control regions decreased with age until adolescence, and variability was stable across development. Error-processing activation in the dorsal anterior cingulate cortex showed continued increases into adulthood and no significant interindividual variability across development, and was uniquely associated with task performance. These findings provide evidence that continued maturation of error-processing abilities supports the protracted development of inhibitory control over adolescence, while motor response control regions provide early-maturing foundational capacities and suggest that some executive control regions may buttress immature networks as error processing continues to mature.

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Global Facilitation of Attended Features Is Obligatory and Restricts Divided Attention

Søren K. Andersen,^{1,2,3} Steven A. Hillyard,² and Matthias M. Müller³

¹School of Psychology, University of Aberdeen, Aberdeen AB24 3FX, United Kingdom, ²Department of Neurosciences, University of California, San Diego, La Jolla, California 92093, and ³Institute of Psychology, University of Leipzig, 04103 Leipzig, Germany

In many common situations such as driving an automobile it is advantageous to attend concurrently to events at different locations (e.g., the car in front, the pedestrian to the side). While spatial attention can be divided effectively between separate locations, studies investigating attention to nonspatial features have often reported a “global effect”, whereby items having the attended feature may be preferentially processed throughout the entire visual field. These findings suggest that spatial and feature-based attention may at times act in direct opposition: spatially divided foci of attention cannot be truly independent if feature attention is spatially global and thereby affects all foci equally.

In two experiments, human observers attended concurrently to one of two overlapping fields of dots of different colors presented in both the left and right visual fields. When the same color or two different colors were attended on the two sides, deviant targets were detected accurately, and visual-cortical potentials elicited by attended dots were enhanced. However, when the attended color on one side matched the ignored color on the opposite side, attentional modulation of cortical potentials was abolished. This loss of feature selectivity could be attributed to enhanced processing of unattended items that shared the color of the attended items in the opposite field. Thus, while it is possible to attend to two different colors at the same time, this ability is fundamentally constrained by spatially global feature enhancement in early visual-cortical areas, which is obligatory and persists even when it explicitly conflicts with task demands.

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Body and Object Effectors: The Organization of Object Representations in High-Level Visual Cortex Reflects Body–Object Interactions

Stefania Bracci and Marius V. Peelen

Center for Mind/Brain Sciences, University of Trento, TN 38068 Rovereto, Italy

The principles driving the functional organization of object representations in high-level visual cortex are not yet fully understood. In four human fMRI experiments, we provide evidence that the organization of high-level visual cortex partly reflects the degree to which objects are typically controlled by the body to interact with the world, thereby extending the body's boundaries. Univariate whole-brain analysis showed an overlap between responses to body effectors (e.g., hands, feet, and limbs) and object effectors (e.g., hammers, combs, and tennis rackets) in lateral occipitotemporal cortex (LOTC) and parietal cortex. Region of interest analyses showed that a hand-selective region in left LOTC responded selectively to object effectors relative to a range of noneffector object control conditions (e.g., graspable objects, “act-on” objects, musical instruments). Object ratings showed that the strong response to object effectors in hand-selective LOTC was not due to general action-related object properties shared with these control conditions, such as hand priming, hand grasping, and hand-action centrality. Finally, whole-brain representational similarity analysis revealed that the similarity of multivoxel object response patterns in left lateral occipitotemporal cortex selectively predicted the degree to which objects were rated as being controlled by and extending the body. Together, these results reveal a clustering of body and object effector representations, indicating that the organization of object representations in high-level visual cortex partly reflects how objects relate to the body.

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Electrical Stimulation of the Human Homolog of the Medial Superior Temporal Area Induces Visual Motion Blindness

Hubertus G. T. Becker,^{1,2*} Thomas Haarmeier,^{1,2,5*} Marcos Tatagiba,⁴ and Alireza Gharabaghi^{3,4}

Departments of ¹General Neurology and ²Cognitive Neurology, Hertie Institute for Clinical Brain Research, and ³Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, 72076 Tübingen, Germany, ⁴Department of Neurosurgery, University Hospital Tübingen, 72076 Tübingen, Germany, and ⁵Department of Neurology, Aachen University, D-52074 Aachen, Germany

Despite tremendous advances in neuroscience research, it is still unclear how neuronal representations of sensory information give rise to the contents of our perception. One of the first and also the most compelling pieces of evidence for direct involvement of cortical signals in perception comes from electrical stimulation experiments addressing the middle temporal (MT) area and the medial superior temporal (MST) area: two neighboring extrastriate cortical areas of the monkey brain housing direction-sensitive neurons. Here we have combined fMRI with electrical stimulation in a patient undergoing awake brain surgery, to separately probe the functional significance of the human homologs, i.e., area hMT and hMST, on motion perception. Both the stimulation of hMT and hMST made it impossible for the patient to perceive the global visual motion of moving random dot patterns. Although visual motion blindness was predominantly observed in the contralateral visual field, stimulation of hMST also affected the ipsilateral hemifield. These results suggest that early visual cortex up to the stage of MT is not sufficient for the perception of global visual motion. Rather, visual motion information must be mediated to higher-tier cortical areas, including hMST, to gain access to conscious perception.

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Role of Somatosensory Cortex in Visuospatial Attention

Daniela Balslev,^{1,2} Bartholomäus Odoj,^{1,2} and Hans-Otto Karnath^{1,3}

¹Center of Neurology, Division of Neuropsychology, Hertie Institute for Clinical Brain Research, University of Tübingen, D-72076 Tübingen, Germany, ²Department of Psychology, University of Copenhagen, DK-1353 Copenhagen, Denmark, and ³Department of Psychology, University of South Carolina, Columbia, South Carolina 29208

The human somatosensory cortex (S1) is not among the brain areas usually associated with visuospatial attention. However, such a function can be presumed, given the recently identified eye proprioceptive input to S1 and the established links between gaze and attention. Here we investigated a rare patient with a focal lesion of the right postcentral gyrus that interferes with the processing of eye proprioception without affecting the ability to locate visual objects relative to her body or to execute eye movements. As a behavioral measure of spatial attention, we recorded fixation time during visual search and reaction time for visual discrimination in lateral displays. In contrast to a group of age-matched controls, the patient showed a gradient in looking time and in visual sensitivity toward the midline. Because an attention bias in the opposite direction, toward the ipsilesional space, occurs in patients with spatial neglect, in a second study, we asked whether the incidental coinjury of S1 together with the neglect-typical perisylvian lesion leads to a milder neglect. A voxelwise lesion behavior mapping analysis of a group of right-hemisphere stroke patients supported this hypothesis. The effect of an isolated S1 lesion on visual exploration and visual sensitivity as well as the modulatory role of S1 in spatial neglect suggest a role of this area in visuospatial attention. We hypothesize that the proprioceptive gaze signal in S1, although playing only a minor role in locating visual objects relative to the body, affects the allocation of attention in the visual space.

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Changes in Brain Function Occur Years before the Onset of Cognitive Impairment

Lori L. Beason-Held,¹ Joshua O. Goh,^{1,2} Yang An,¹ Michael A. Kraut,³ Richard J. O'Brien,⁴ Luigi Ferrucci,¹ and Susan M. Resnick¹

¹Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224, ²Graduate Institute of Brain and Mind Sciences, National Taiwan University College of Medicine, Taipei 100, Taiwan, ³Department of Radiology, Johns Hopkins Hospital, Baltimore, Maryland 21287, and ⁴Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

To develop targeted intervention strategies for the treatment of Alzheimer's disease, we first need to identify early markers of brain changes that occur before the onset of cognitive impairment. Here, we examine changes in resting-state brain function in humans from the Baltimore Longitudinal Study of Aging. We compared longitudinal changes in regional cerebral blood flow (rCBF), assessed by ¹⁵O-water PET, over a mean 7 year period between participants who eventually developed cognitive impairment ($n = 22$) and those who remained cognitively normal ($n = 99$). Annual PET assessments began an average of 11 years before the onset of cognitive impairment in the subsequently impaired group, so all participants were cognitively normal during the scanning interval. A voxel-based mixed model analysis was used to compare groups with and without subsequent impairment. Participants with subsequent impairment showed significantly greater longitudinal rCBF increases in orbitofrontal, medial frontal, and anterior cingulate regions, and greater longitudinal decreases in parietal, temporal, and thalamic regions compared with those who maintained cognitive health. These changes were linear in nature and were not influenced by longitudinal changes in regional tissue volume. Although all participants were cognitively normal during the scanning interval, most of the accelerated rCBF changes seen in the subsequently impaired group occurred within regions thought to be critical for the maintenance of cognitive function. These changes also occurred within regions that show early accumulation of pathology in Alzheimer's disease, suggesting that there may be a connection between early pathologic change and early changes in brain function.

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CLN3 Loss Disturbs Membrane Microdomain Properties and Protein Transport in Brain Endothelial Cells

Luis Tecedor,¹ Colleen S. Stein,¹ Mark L. Schultz,⁴ Hany Farwanah,⁵ Konrad Sandhoff,⁵ and Beverly L. Davidson^{1,2,3,4}

Departments of ¹Internal Medicine, ²Molecular Physiology and Biophysics, ³Neurology, and ⁴Molecular and Cell Biology Program, University of Iowa, Iowa City, Iowa 52242, and ⁵Life and Medical Sciences Institute, Membrane Biology and Lipid Biochemistry Unit, University of Bonn, D-53121 Bonn, Germany

Juvenile neuronal ceroid lipofuscinosis (JNCL) is a fatal childhood-onset neurodegenerative disorder caused by mutations in ceroid lipofuscinosis neuronal-3 (CLN3), a hydrophobic transmembrane protein of unresolved function. Previous studies indicate blood–brain barrier (BBB) defects in JNCL, and our earlier report showed prominent *Cln3* expression in mouse brain endothelium. Here we find that CLN3 is necessary for normal trafficking of the microdomain-associated proteins caveolin-1, syntaxin-6, and multidrug resistance protein 1 (MDR1) in brain endothelial cells. Correspondingly, CLN3-null cells have reduced caveolae, and impaired caveolae- and MDR1-related functions including endocytosis, drug efflux, and cell volume regulation. We also detected an abnormal blood–brain barrier response to osmotic stress *in vivo*. Evaluation of the plasma membrane with fluorescent sphingolipid probes suggests microdomain destabilization and enhanced fluidity in CLN3-null cells. In further work we found that application of the glycosphingolipid lactosylceramide to CLN3-deficient cells rescues protein transport and caveolar endocytosis. Last, we show that CLN3 localizes to the trans-Golgi network (TGN) and partitions with buoyant microdomain fractions. We propose that CLN3 facilitates TGN-to-plasma membrane transport of microdomain-associated proteins. Insult to this pathway may underlie BBB dysfunction and contribute to JNCL pathogenesis.

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Autoantibodies to Epilepsy-Related LGI1 in Limbic Encephalitis Neutralize LGI1-ADAM22 Interaction and Reduce Synaptic AMPA Receptors

Toshika Ohkawa,^{1,2} Yuko Fukata,^{1,2} Miwako Yamasaki,³ Taisuke Miyazaki,³ Norihiko Yokoi,^{1,2} Hiroshi Takashima,⁴ Masahiko Watanabe,^{3,5} Osamu Watanabe,^{4*} and Masaki Fukata^{1,2*}

¹Division of Membrane Physiology, Department of Cell Physiology, National Institute for Physiological Sciences (NIPS), Okazaki 444-8787, Japan; ²Department of Physiological Sciences, School of Life Science, The Graduate University for Advanced Studies (SOKENDAI), Okazaki 444-8787, Japan; ³Department of Anatomy, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan; ⁴Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Science, Kagoshima 890-8544, Japan; and ⁵Japan Science and Technology Agency, Core Research for Evolutional Science and Technology, Chiyoda-ku, Tokyo 102-0075, Japan

More than 30 mutations in LGI1, a secreted neuronal protein, have been reported with autosomal dominant lateral temporal lobe epilepsy (ADLTE). Although LGI1 haploinsufficiency is thought to cause ADLTE, the underlying molecular mechanism that results in abnormal brain excitability remains mysterious. Here, we focused on a mode of action of LGI1 autoantibodies associated with limbic encephalitis (LE), which is one of acquired epileptic disorders characterized by subacute onset of amnesia and seizures. We comprehensively screened human sera from patients with immune-mediated neurological disorders for LGI1 autoantibodies, which also uncovered novel autoantibodies against six cell surface antigens including DCC, DPP10, and ADAM23. Our developed ELISA arrays revealed a specific role for LGI1 antibodies in LE and concomitant involvement of multiple antibodies, including LGI1 antibodies in neuromyotonia, a peripheral nerve disorder. LGI1 antibodies associated with LE specifically inhibited the ligand–receptor interaction between LGI1 and ADAM22/23 by targeting the EPTP repeat domain of LGI1 and reversibly reduced synaptic AMPA receptor clusters in rat hippocampal neurons. Furthermore, we found that disruption of LGI1–ADAM22 interaction by soluble extracellular domain of ADAM22 was sufficient to

reduce synaptic AMPA receptors in rat hippocampal neurons and that levels of AMPA receptor were greatly reduced in the hippocampal dentate gyrus in the epileptic LGI1 knock-out mouse. Therefore, either genetic or acquired loss of the LGI1-ADAM22 interaction reduces the AMPA receptor function, causing epileptic disorders. These results suggest that by finely regulating the synaptic AMPA receptors, the LGI1-ADAM22 interaction maintains physiological brain excitability throughout life.

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Tau Pathology is Present *In Vivo* and Develops *In Vitro* in Sensory Neurons from Human P301S Tau Transgenic Mice: A System for Screening Drugs against Tauopathies

Manuela Mellone,¹ Dimitra Kestoras,¹ Melissa R. Andrews,¹ Elisa Dassie,¹ R. Anthony Crowther,² Gorazd B. Stokin,³ Jon Tinsley,⁴ Graeme Horne,⁴ Michel Goedert,² Aviva M. Tolkovsky,¹ and Maria Grazia Spillantini¹

¹John Van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, Cambridge, CB2 0PY, United Kingdom, ²Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom, ³Division of Neurology, University Psychiatric Hospital, SI-1000 Ljubljana, Slovenia, and ⁴Summit plc, Abingdon, OX14 4RY, United Kingdom

Intracellular tau aggregates are the neuropathological hallmark of several neurodegenerative diseases, including Alzheimer's disease, progressive supranuclear palsy, and cases of frontotemporal dementia, but the link between these aggregates and neurodegeneration remains unclear. Neuronal models recapitulating the main features of tau pathology are necessary to investigate the molecular mechanisms of tau malfunction, but current models show little and inconsistent spontaneous tau aggregation. We show that dorsal root ganglion (DRG) neurons in transgenic mice expressing human P301S tau (P301S-htau) develop tau pathology similar to that found in brain and spinal cord and a significant reduction in mechanosensation occurs before detectable fibrillar tau formation. DRG neuronal cultures established from adult P301S-htau mice at different ages retained the pattern of aberrant tau found *in vivo*. Moreover, htau became progressively hyperphosphorylated over 2 months *in vitro* beginning with nonsymptomatic neurons, while hyperphosphorylated P301S-htau-positive neurons from 5-month-old mice cultured for 2 months died preferentially. P301S-htau-positive neurons grew aberrant axons, including spheroids, typically found in human tauopathies. Neurons cultured at advanced stages of tau pathology showed a 60% decrease in the fraction of moving mitochondria. SEG28019, a novel O-GlcNAcase inhibitor, reduced steady-state pSer396/pSer404 phosphorylation over 7 weeks in a significant proportion of DRG neurons showing for the first time the possible beneficial effect of prolonged dosing of O-GlcNAcase inhibitor *in vitro*. Our system is unique in that fibrillar tau forms without external manipulation and provides an important new tool for understanding the mechanisms of tau dysfunction and for screening of compounds for treatment of tauopathies.

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Cerebral Arterial Pulsation Drives Paravascular CSF–Interstitial Fluid Exchange in the Murine Brain

Jeffrey J. Iliff,^{1,2} Minghuan Wang,^{1,3} Douglas M. Zeppenfeld,^{1,2} Arun Venkataraman,¹ Benjamin A. Plog,¹ Yonghong Liao,¹ Rashid Deane,¹ and Maiken Nedergaard¹

¹Division of Glial Disease and Therapeutics, Center for Translational Neuromedicine, Department of Neurosurgery, University of Rochester Medical Center, Rochester, New York 14642, ²Department of Anesthesiology and Peri-Operative Medicine, Oregon Health & Science University, Portland, Oregon 97239, and ³Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China 430030

CSF from the subarachnoid space moves rapidly into the brain along paravascular routes surrounding penetrating cerebral arteries, exchanging with brain interstitial fluid (ISF) and facilitating the clearance of interstitial solutes, such as amyloid β , in a pathway that we have termed the “glymphatic” system. Prior reports have suggested that paravascular bulk flow of CSF or ISF may be driven by arterial pulsation. However, cerebral arterial pulsation could not be directly assessed. In the present study, we use *in vivo* two-photon microscopy in mice to visualize vascular wall pulsatility in penetrating intracortical arteries. We observed that unilateral ligation of the internal carotid artery significantly reduced arterial pulsatility by ~50%, while systemic administration of the adrenergic agonist dobutamine increased pulsatility of penetrating arteries by ~60%. When paravascular CSF–ISF exchange was evaluated in real time using *in vivo* two-photon and *ex vivo* fluorescence imaging, we observed that internal carotid artery ligation slowed the rate of paravascular CSF–ISF exchange, while dobutamine increased the rate of paravascular CSF–ISF exchange. These findings demonstrate that cerebral arterial pulsatility is a key driver of paravascular CSF influx into and through the brain parenchyma, and suggest that changes in arterial pulsatility may contribute to accumulation and deposition of toxic solutes, including amyloid β , in the aging brain.

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Synapsin II and Rab3a Cooperate in the Regulation of Epileptic and Synaptic Activity in the CA1 Region of the Hippocampus

Pedro Feliciano,¹ Rodrigo Andrade,² and Maria Bykhovskaia¹

¹Neuroscience Department, Universidad Central del Caribe, Bayamon, Puerto Rico 00956, and ²Department of Pharmacology Wayne State University School of Medicine, Detroit, Michigan 48001

Some forms of idiopathic epilepsy in animals and humans are associated with deficiency of synapsin, a phosphoprotein that reversibly associates with synaptic vesicles. We have previously shown that the epileptic phenotype seen in synapsin II knock-out mice (SynII(–)) can be rescued by the genetic deletion of the Rab3a protein. Here we have examined the cellular basis for this rescue using whole-cell recordings from CA1 hippocampal pyramidal cells in brain slices. We find that SynII(–) neurons have increased spontaneous activity and a reduced threshold for the induction of epileptiform activity by 4-aminopyridine (4-AP). Using selective recordings of glutamatergic and

GABAergic activity we show that in wild-type neurons low concentrations of 4-AP facilitate glutamatergic and GABAergic transmission in a balanced way, whereas in SynII(−) neurons this balance is shifted toward excitation. This imbalance reflects a deficit in inhibitory synaptic transmission that appears to be secondary to reduced Ca^{2+} sensitivity in SynII(−) neurons. This suggestion is supported by our finding that synaptic and epileptiform activity at SynII(−) and wild-type synapses is similar when GABAergic transmission is blocked. Deletion of Rab3a results in glutamatergic synapses that have a compromised responsiveness to either low 4-AP concentrations or elevated extracellular Ca^{2+} . These changes mitigate the overexcitable phenotype observed in SynII(−) neurons. Thus, Rab3a deletion appears to restore the excitatory/inhibitory imbalance observed in SynII(−) hippocampal slices indirectly, not by correcting the deficit in GABAergic synaptic transmission but rather by impairing excitatory glutamatergic synaptic transmission.

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