

This Week in The Journal

D2 Dopamine Receptors Couple to $G\alpha_s$ in Prefrontal Cortex

Sarah E. Robinson and Vikaas S. Sohal

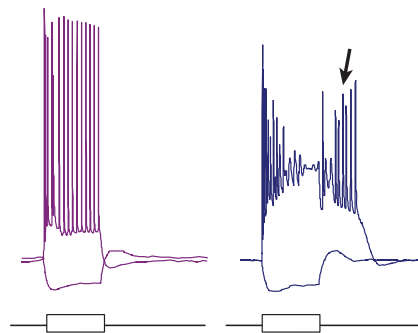
(see pages 10063–10073)

Dopaminergic input to the prefrontal cortex (PFC) regulates multiple cognitive functions, including working memory, decision making, and attention. The effects of dopamine on PFC neurons depend on which type of dopamine receptor they express. Previous work (Gee et al. 2012 *Neurosci* 32:4959) indicated that in PFC layer 5, D2 dopamine receptors (D2Rs) are expressed selectively in a subpopulation of neurons that project subcortically (to the thalamus). Co-activation of D2Rs with NMDA receptors or L-type calcium channels altered the electrophysiological properties of these neurons, such that single depolarizing electrical pulses evoked a long-lasting afterdepolarization (ADP), during which neurons continued to spike.

Dopamine receptors are G-protein-coupled receptors that exert different effects by interacting with different $G\alpha$ subunits, as well as by interacting directly with other receptors and ion channels. In the striatum and other brain regions, D1Rs are coupled to $G\alpha_s$ or $G\alpha_{olf}$, through which they increase intracellular cAMP levels, promote the activity of protein kinase A (PKA), and increase neuronal excitability. In contrast, D2Rs are coupled to $G\alpha_i$ or $G\alpha_o$; their activation reduces cAMP levels, PKA activity, and neuronal excitability. Although D2Rs were shown to increase excitability in subcortically projecting L5 neurons in mouse PFC, Robinson and Sohal expected the receptors to exert these effects through $G\alpha_i$ or $G\alpha_o$. If this were true, PKA inhibitors should mimic the effects of D2R activation in these neurons. Surprisingly, however, this was not the case. In fact, PKA inhibitors blocked the effect of the D2R agonist quinpirole, whereas blocking $G\alpha_i$ -mediated signaling had no effect. Moreover, activation of a $G\alpha_s$ -coupled designer receptor produced an ADP similar to that produced by quinpirole, whereas neither $G\alpha_i$ - nor $G\alpha_o$ -coupled receptors produced an ADP. Finally, quinpirole did not induce an ADP

in subcortically projecting neurons after D2Rs were knocked out.

These results suggest that unlike in other brain areas, D2Rs in PFC couple to $G\alpha_s$, thus promoting activation of PKA and increasing neuronal excitability. Given that D2Rs might have a higher affinity for dopamine than D1Rs, the coupling of D1Rs and D2Rs to the same G-protein might allow dopamine to increase excitability in different populations of PFC neurons depending on its concentration. How this increased excitability relates to PFC functions remains to be determined.



In the presence of NMDA and a D2R agonist (right), subcortically projecting PFC neurons fire spikes during depolarizing current pulses, as well as during an afterdepolarization (arrow) that was present after pulse termination. No afterdepolarization occurred in the presence of NMDA alone (left). See Robinson and Sohal for details.

Circadian Clock Gene Affects Pericytes, Blood–Brain Barrier

Ryota Nakazato, Kenji Kawabe, Daisuke Yamada, Shinsuke Ikeno, Michihiro Mieda, et al.

(see pages 10052–10062)

Nearly every cell in the body exhibits circadian oscillations in gene expression and function, with 2–10% of genes being under circadian control. In each cell, oscillations are driven by the *Bmal1*/Clock protein complex, whose targets include genes encoding *Per* and *Cry*, which eventually inhibit *Bmal1*/Clock-induced transcription. Degradation of *Per* and *Cry* then allow the cycle to resume. In mammals, oscillators in each cell are synchronized by inputs stemming directly or indirectly from the suprachias-

matic nucleus, whose activity is synchronized to the daily light/dark cycle. This synchronization helps to ensure that various organ systems, such as those regulating circulation and metabolism, interact efficiently and are coordinated with daily activity cycles.

Chronic disruption of circadian rhythms has been linked to several diseases, including cardiovascular diseases, metabolic disorders, and cancer. Furthermore, dampening of circadian oscillations during aging is thought to contribute to cognitive decline and neurodegeneration. Consistent with this, deletion of *Bmal1* in most of the brain (excluding the suprachiasmatic nuclei, thus leaving sleep/wake cycles intact) led to loss of axonal terminals, as well as neuroinflammation and reactive astrogliosis (Musiek et al. 2013 *J Clin Invest* 123:5389). Nakazato et al. suggest these effects stem from disruption of the blood–brain barrier resulting from pericyte dysfunction.

The authors showed that knocking out *Bmal1* in nestin-expressing cells led to increased permeability of the blood–brain barrier in addition to astrogliosis and neuroinflammation. This increased permeability was accompanied by progressive loss of pericyte coverage of blood vessels in the cerebral cortex. Furthermore, expression of platelet-derived growth factor receptor β (PDGFR β)—a protein previously shown to be required for maintenance of the blood–brain barrier—was greatly reduced in pericytes. Importantly, blood–brain barrier breakdown and astrogliosis did not occur in mice in which *Bmal1* was knocked out selectively in neurons or astrocytes, suggesting that these effects stemmed from loss of *Bmal1* in pericytes.

These results suggest that by regulating expression of PDGFR β in pericytes, *Bmal1* helps to maintain the integrity of the blood–brain barrier. They also suggest that PDGFR β expression is under circadian control. Whether circadian oscillation in PDGFR β leads to circadian variation in the integrity of the blood–brain barrier should be examined in future studies.

This Week in The Journal was written by Teresa Esch, Ph.D.



Cover legend: Spinal microglia are critical mediators in the development of opioid tolerance. In this image, a variety of individual Iba1-labelled microglia from rat spinal dorsal horn sections are re-colored and compiled to form a cross-section of the lumbar spinal cord. Each microglia cell is unique and they show a variety of morphologies representative of the dynamic and reactive nature of microglia. For more information, see the article by Leduc-Pessah et al. (pages 10154–10172).

i This Week in The Journal

Viewpoints

- 10023 **On Myelinated Axon Plasticity and Neuronal Circuit Formation and Function**
Rafael G. Almeida and David A. Lyons

Journal Club

- 10035 **Stress and Exploitative Decision-Making**
Madeline B. Harms

Research Articles

CELLULAR/MOLECULAR

- 10038 **Conditional Deletion of the L-Type Calcium Channel Cav1.2 in NG2-Positive Cells Impairs Remyelination in Mice**
Diara A. Santiago González, Veronica T. Cheli, Norma N. Zamora, Tenzing N. Lama, Vilma Spreuer, Geoffrey G. Murphy, and Pablo M. Paez
- 10063 **Dopamine D2 Receptors Modulate Pyramidal Neurons in Mouse Medial Prefrontal Cortex through a Stimulatory G-Protein Pathway**
Sarah E. Robinson and Vikaas S. Sohal

DEVELOPMENT/PLASTICITY/REPAIR

- 10097 **Visual Experience Shapes the Neural Networks Remapping Touch into External Space**
Virginie Crollen, Latifa Lazzouni, Mohamed Rezk, Antoine Bellemare, Franco Lepore, and Olivier Collignon

SYSTEMS/CIRCUITS

- 10074 **Visual Motion Discrimination by Propagating Patterns in Primate Cerebral Cortex**
Rory G. Townsend, Selina S. Solomon, Paul R. Martin, Samuel G. Solomon, and Pulin Gong
- 10085 **Rubrocerebellar Feedback Loop Isolates the Interposed Nucleus as an Independent Processor of Corollary Discharge Information in Mice**
Christy S. Beitzel, Brenda D. Houck, Samantha M. Lewis, and Abigail L. Person
- 10114 **The Interplay between Long- and Short-Range Temporal Correlations Shapes Cortex Dynamics across Vigilance States**
Christian Meisel, Andreas Klaus, Vladyslav V. Vyazovskiy, and Dietmar Plenz

- 10125 **Contralateral Bias of High Spatial Frequency Tuning and Cardinal Direction Selectivity in Mouse Visual Cortex**
Kirstie J. Salinas, Dario X. Figueroa Velez, Jack H. Zeitoun, Hyungtae Kim, and Sunil P. Gandhi
- 10200 **Sublaminar Subdivision of Mouse Auditory Cortex Layer 2/3 Based on Functional Translaminar Connections**
Xiangying Meng, Daniel E. Winkowski, Joseph P.Y. Kao, and Patrick O. Kanold

BEHAVIORAL/COGNITIVE

- 10104 **Approaching the Ground Truth: Revealing the Functional Organization of Human Multisensory STC Using Ultra-High Field fMRI**
Francesco Gentile, Nienke van Atteveldt, Federico De Martino, and Rainer Goebel
- 10139 **Primary Generators of Visually Evoked Field Potentials Recorded in the Macaque Auditory Cortex**
Yoshinao Kajikawa, John F. Smiley, and Charles E. Schroeder
- 10173 **Individual Alpha Peak Frequency Predicts 10 Hz Flicker Effects on Selective Attention**
Rasa Gulbinaite, Tara van Viegen, Martijn Wieling, Michael X Cohen, and Rufin VanRullen
- 10215 **The Neural Basis of Aversive Pavlovian Guidance during Planning**
Níall Lally, Quentin J.M. Huys, Neir Eshel, Paul Faulkner, Peter Dayan, and Jonathan P. Roiser
- 10240 **Multiple Signaling Pathways Coordinately Regulate Forgetting of Olfactory Adaptation through Control of Sensory Responses in *Caenorhabditis elegans***
Tomohiro Kitazono, Sayuri Hara-Kuge, Osamu Matsuda, Akitoshi Inoue, Manabi Fujiwara, and Takeshi Ishihara

NEUROBIOLOGY OF DISEASE

- 10052 **Disruption of Bmal1 Impairs Blood–Brain Barrier Integrity via Pericyte Dysfunction**
Ryota Nakazato, Kenji Kawabe, Daisuke Yamada, Shinsuke Ikeno, Michihiro Mieda, Shigeki Shimba, Eiichi Hinoi, Yukio Yoneda, and Takeshi Takarada
- 10154 **Site-Specific Regulation of P2X7 Receptor Function in Microglia Gates Morphine Analgesic Tolerance**
Heather Leduc-Pessah, Nicholas L. Weiling, Churmy Y. Fan, Nicole E. Burma, Roger J. Thompson, and Tuan Trang
- 10185 **Mitochondrial DNA Double-Strand Breaks in Oligodendrocytes Cause Demyelination, Axonal Injury, and CNS Inflammation**
Pernille M. Madsen, Milena Pinto, Shreyans Patel, Stephanie McCarthy, Han Gao, Mehran Taherian, Shaffiat Karmally, Claudia V. Pereira, Galina Dvorianchikova, Dmitry Ivanov, Kenji F. Tanaka, Carlos T. Moraes, and Roberta Brambilla
- 10230 **Enhanced Nociception in Angelman Syndrome Model Mice**
Eric S. McCoy, Bonnie Taylor-Blake, Megumi Aita, Jeremy M. Simon, Benjamin D. Philpot, and Mark J. Zylka

- 10252** ***Correction:*** The article “AMPA Receptor-Induced Local Brain-Derived Neurotrophic Factor Signaling Mediates Motor Recovery after Stroke” by Andrew N. Clarkson, Justine J. Overman, Sheng Zhong, Rudolf Mueller, Gary Lynch, and S. Thomas Carmichael, appeared on pages 3766–3775 of the March 9, 2011 issue. A correction for this article appears on page 10252.
- 10253** ***Erratum:*** The article “UPF1 Governs Synaptic Plasticity through Association with a STAU2 RNA Granule” by Tyson E. Graber, Erika Freemantle, Mina N. Anadolu, Sarah Hébert-Seropian, Robyn L. MacAdam, Unkyung Shin, Huy-Dung Hoang, Tommy Alain, Jean-Claude Lacaille, and Wayne S. Sossin, appeared on pages 9116–9131 of the September 20, 2017 issue. An erratum for this article appears on page 10253.

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On Myelinated Axon Plasticity and Neuronal Circuit Formation and Function

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Studies of activity-driven nervous system plasticity have primarily focused on the gray matter. However, MRI-based imaging studies have shown that white matter, primarily composed of myelinated axons, can also be dynamically regulated by activity of the healthy brain. Myelination in the CNS is an ongoing process that starts around birth and continues throughout life. Myelin in the CNS is generated by oligodendrocytes and recent evidence has shown that many aspects of oligodendrocyte development and myelination can be modulated by extrinsic signals including neuronal activity. Because modulation of myelin can, in turn, affect several aspects of conduction, the concept has emerged that activity-regulated myelination represents an important form of nervous system plasticity. Here we review our increasing understanding of how neuronal activity regulates oligodendrocytes and myelinated axons *in vivo*, with a focus on the timing of relevant processes. We highlight the observations that neuronal activity can rapidly tune axonal diameter, promote re-entry of oligodendrocyte progenitor cells into the cell cycle, or drive their direct differentiation into oligodendrocytes. We suggest that activity-regulated myelin formation and remodeling that significantly change axonal conduction properties are most likely to occur over timescales of days to weeks. Finally, we propose that precise fine-tuning of conduction along already-myelinated axons may also be mediated by alterations to the axon itself. We conclude that future studies need to analyze activity-driven adaptations to both axons and their myelin sheaths to fully understand how myelinated axon plasticity contributes to neuronal circuit formation and function.

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Articles

CELLULAR/MOLECULAR

Conditional Deletion of the L-Type Calcium Channel Cav1.2 in NG2-Positive Cells Impairs Remyelination in Mice

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Exploring the molecular mechanisms that drive the maturation of oligodendrocyte progenitor cells (OPCs) during the remyelination process is essential to developing new therapeutic tools to intervene in demyelinating diseases such as multiple sclerosis. To determine whether L-type voltage-gated calcium channels (L-VGCCs) are required for OPC development during remyelination, we generated an inducible conditional knock-out mouse in which the L-VGCC isoform Cav1.2 was deleted in NG2-positive OPCs (Cav1.2^{KO}). Using the cuprizone (CPZ) model of demyelination and mice of either sex, we establish that Cav1.2 deletion in OPCs leads to less efficient remyelination of the adult brain. Specifically, Cav1.2^{KO} OPCs mature slower and produce less myelin than control oligodendrocytes during the recovery period after CPZ intoxication. This reduced remyelination was accompanied by an important decline in the number of myelinating oligodendrocytes and in the rate of OPC proliferation. Furthermore, during the remyelination phase of the CPZ model, the corpus callosum of Cav1.2^{KO} animals presented a significant decrease in the percentage of myelinated axons and a substantial increase in the mean g-ratio of myelinated axons compared with controls. In addition, in a mouse line in which the Cav1.2^{KO} OPCs were identified by a *Cre* reporter, we establish that Cav1.2^{KO} OPCs display a reduced maturational rate through the entire remyelination process. These results suggest that Ca²⁺ influx mediated by L-VGCCs in oligodendroglial cells is necessary for normal remyelination and is an essential Ca²⁺ channel for OPC maturation during the remyelination of the adult brain.

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Dopamine D2 Receptors Modulate Pyramidal Neurons in Mouse Medial Prefrontal Cortex through a Stimulatory G-Protein Pathway

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Dopaminergic modulation of prefrontal cortex (PFC) is thought to play key roles in many cognitive functions and to be disrupted in pathological conditions, such as schizophrenia. We have previously described a phenomenon whereby dopamine D2 receptor (D2R) activation elicits afterdepolarizations (ADPs) in subcortically projecting (SC) pyramidal neurons within L5 of the PFC. These D2R-induced ADPs only occur following synaptic input, which activates NMDARs, even when the delay between the synaptic input and ADPs is relatively long (e.g., several hundred milliseconds). Here, we use a combination of electrophysiological, optogenetic, pharmacological, transgenic, and chemogenetic approaches to elucidate cellular mechanisms underlying this phenomenon in male and female mice. We find that knocking out D2Rs eliminates

the ADP in a cell-autonomous fashion, confirming that this ADP depends on D2Rs. Hyperpolarizing current injection, but not AMPA receptor blockade, prevents synaptic stimulation from facilitating D2R-induced ADPs, suggesting that this phenomenon depends on the recruitment of voltage-dependent currents (e.g., NMDAR-mediated Ca^{2+} influx) by synaptic input. Finally, the D2R-induced ADP is blocked by inhibitors of cAMP/PKA signaling, insensitive to pertussis toxin or β -arrestin knock-out, and mimicked by G_s -DREADD stimulation, suggesting that D2R activation elicits the ADP by stimulating cAMP/PKA signaling. These results show that this unusual physiological phenomenon, in which D2Rs enhance cellular excitability in a manner that depends on synaptic input, is mediated at the cellular level through the recruitment of signaling pathways associated with G_s , rather than the $G_{i/o}$ -associated mechanisms that have classically been ascribed to D2Rs.

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DEVELOPMENT/PLASTICITY/REPAIR

Visual Experience Shapes the Neural Networks Remapping Touch into External Space

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Localizing touch relies on the activation of skin-based and externally defined spatial frames of reference. Psychophysical studies have demonstrated that early visual deprivation prevents the automatic remapping of touch into external space. We used fMRI to characterize how visual experience impacts the brain circuits dedicated to the spatial processing of touch. Sighted and congenitally blind humans performed a tactile temporal order judgment (TOJ) task, either with the hands uncrossed or crossed over the body midline. Behavioral data confirmed that crossing the hands has a detrimental effect on TOJ judgments in sighted but not in early blind people. Crucially, the crossed hand posture elicited enhanced activity, when compared with the uncrossed posture, in a frontoparietal network in the sighted group only. Psychophysiological interaction analysis revealed, however, that the congenitally blind showed enhanced functional connectivity between parietal and frontal regions in the crossed versus uncrossed hand postures. Our results demonstrate that visual experience scaffolds the neural implementation of the location of touch in space.

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SYSTEMS/CIRCUITS

Visual Motion Discrimination by Propagating Patterns in Primate Cerebral Cortex

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Visual stimuli can evoke waves of neural activity that propagate across the surface of visual cortical areas. The relevance of these waves for visual processing is unknown. Here, we measured the phase and amplitude of local field potentials (LFPs) in electrode array recordings from the motion-processing medial temporal (MT) area of anesthetized male marmosets. Animals viewed grating or dot-field stimuli drifting in different directions. We found that, on individual trials, the direction of LFP wave propagation is sensitive to the direction of stimulus motion. Propagating LFP patterns are also detectable in trial-averaged activity, but the trial-averaged patterns exhibit different dynamics and behaviors from those in single trials and are similar across motion directions. We show that this difference arises because stimulus-sensitive propagating patterns are present in the phase of single-trial oscillations, whereas the trial-averaged signal is dominated by additive amplitude effects. Our results demonstrate that propagating LFP patterns can represent sensory inputs at timescales relevant to visually guided behaviors and raise the possibility that propagating activity patterns serve neural information processing in area MT and other cortical areas.

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Rubrocerebellar Feedback Loop Isolates the Interposed Nucleus as an Independent Processor of Corollary Discharge Information in Mice

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Understanding cerebellar contributions to motor coordination requires deeper insight into how the output structures of the cerebellum, the cerebellar nuclei, integrate their inputs and influence downstream motor pathways. The magnocellular red nucleus (RNm), a brainstem premotor structure, is a major target of the interposed nucleus (IN), and has also been described in previous studies to send feedback collaterals to the cerebellum. Because such a pathway is in a key position to provide motor efferent information to the cerebellum, satisfying predictions about the use of corollary discharge in cerebellar computations, we studied it in mice of both sexes. Using anterograde viral tracing, we show that innervation of cerebellum by rubrospinal neuron collaterals is remarkably selective for the IN compared with the cerebellar cortex. Optogenetic activation of the pathway in acute mouse brain slices drove IN activity despite small amplitude synaptic currents, suggesting an active role in IN information processing. Monosynaptic transsynaptic rabies tracing indicated the pathway contacts multiple cell types within the IN. By contrast, IN inputs to the RNm targeted a region that lacked

inhibitory neurons. Optogenetic drive of IN inputs to the RNm revealed strong, direct excitation but no inhibition of RNm neurons. Together, these data indicate that the cerebellar nuclei are under afferent control independent of the cerebellar cortex, potentially diversifying its roles in motor control.
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The Interplay between Long- and Short-Range Temporal Correlations Shapes Cortex Dynamics across Vigilance States

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Increasing evidence suggests that cortical dynamics during wake exhibits long-range temporal correlations suitable to integrate inputs over extended periods of time to increase the signal-to-noise ratio in decision making and working memory tasks. Accordingly, sleep has been suggested as a state characterized by a breakdown of long-range correlations. However, detailed measurements of neuronal timescales that support this view have so far been lacking. Here, we show that the cortical timescales measured at the individual neuron level in freely behaving male rats change as a function of vigilance state and time awake. Although quiet wake and rapid eye movement (REM) sleep are characterized by similar, long timescales, these long timescales are abrogated in non-REM sleep. We observe that cortex dynamics exhibits rapid transitions between long-timescale states and sleep-like states governed by short timescales even during wake. This becomes particularly evident during sleep deprivation, when the interplay between these states can lead to an increasing disruption of long timescales that are restored after sleep. Experiments and modeling identify the intrusion of neuronal offline periods as a mechanism that disrupts the long timescales arising from reverberating cortical network activity. Our results provide novel mechanistic and functional links among behavioral manifestations of sleep, wake, and sleep deprivation and specific measurable changes in the network dynamics relevant for characterizing the brain's changing information-processing capabilities. They suggest a network-level function of sleep to reorganize cortical networks toward states governed by long timescales to ensure efficient information integration for the time awake.

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Contralateral Bias of High Spatial Frequency Tuning and Cardinal Direction Selectivity in Mouse Visual Cortex

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Binocular mechanisms for visual processing are thought to enhance spatial acuity by combining matched input from the two eyes. Studies in the primary visual cortex of carnivores and primates have confirmed that eye-specific neuronal response properties are largely matched. In recent years, the mouse has emerged as a prominent model for binocular visual processing, yet little is known about the spatial frequency tuning of binocular responses in mouse visual cortex. Using calcium imaging in awake mice of both sexes, we show that the spatial frequency preference of cortical responses to the contralateral eye is ~35% higher than responses to the ipsilateral eye. Furthermore, we find that neurons in binocular visual cortex that respond only to the contralateral eye are tuned to higher spatial frequencies. Binocular neurons that are well matched in spatial frequency preference are also matched in orientation preference. In contrast, we observe that binocularly mismatched cells are more mismatched in orientation tuning. Furthermore, we find that contralateral responses are more direction-selective than ipsilateral responses and are strongly biased to the cardinal directions. The contralateral bias of high spatial frequency tuning was found in both awake and anesthetized recordings. The distinct properties of contralateral cortical responses may reflect the functional segregation of direction-selective, high spatial frequency-preferring neurons in earlier stages of the central visual pathway. Moreover, these results suggest that the development of binocularity and visual acuity may engage distinct circuits in the mouse visual system.

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Sublaminar Subdivision of Mouse Auditory Cortex Layer 2/3 Based on Functional Translaminar Connections

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The cerebral cortex is subdivided into six layers based on morphological features. The supragranular layers 2/3 (L2/3) contain morphologically and genetically diverse populations of neurons, suggesting the existence of discrete classes of cells. In primates and carnivores L2/3 can be subdivided morphologically, but cytoarchitectonic divisions are less clear in rodents. Nevertheless, discrete classes of cells could exist based on their computational requirement, which might be linked to their associated functional microcircuits. Through *in vitro* slice recordings coupled with laser-scanning photostimulation we investigated whether L2/3 of male mouse auditory cortex contains discrete subpopulations of cells with specific functional microcircuits. We use hierarchical clustering on the laminar connection patterns to reveal the existence of multiple distinct classes of L2/3 neurons. The classes of L2/3 neurons are distinguished by the pattern of their laminar and columnar inputs from within A1 and their location within L2/3. Cells in superficial L2 show more extensive columnar integration than deeper L3 cells. Moreover, L3 cells receive more translaminar input from L4. *In vivo* imaging in awake mice revealed that L2 cells had higher bandwidth than L3 cells, consistent with the laminar differences in columnar integration. These results suggest that similar to higher mammals, rodent L2/3 is not a homogenous layer but contains several parallel microcircuits.

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Approaching the Ground Truth: Revealing the Functional Organization of Human Multisensory STC Using Ultra-High Field fMRI

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Integrating inputs across sensory systems is a property of the brain that is vitally important in everyday life. More than two decades of fMRI research have revealed crucial insights on multisensory processing, yet the multisensory operations at the neuronal level in humans have remained largely unknown. Understanding the fine-scale spatial organization of multisensory brain regions is fundamental to shed light on their neuronal operations. Monkey electrophysiology revealed that the bimodal superior temporal cortex (bSTC) is topographically organized according to the modality preference (visual, auditory, and bimodal) of its neurons. In line with invasive studies, a previous 3 Tesla fMRI study suggests that the human bSTC is also topographically organized according to modality preference (visual, auditory, and bimodal) when analyzed at $1.6 \times 1.6 \times 1.6 \text{ mm}^3$ voxel resolution. However, it is still unclear whether this resolution is able to unveil an accurate spatial organization of the human bSTC. This issue was addressed in the present study by investigating the spatial organization of functional responses of the bSTC in 10 participants (from both sexes) at $1.5 \times 1.5 \times 1.5 \text{ mm}^3$ and $1.1 \times 1.1 \times 1.1 \text{ mm}^3$ using ultra-high field fMRI (at 7 Tesla). Relative to $1.5 \times 1.5 \times 1.5 \text{ mm}^3$, the bSTC at $1.1 \times 1.1 \times 1.1 \text{ mm}^3$ resolution was characterized by a larger selectivity for visual and auditory modalities, stronger integrative responses in bimodal voxels, and it was organized in more distinct functional clusters indicating a more precise separation of underlying neuronal clusters. Our findings indicate that increasing the spatial resolution may be necessary and sufficient to achieve a more accurate functional topography of human multisensory integration.

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Primary Generators of Visually Evoked Field Potentials Recorded in the Macaque Auditory Cortex

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Prior studies have reported “local” field potential (LFP) responses to faces in the macaque auditory cortex and have suggested that such face-LFPs may be substrates of audiovisual integration. However, although field potentials (FPs) may reflect the synaptic currents of neurons near the recording electrode, due to the use of a distant reference electrode, they often reflect those of synaptic activity occurring in distant sites as well. Thus, FP recordings within a given brain region (e.g., auditory cortex) may be “contaminated” by activity generated elsewhere in the brain. To determine whether face responses are indeed generated within macaque auditory cortex, we recorded FPs and concomitant multiunit activity with linear array multielectrodes across auditory cortex in three macaques (one female), and applied current source density (CSD) analysis to the laminar FP profile. CSD analysis revealed no appreciable local generator contribution to the visual FP in auditory cortex, although we did note an increase in the amplitude of visual FP with cortical depth, suggesting that their generators are located below auditory cortex. In the underlying inferotemporal cortex, we found polarity inversions of the main visual FP components accompanied by robust CSD responses and large-amplitude multiunit activity. These results indicate that face-evoked FP responses in auditory cortex are not generated locally but are volume-conducted from other face-responsive regions. In broader terms, our results underscore the caution that, unless far-field contamination is removed, LFPs in general may reflect such “far-field” activity, in addition to, or in absence of, local synaptic responses.

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Individual Alpha Peak Frequency Predicts 10 Hz Flicker Effects on Selective Attention

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Rhythmic visual stimulation (“flicker”) is primarily used to “tag” processing of low-level visual and high-level cognitive phenomena. However, preliminary evidence suggests that flicker may also entrain endogenous brain oscillations, thereby modulating cognitive processes supported by those brain rhythms. Here we tested the interaction between 10 Hz flicker and endogenous alpha-band ($\sim 10 \text{ Hz}$) oscillations during a selective visuospatial attention task. We recorded EEG from human participants (both genders) while they performed a modified Eriksen flanker task in which distractors and targets flickered within (10 Hz) or outside (7.5 or 15 Hz) the alpha band. By using a combination of EEG source separation, time-frequency, and single-trial linear mixed-effects modeling, we demonstrate that 10 Hz flicker interfered with stimulus processing more on incongruent than congruent trials (high vs low selective attention demands). Crucially, the effect of 10 Hz flicker on task performance was

predicted by the distance between 10 Hz and individual alpha peak frequency (estimated during the task). Finally, the flicker effect on task performance was more strongly predicted by EEG flicker responses during stimulus processing than during preparation for the upcoming stimulus, suggesting that 10 Hz flicker interfered more with reactive than proactive selective attention. These findings are consistent with our hypothesis that visual flicker entrained endogenous alpha-band networks, which in turn impaired task performance. Our findings also provide novel evidence for frequency-dependent exogenous modulation of cognition that is determined by the correspondence between the exogenous flicker frequency and the endogenous brain rhythms.

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The Neural Basis of Aversive Pavlovian Guidance during Planning

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Important real-world decisions are often arduous as they frequently involve sequences of choices, with initial selections affecting future options. Evaluating every possible combination of choices is computationally intractable, particularly for longer multistep decisions. Therefore, humans frequently use heuristics to reduce the complexity of decisions. We recently used a goal-directed planning task to demonstrate the profound behavioral influence and ubiquity of one such shortcut, namely aversive pruning, a reflexive Pavlovian process that involves neglecting parts of the decision space residing beyond salient negative outcomes. However, how the brain implements this important decision heuristic and what underlies individual differences have hitherto remained unanswered. Therefore, we administered an adapted version of the same planning task to healthy male and female volunteers undergoing functional magnetic resonance imaging (fMRI) to determine the neural basis of aversive pruning. Through both computational and standard categorical fMRI analyses, we show that when planning was influenced by aversive pruning, the subgenual cingulate cortex was robustly recruited. This neural signature was distinct from those associated with general planning and valuation, two fundamental cognitive components elicited by our task but which are complementary to aversive pruning. Furthermore, we found that individual variation in levels of aversive pruning was associated with the responses of insula and dorsolateral prefrontal cortices to the receipt of large monetary losses, and also with subclinical levels of anxiety. In summary, our data reveal the neural signatures of an important reflexive Pavlovian process that shapes goal-directed evaluations and thereby determines the outcome of high-level sequential cognitive processes.

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Multiple Signaling Pathways Coordinately Regulate Forgetting of Olfactory Adaptation through Control of Sensory Responses in *Caenorhabditis elegans*

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Forgetting memories is important for animals to properly respond to continuously changing environments. To elucidate the mechanisms of forgetting, we used one of the behavioral plasticities of *Caenorhabditis elegans* hermaphrodite, olfactory adaptation to an attractive odorant, diacetyl, as a simple model of learning. In *C. elegans*, the TIR-1/JNK-1 pathway accelerates forgetting of olfactory adaptation by facilitating neural secretion from AWC sensory neurons. In this study, to identify the downstream effectors of the TIR-1/JNK-1 pathway, we conducted a genetic screen for suppressors of the gain-of-function mutant of *tir-1* (*ok1052*), which shows excessive forgetting. Our screening showed that three proteins—a membrane protein, MACO-1; a receptor tyrosine kinase, SCD-2; and its putative ligand, HEN-1—regulated forgetting downstream of the TIR-1/JNK-1 pathway. We further demonstrated that MACO-1 and SCD-2/HEN-1 functioned in parallel genetic pathways, and only MACO-1 regulated forgetting of olfactory adaptation to isoamyl alcohol, which is an attractive odorant sensed by different types of sensory neurons. In olfactory adaptation, odor-evoked Ca^{2+} responses in olfactory neurons are attenuated by conditioning and recovered thereafter. A Ca^{2+} imaging study revealed that this attenuation is sustained longer in *maco-1* and *scd-2* mutant animals than in wild-type animals like the TIR-1/JNK-1 pathway mutants. Furthermore, temporal silencing by histamine-gated chloride channels revealed that the neuronal activity of AWC neurons after conditioning is important for proper forgetting. We propose that distinct signaling pathways, each of which has a specific function, may coordinately and temporally regulate forgetting by controlling sensory responses.

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Disruption of Bmal1 Impairs Blood–Brain Barrier Integrity via Pericyte Dysfunction

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Circadian rhythm disturbances are well established in neurological diseases. However, how these disruptions cause homeostatic imbalances remains poorly understood. Brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1) is a major circadian clock transcriptional activator, and Bmal1 deficiency in male *Bmal1^{nestin}^{−/−}* mice induced marked astroglial activation without affecting the number of astrocytes in the brain and spinal cord. Bmal1 deletion caused blood–brain barrier (BBB) hyperpermeability with an age-dependent loss of pericyte coverage of blood vessels in the brain. Using Nestin-green fluorescent protein (GFP) transgenic mice, we determined that pericytes are Nestin-GFP⁺ in the adult brain. Bmal1 deletion caused Nestin-GFP⁺ pericyte dysfunction, including the downregulation of platelet-derived growth factor receptor β (PDGFR β), a protein necessary for maintaining BBB integrity. Knockdown of Bmal1 downregulated PDGFR β transcription in the brain pericyte cell line. Thus, the circadian clock component Bmal1 maintains BBB integrity via regulating pericytes.

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Site-Specific Regulation of P2X7 Receptor Function in Microglia Gates Morphine Analgesic Tolerance

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Tolerance to the analgesic effects of opioids is a major problem in chronic pain management. Microglia are implicated in opioid tolerance, but the core mechanisms regulating their response to opioids remain obscure. By selectively ablating microglia in the spinal cord using a saporin-conjugated antibody to Mac1, we demonstrate a causal role for microglia in the development, but not maintenance, of morphine tolerance in male rats. Increased P2X7 receptor (P2X7R) activity is a cardinal feature of microglial activation, and in this study we found that morphine potentiates P2X7R-mediated Ca²⁺ responses in resident spinal microglia acutely isolated from morphine tolerant rats. The increased P2X7R function was blocked in cultured microglia by PP2, a Src family protein tyrosine kinase inhibitor. We identified Src family kinase activation mediated by μ -receptors as a key mechanistic step required for morphine potentiation of P2X7R function. Furthermore, we show by site-directed mutagenesis that tyrosine (Y_{382–384}) within the P2X7R C-terminus is differentially modulated by repeated morphine treatment and has no bearing on normal P2X7R function. Intrathecal administration of a palmitoylated peptide corresponding to the Y_{382–384} site suppressed morphine-induced microglial reactivity and preserved the antinociceptive effects of morphine in male rats. Thus, site-specific regulation of P2X7R function mediated by Y_{382–384} is a novel cellular determinant of the microglial response to morphine that critically underlies the development of morphine analgesic tolerance.

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Mitochondrial DNA Double-Strand Breaks in Oligodendrocytes Cause Demyelination, Axonal Injury, and CNS Inflammation

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Mitochondrial dysfunction has been implicated in the pathophysiology of neurodegenerative disorders, including multiple sclerosis (MS). To date, the investigation of mitochondrial dysfunction in MS has focused exclusively on neurons, with no studies exploring whether dysregulation of mitochondrial bioenergetics and/or genetics in oligodendrocytes might be associated with the etiopathogenesis of MS and other demyelinating syndromes. To address this question, we established a mouse model where mitochondrial DNA (mtDNA) double-strand breaks (DSBs) were specifically induced in myelinating oligodendrocytes (PLP:mtPstI mice) by expressing a mitochondrial-targeted endonuclease, mtPstI, starting at 3 weeks of age. In both female and male mice, DSBs of oligodendroglial mtDNA caused impairment of locomotor function, chronic demyelination, glial activation, and axonal degeneration, which became more severe with time of induction. In addition, after short transient induction of mtDNA DSBs, PLP:mtPstI mice showed an exacerbated response to experimental autoimmune encephalomyelitis. Together, our data demonstrate that mtDNA damage can cause primary oligodendropathy, which in turn

triggers demyelination, proving PLP:mtPstI mice to be a useful tool to study the pathological consequences of mitochondrial dysfunction in oligodendrocytes. In addition, the demyelination and axonal loss displayed by PLP:mtPstI mice recapitulate some of the key features of chronic demyelinating syndromes, including progressive MS forms, which are not accurately reproduced in the models currently available. For this reason, the PLP:mtPstI mouse represents a unique and much needed platform for testing remyelinating therapies.

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Enhanced Nociception in Angelman Syndrome Model Mice

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Angelman syndrome (AS) is a severe neurodevelopmental disorder caused by mutation or deletion of the maternal *UBE3A* allele. The maternal *UBE3A* allele is expressed in nearly all neurons of the brain and spinal cord, whereas the paternal *UBE3A* allele is repressed by an extremely long antisense transcript (*UBE3A-ATS*). Little is known about expression of *UBE3A* in the peripheral nervous system, where loss of maternal *UBE3A* might contribute to AS phenotypes. Here we sought to examine maternal and paternal *Ube3a* expression in DRGs neurons and to evaluate whether nociceptive responses were affected in AS model mice (global deletion of maternal *Ube3a* allele; *Ube3a*^{m-/p+}). We found that most large-diameter proprioceptive and mechanosensitive DRG neurons expressed maternal *Ube3a* and paternal *Ube3a-ATS*. In contrast, most small-diameter neurons expressed *Ube3a* biallelically and had low to undetectable levels of *Ube3a-ATS*. Analysis of single-cell DRG transcriptomes further suggested that *Ube3a* is expressed monoallelically in myelinated large-diameter neurons and biallelically in unmyelinated small-diameter neurons. Behavioral responses to some noxious thermal and mechanical stimuli were enhanced in male and female AS model mice; however, nociceptive responses were not altered by the conditional deletion of maternal *Ube3a* in the DRG. These data suggest that the enhanced nociceptive responses in AS model mice are due to loss of maternal *Ube3a* in the central, but not peripheral, nervous system. Our study provides new insights into sensory processing deficits associated with AS.

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