

# This Week in The Journal

## ● Cellular/Molecular

### *TRPV1 and Osmoregulation*

Sorana Ciura and Charles W. Bourque

(see pages 9069–9075)

Central control of systemic osmoregulation is the job of the organum vasculosum lamina terminalis (OVLT), located at the rostral ventral edge of the third ventricle. In this week's Journal, Ciura and Bourque demonstrate that OVLT neurons are intrinsically osmosensitive because they express transient receptor potential vanilloid 1 (TRPV1) channels. The authors compared the neuronal activity of hypothalamic explants from wild-type and TRPV1-deficient mice. OVLT neurons in wild-type mice responded to increased osmolality with an increased firing rate, whereas TRPV1<sup>-/-</sup> neurons did not. OVLT neurons responded directly to the change in osmolality with membrane depolarization. Ruthenium red, a blocker of nonselective cation channels, prevented hypertonic solution-induced depolarization, pointing to TRPV1 as the transducer. *In vivo*, TRPV1<sup>-/-</sup> mice drank less water after an osmotic challenge than did their WT counterparts, placing TRPV1 in the pathway that triggers thirst.

## ▲ Development/Plasticity/Repair

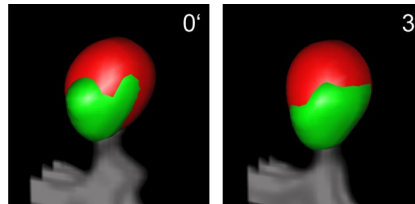
### *Astrocyte Processes and Dendritic Spines in Motion*

Michael Haber, Lei Zhou, and Keith K. Murai

(see pages 8881–8891)

Glial cells sure aren't what they were thought to be. They have calcium signals, release transmitters, and receive "synaptic" input. Now Haber et al. provide evidence that they can even move faster than neurons. The authors used time-lapse confocal imaging of organotypic hippocampal explants to monitor the movements of dendritic spines and their associated glial processes. Two Semliki Forest viral vectors were used to selectively infect glia and neurons with membrane-tethered enhanced green fluorescent protein

and red fluorescent protein. The membrane-tethered probes facilitated imaging of fine glial processes. In the hippocampal CA1 area, astrocytes and dendrites showed complex interactions, including astrocytic processes that encapsulated some dendritic spines. Astrocytic processes extended and retracted considerable distances (5  $\mu$ m) during a 30 min observation period. The GABA<sub>A</sub> receptor antagonist bicuculline, which increases neural activity, stabilized spines but had no apparent effect on astrocyte motility. In general, interactions between astrocyte processes and larger spines were more stable than those with smaller spines.



This reconstruction shows the association between a dendritic spine (red) and an astrocyte process (green) over a 3 min imaging period. See Haber et al. for details.

## ■ Behavioral/Systems/Cognitive

### *To Burst or Not to Burst in the Medial Septum In Vivo*

Axelle Pascale Simon, Frédérique Poindessous-Jazat, Patrick Dutar, Jacques Epelbaum, and Marie-Hélène Bassant

(see pages 9038–9046)

The neurons in the medial septum-diagonal band of Broca (MS-DB) have long been implicated in hippocampal theta rhythms. In this week's Journal, Simon et al. sought to differentiate the firing patterns of GABAergic and cholinergic MS-DB neurons. Their results indicate that GABAergic cells rather than cholinergic neurons display theta-related bursting or tonic activity in the MS-DB. Parvalbumin (PV) labels most GABAergic septohippocampal neurons, whereas other GABAergic neurons express glutamic acid decarboxylase (GAD). The au-

thors recorded from MS-DB neurons in anesthetized and unanesthetized rats, and labeled the recorded neurons with neurobiotin. Of 90 neurons, three-quarters expressed GAD, and PV labeled approximately one-third of those. Only eight neurons, negative for GAD and PV, were cholinergic. GABAergic neurons displayed tonic, cluster, or burst-firing discharge patterns that varied with the sleep-wake cycle, but PV-positive neurons displayed more pronounced rhythmic bursting activity. Cholinergic neurons all displayed a low discharge rate.

## ◆ Neurobiology of Disease

### *A KCC2 Homolog and Seizures in Drosophila*

Daria S. Hekmat-Scafe, Miriam Y. Lundy, Rakhee Ranga, and Mark A. Tanouye

(see pages 8943–8954)

By setting the chloride gradient across the membrane, the potassium/chloride cotransporter KCC2 controls whether GABA<sub>A</sub> receptor signaling in mammalian neurons is depolarizing or hyperpolarizing. In this week's Journal, Hekmat-Scafe et al. explore the homologous K<sup>+</sup>/Cl<sup>-</sup> cotransporter in *Drosophila*. Null mutations in the *kazachoc* (*kcc*) gene were lethal, but partial loss-of-function mutations resulted in increased seizure sensitivity. Why the name *kazachoc*? Well, this is a Slavic dance that involves squatting and kicking, which reminded the authors of flies with the bang-sensitive (BS) phenotype, a behavior that correlates with seizure sensitivity. And what is a BS phenotype you ask? Well, flies are vortexed for 10 s at high speed, and BS-sensitive flies display a period of paralysis followed by hyperactivity. For *kcc* mutants, BS sensitivity showed incomplete penetrance and was more prominent at lower temperatures and younger ages. *Kcc*-related seizure susceptibility was also reduced in flies treated with the GABA<sub>A</sub> receptor antagonist picrotoxin and in flies that expressed reduced levels of the GABA<sub>A</sub> receptor.

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**Cover legend:** The image portrays the timing of discriminating activity during perceptual decision-making. The discriminating components, identified by single-trial analysis of electroencephalograms (EEGs), were used to develop a timing diagram. The components correlated with visual perception (170 ms), decision difficulty (220 ms), and decision-making (300 ms), as indicated by color-coded scalp projections of the EEG activity. For details, see the article by Philiastides et al. (pages 8965–8975). Cover art by Mimi Duvall and John-Paul Layedra.

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**Correction:** In the article "Chemokines Regulate the Migration of Neural Progenitors to Sites of Neuroinflammation" by Abdelhak Belmadani, Phuong B. Tran, Dongjun Ren and Richard J. Miller, which appeared on pages 3182–3191 of the March 22, 2006, the construct reported as a recombinant adenovirus coexpressing EGFP and  $\beta$ -amyloid is incorrectly described. The correct description should have been stated as the adenovirus coexpressed GFP and the Swedish familial AD mutant of myc-tagged APP695 as described in the paper by Ikezu T et al. (2003), J Neurochem 85:925–934.

To the article "Multiprotein Complexes of the Survival of Motor Neuron Protein SMN with Gemins Traffic to Neuronal Processes and Growth Cones of Motor Neurons" by Honglai Zhang, Lei Xing, Wilfried Rossoll, Hynek Wichterle, Robert H. Singer, and Gary J. Bassell, which appeared on pages 8622–8632 of the August 16, 2006 issue, the authors would also like to add acknowledgment of support from NIH AR41480 to Robert H. Singer.

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## EphB Receptors and Ephrin-B3 Regulate Axon Guidance at the Ventral Midline of the Embryonic Mouse Spinal Cord

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EphB receptors and their ephrin-B ligands are required for midline guidance decisions at several rostrocaudal levels of the developing CNS. In the embryonic vertebrate spinal cord, ephrin-B3 is localized to the floor plate (FP) at the ventral midline (VM), ephrin-B1 and ephrin-B2 are expressed in the dorsal spinal cord, and decussated EphB receptor-bearing commissural axons navigate between these ventral and dorsal ephrin-B domains. Despite these compelling expression patterns, the *in vivo* role(s) for EphB and ephrin-B proteins in regulating the guidance of spinal commissural axons has not been established. Here, we use DiI (1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate) labeling to assess the pathfinding of commissural axons in the spinal cords of ephrin-B and EphB mutant mouse embryos. In mice lacking ephrin-B3 or multiple EphB receptors, a significant number of axons followed aberrant trajectories in the immediate vicinity of the VM. Furthermore, forked transverse commissural (FTC) axons, a unique class of commissural axons that continues to project in the transverse plane on the contralateral side of the FP, were present at a markedly higher frequency in ephrin-B3 and EphB mutants, compared with wild-type embryos. Neither the midline guidance errors nor excessive numbers of FTC axons were observed in the spinal cords of *ephrin-B3<sup>lacZ</sup>* mice that express a truncated form of ephrin-B3, which is capable of forward but not reverse signaling. In contrast to the midline guidance defects observed in EphB and ephrin-B3 mutant embryos, wild-type-like contralateral projections were observed in mice lacking ephrin-B1 and/or ephrin-B2.

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## Altered Hippocampal Synaptic Potentiation in P2X<sub>4</sub> Knock-Out Mice

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P2X<sub>4</sub> purinergic receptors are calcium-permeable, ATP-activated ion channels. In the CA1 area of the hippocampus, they are located at the subsynaptic membrane somewhat peripherally to AMPA receptors. The possible role of P2X<sub>4</sub> receptors has been difficult to elucidate because of the lack of selective antagonists. Here we report the generation of a P2X<sub>4</sub> receptor knock-out mouse and show that long-term potentiation (LTP) at Schaffer collateral synapses is reduced relative to that in wild-type mice. Ivermectin, which selectively potentiates currents at P2X<sub>4</sub>, was found to increase LTP in wild-type mice but had no effect in P2X<sub>4</sub> knock-out mice. We suggest that calcium entry through subsynaptic P2X<sub>4</sub> receptors during high-frequency stimulation contributes to synaptic strengthening.

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## Social Context-Dependent Singing-Regulated Dopamine

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Like the mammalian striatum, the songbird striatum receives dense dopaminergic input from the midbrain ventral tegmental area–substantia nigra pars compacta complex. The songbird striatum also contains a unique vocal nucleus, Area X, which has been implicated in song learning and social context-dependent song production. Area X shows increased neural firing and activity-dependent gene expression when birds sing, and the level of activation is higher and more variable during undirected singing relative to directed singing to other birds. Here we show in the first report of *in vivo* microdialysis in awake, behaving songbirds that singing is associated with increased dopamine levels in Area X. Dopamine levels are significantly higher with directed relative to undirected singing. This social context-dependent difference in dopamine levels requires the dopamine transporter, because local *in vivo* blockade of the transporter caused dopamine levels for undirected singing to increase to levels similar to that for directed singing, eliminating the social context-dependent difference. The increase in dopamine is presumably depolarization and vesicular release dependent, because adding of high K<sup>+</sup> increased and removal of Ca<sup>2+</sup> increased and decreased extracellular DA levels. Our findings implicate DA and molecules that control DA kinetics in singing behavior and social context-dependent brain function.

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## Involvement of Protein Kinase C- $\epsilon$ in Activity-Dependent Potentiation of Large Dense-Core Vesicle Exocytosis in Chromaffin Cells

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Neurotransmitter release is modulated in an activity-dependent manner. We showed previously that repetitive stimulation of nicotinic acetylcholine receptor (nAChR) induced activity-dependent potentiation (ADP) of large dense-core vesicle (LDCV) exocytosis in chromaffin cells. Here we report that protein kinase C (PKC)- $\epsilon$  is critically involved in ADP. Stimulation of nAChR induced activation of PKC- $\epsilon$ , and inhibition of PKC- $\epsilon$  by expression of the dominant-negative mutant of PKC- $\epsilon$  (DN-PKC- $\epsilon$ ) or short interfering (siRNA) against PKC- $\epsilon$  abolished ADP via decreasing the frequency and quantal size of fused vesicles without affecting basal exocytosis, suggesting that PKC- $\epsilon$  is specifically involved in ADP. Electron microscopy revealed that inhibition of PKC- $\epsilon$  disrupts activity-induced vesicle translocation required for ADP. We also suggest the involvement of myristoylated alanine-rich C kinase substrate (MARCKS), which is known as a downstream target of PKC- $\epsilon$ , in ADP of LDCV exocytosis. The level of phospho-MARCKS correlated with the time course of ADP and was reduced by transfection with DN-PKC- $\epsilon$ . Actin filament disassembly induced by MARCKS phosphorylation was also significantly blocked by transfection of DN-PKC- $\epsilon$ . Furthermore, knockdown of MARCKS by siRNA resulted in inhibition of ADP and reduction of the number of fused vesicles. Together, we provide evidence that ADP of LDCV exocytosis is regulated by PKC- $\epsilon$  and its downstream target MARCKS via modulating vesicle translocation.

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## Transient Receptor Potential Vanilloid 1 Is Required for Intrinsic Osmoreception in Organum Vasculosum Lamina Terminalis Neurons and for Normal Thirst Responses to Systemic Hyperosmolality

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Recent studies have indicated that members of the transient receptor potential vanilloid (TRPV) family of cation channels are required for the generation of normal osmoregulatory responses, yet the mechanism of osmosensory transduction in primary osmoreceptor neurons of the CNS remains to be defined. Indeed, despite ample evidence suggesting that the organum vasculosum lamina terminalis (OVLT) serves as the primary locus of the brain for the detection of osmotic stimuli, evidence that neurons in the OVLT are intrinsically osmosensitive has remained elusive. Here we show that murine OVLT neurons are intrinsically sensitive to increases in the osmolality of the extracellular fluid. Hypertonic conditions provoked increases in membrane cation conductance that resulted in the generation of an inward current, depolarizing osmoreceptor potentials, and enhanced action potential discharge. Moreover, we found that this osmosensory signal transduction cascade was absent in OVLT neurons from TRPV1 knock-out (*TRPV1*<sup>-/-</sup>) mice and that responses of wild type (WT) OVLT neurons could be blocked by ruthenium red, an inhibitor of TRPV channels. Finally, *TRPV1*<sup>-/-</sup> mice showed significantly attenuated water intake in response to systemic hypertonicity compared with WT controls. These findings indicate that OVLT neurons act as primary osmoreceptors and that a product of the *trpv1* gene is required for osmosensory transduction.

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## DEVELOPMENT/PLASTICITY/REPAIR

## Cooperative Astrocyte and Dendritic Spine Dynamics at Hippocampal Excitatory Synapses

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Accumulating evidence is redefining the importance of neuron–glial interactions at synapses in the CNS. Astrocytes form “tripartite” complexes with presynaptic and postsynaptic structures and regulate synaptic transmission and plasticity. Despite our understanding of the importance of neuron–glial relationships in physiological contexts, little is known about the structural interplay between astrocytes and synapses. In the past, this has been difficult to explore because studies have been hampered by the lack of a system that preserves complex neuron–glial relationships observed in the brain. Here we present a system that can be used to characterize the intricate relationship between astrocytic processes and synaptic structures *in situ* using organotypic hippocampal slices, a preparation that retains the three-dimensional architecture of astrocyte–synapse interactions. Using time-lapse confocal imaging, we demonstrate that astrocytes can rapidly extend and retract fine processes to engage and disengage from motile postsynaptic dendritic spines. Surprisingly, astrocytic motility is, on average, higher than its dendritic spine counterparts and likely relies on actin-based cytoskeletal reorganization. Changes in astrocytic processes are typically coordinated with changes in spines, and astrocyte–spine interactions are stabilized at larger spines. Our results suggest that dynamic structural changes in astrocytes help control the degree of neuron–glial communication at hippocampal synapses.

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# Temporal Coding Mediates Discrimination of “Bitter” Taste Stimuli by an Insect

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The mechanisms that mediate discriminative taste processing in insects are poorly understood. We asked whether temporal patterns of discharge from the peripheral taste system of an insect (*Manduca sexta* caterpillars; Sphingidae) contribute to the discrimination of three “bitter” taste stimuli: salicin, caffeine, and aristolochic acid. The gustatory response to these stimuli is mediated exclusively by three pairs of bitter-sensitive taste cell, which are located in the medial, lateral, and epipharyngeal sensilla. We tested for discrimination by habituating the caterpillars to salicin and then determining whether the habituation generalized to caffeine or aristolochic acid. We ran habituation-generalization tests in caterpillars with their full complement of taste sensilla (i.e., intact) and in caterpillars with ablated lateral sensilla (i.e., lat-ablated). The latter perturbation enabled us to examine discrimination in caterpillars with a modified peripheral taste profile. We found that the intact and lat-ablated caterpillars both generalized the salicin-habituation to caffeine but not aristolochic acid. Next, we determined whether this pattern of stimulus-generalization could be explained by salicin and aristolochic acid generating distinct ensemble, rate, temporal, or spatiotemporal codes. To this end, we recorded excitatory responses from the bitter-sensitive taste cells and then used these responses to formulate predictions about whether the salicin-habituation should generalize to caffeine or aristolochic acid, separately for each coding framework. We found that the pattern of stimulus generalization in both intact and lat-ablated caterpillars could only be predicted by temporal coding. We conclude that temporal codes from the periphery can mediate discriminative taste processing.

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# Prolongation of Evoked and Spontaneous Synaptic Currents at the Neuromuscular Junction after Activity Blockade Is Caused by the Upregulation of Fetal Acetylcholine Receptors

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It has been shown previously in a number of systems that after an extended block of activity, synaptic strength is increased. We found that an extended block of synaptic activity at the mouse neuromuscular junction, using a tetrodotoxin cuff *in vivo*, increased synaptic strength by prolonging the evoked endplate current (EPC) decay. Prolongation of EPC decay was accompanied by only modest prolongation of spontaneous miniature EPC (MEPC) decay. Prolongation of EPC decay was reversed when quantal content was lowered by reducing extracellular calcium. These findings suggested that the cause of EPC prolongation was presynaptic in origin. However, when we acutely inhibited fetal-type acetylcholine receptors (AChRs) using a novel peptide toxin ( $\alpha$ A-conotoxin OIVA[K15N]), prolongation of both EPC and MEPC decay were reversed. We also blocked synaptic activity in a mutant strain of mice in which persistent muscle activity prevents upregulation of fetal-type AChRs. In these mice, there was no prolongation of EPC decay. We conclude that upregulation of fetal-type AChRs after blocking synaptic activity causes modest prolongation of MEPC decay that is accompanied by much greater prolongation of EPC decay. This might occur if acetylcholine escapes from endplates and binds to extrajunctional fetal-type AChRs only during large, evoked EPCs. Our study is the first to demonstrate a functional role for upregulation of extrajunctional AChRs.

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## BEHAVIORAL/SYSTEMS/COGNITIVE

# Extinction Training in Conjunction with a Partial Agonist of the Glycine Site on the NMDA Receptor Erases Memory Trace

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Much evidence indicates that extinction training does not erase memory traces but instead forms inhibitory learning that prevents the expression of original memory. Fear conditioning induces long-term potentiation and drives synaptic insertion of AMPA receptors into the amygdala. Here we show that extinction training applied 1 h after training reversed the conditioning-induced increase in surface glutamate receptor subunit 1 (GluR1) in parallel with the inhibition of startle potentiation. However, if applied 24 h after training, extinction training reduced startle potentiation without influencing the GluR1 increase. We infused D-cycloserine (DCS), a partial agonist of the glycine site on the NMDA receptor, bilaterally into the amygdala 30 min before extinction training. This augmented the extinction training-elicited reduction in startle and reversed the conditioning-induced increase in GluR1. Delivery of five sets of tetanic stimulation (TS) to the external capsule produced a robust enhancement of synaptic responses in the lateral amygdala neurons that persisted for >2 h. Low-frequency stimulation applied 1 h after TS had no long-lasting effect on synaptic responses. The same treatments, however, induced depotentiation in the presence of DCS and reversed TS-induced increase in surface GluR1. Together, this study has two important findings: (1) whether a memory trace remains intact or is erased depends on the interval between conditioning and extinction training and (2) DCS facilitates the reversal of memory trace. DCS-induced augmentation of extinction and reversal of GluR1 surface expression are likely mediated by DCS-facilitated endocytosis of AMPA receptors.

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# Amygdala Response to Facial Expressions Reflects Emotional Learning

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The functional role of the human amygdala in the evaluation of emotional facial expressions is unclear. Previous animal and human research shows that the amygdala participates in processing positive and negative reinforcement as well as in learning predictive associations between stimuli and subsequent reinforcement. Thus, amygdala response to facial expressions could reflect the processing of primary reinforcement or emotional learning. Here, using functional magnetic resonance imaging, we tested the hypothesis that amygdala response to facial expressions is driven by emotional association learning. We show that the amygdala is more responsive to learning object-emotion associations from happy and fearful facial expressions than it is to the presentation of happy and fearful facial expressions alone. The results provide evidence that the amygdala uses social signals to rapidly and flexibly learn threatening and rewarding associations that ultimately serve to enhance survival.

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# Activation of Pedunculopontine Tegmental Protein Kinase A: A Mechanism for Rapid Eye Movement Sleep Generation in the Freely Moving Rat

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Cells in the pedunculopontine tegmentum (PPT) play a key role in the generation of rapid eye movement (REM) sleep, but its intracellular signaling mechanisms remain unknown. In the current studies, the role of PPT intracellular protein kinase A (PKA) in the regulation of REM sleep was evaluated by comparing PKA subunit [catalytic (PKA<sub>Cα</sub>) and regulatory (PKA<sub>RI</sub>, PKA<sub>RIIα</sub>, and PKA<sub>RIIβ</sub>) types] expression and activity in the PPT at normal, high, and low REM sleep conditions. To compare anatomical specificity, REM sleep-dependent expressions of these PKA subunits were also measured in the medial pontine reticular formation (mPRF), medial prefrontal cortex (mPFC), and anterior hypothalamus (AHTh). The results of these PKA subunit expression and activity studies demonstrated that the expression of PKA<sub>Cα</sub> and PKA activity in the PPT increased and decreased during high and low REM sleep, respectively. Conversely, PKA<sub>Cα</sub> expression and PKA activity decreased with high REM sleep in the mPRF. Expression of PKA<sub>Cα</sub> also decreased in the mPFC and remained unchanged in the AHTh with high REM sleep. These subunit expression and PKA activity data reveal a positive relationship between REM sleep and increased PKA activity in the PPT. To test this molecular evidence, localized activation of cAMP-dependent PKA activity was blocked using a pharmacological technique. The results of this pharmacological study demonstrated that the localized inhibition of cAMP-dependent PKA activation in the PPT dose-dependently suppressed REM sleep. Together, these results provide the first evidence that the activation of the PPT intracellular PKA system is involved in the generation of REM sleep.

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# Increased Expression of the 5-HT Transporter Confers a Low-Anxiety Phenotype Linked to Decreased 5-HT Transmission

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A commonly occurring polymorphic variant of the human 5-hydroxytryptamine (5-HT) transporter (5-HTT) gene that increases 5-HTT expression has been associated with reduced anxiety levels in human volunteer and patient populations. However, it is not known whether this linkage between genotype and anxiety relates to variation in 5-HTT expression and consequent changes in 5-HT transmission. Here we test this hypothesis by measuring the neurochemical and behavioral characteristics of a mouse genetically engineered to overexpress the 5-HTT. Transgenic mice overexpressing the human 5-HTT (h5-HTT) were produced from a 500 kb yeast artificial chromosome construct. These transgenic mice showed the presence of h5-HTT mRNA in the midbrain raphe nuclei, as well as a twofold to threefold increase in 5-HTT binding sites in the raphe nuclei and a range of forebrain regions. The transgenic mice had reduced regional brain whole-tissue levels of 5-HT and, in microdialysis experiments, decreased brain extracellular 5-HT, which reversed on administration of the 5-HTT inhibitor paroxetine. Compared with wild-type mice, the transgenic mice exhibited a low-anxiety phenotype in plus maze and hyponeophagia tests. Furthermore, in the plus maze test, the low-anxiety phenotype of the transgenic mice was reversed by acute administration of paroxetine, suggesting a direct link between the behavior, 5-HTT overexpression, and low extracellular 5-HT. *In toto*, these findings demonstrate that associations between increased 5-HTT expression and anxiety can be modeled in mice and may be specifically mediated by decreases in 5-HT transmission.

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## Neural Representation of Task Difficulty and Decision Making during Perceptual Categorization: A Timing Diagram

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When does the brain know that a decision is difficult to make? How does decision difficulty affect the allocation of neural resources and timing of constituent cortical processing? Here, we use single-trial analysis of electroencephalography (EEG) to identify neural correlates of decision difficulty and relate these to neural correlates of decision accuracy. Using a cued paradigm, we show that we can identify a component in the EEG that reflects the inherent task difficulty and not simply a correlation with the stimulus. We find that this decision difficulty component arises  $\approx 220$  ms after stimulus presentation, between two EEG components that are predictive of decision accuracy [an “early” (170 ms) and a “late” ( $\approx 300$  ms) component]. We use these results to develop a timing diagram for perceptual decision making and relate the component activities to parameters of a diffusion model for decision making.

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## Encoding Difficulty Promotes Postlearning Changes in Sleep Spindle Activity during Napping

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Learning-dependent increases in sleep spindle density have been reported during nocturnal sleep immediately after the learning session. Here, we investigated experience-dependent changes in daytime sleep EEG activity after declarative learning of unrelated word pairs. At weekly intervals, 13 young male volunteers spent three 24 h sessions in the laboratory under carefully controlled homeostatic and circadian conditions. At approximately midday, subjects performed either one of two word-pair learning tasks or a matched nonlearning control task, in a counterbalanced order. The two learning lists differed in the level of concreteness of the words used, resulting in an easier and a more difficult associative encoding condition, as confirmed by performance at immediate cued recall. Subjects were then allowed to sleep for 4 h; afterward, delayed cued recall was tested. Compared with the control condition, sleep EEG spectral activity in the low spindle frequency range and the density of low-frequency sleep spindles (11.25–13.75 Hz) were both significantly increased in the left frontal cortex after the difficult but not after the easy encoding condition. Furthermore, we found positive correlations between these EEG changes during sleep and changes in memory performance between pre-nap and post-nap recall sessions. These results indicate that, like during nocturnal sleep, daytime sleep EEG oscillations including spindle activity are modified after declarative learning of word pairs. Furthermore, we demonstrate here that the nature of the learning material is a determinant factor for sleep-related alterations after declarative learning.

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## Interactions between Speed and Contrast Tuning in the Middle Temporal Area: Implications for the Neural Code for Speed

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A car driving through the fog appears to move more slowly than one driving on a clear and sunny day. In the laboratory, this observation has been confirmed as a pronounced reduction of perceived speed caused by a reduction in contrast.

We measured the influence of contrast on cells in the middle temporal area (MT) of the macaque, which has been hypothesized to underlie the perception of speed. The influence of contrast on the responsiveness and speed tuning of these cells was pervasive and highly regular. As expected, most cells responded less at low contrast. More importantly, the preferred speed of most cells shifted to lower speeds at lower contrasts. Moreover, approximately one-third of cells surprisingly responded more strongly to slow low-contrast stimuli than to slow high-contrast stimuli.

Current models of speed perception suggest that each MT cell votes for its preferred speed, with a vote determined by its firing rate. We tested a number of these labeled-line models by entering the neural responses we recorded from MT and comparing the predictions of the models with the perceptual reports of human subjects and monkeys. Contrary to the perceptual reports, the labeled-line models predicted that perceived speed should increase when contrast is decreased. We therefore conclude that perceived speed is not based on a labeled-line interpretation of MT cells.

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# Lighter or Heavier Than Predicted: Neural Correlates of Corrective Mechanisms during Erroneously Programmed Lifts

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A central concept in neuroscience is that the CNS signals the sensory discrepancy between the predicted and actual sensory consequences of action. It has been proposed that the cerebellum and parietal cortex are involved in this process. A discrepancy will trigger preprogrammed corrective responses and update the engaged sensorimotor memories. Here we use functional magnetic resonance imaging with an event-related design to investigate the neuronal correlates of such discrepancies. Healthy adults repeatedly lifted an object between their right index fingers and thumbs, and on some lifting trials, the weight of the object was unpredictably changed between light (230 g) and heavy (830 g). Regardless of whether the weight was heavier or lighter than predicted, activity was found in the right inferior parietal cortex (supramarginal gyrus). This suggests that this region is involved in the comparison of the predicted and actual sensory input and the updating of the sensorimotor memories. When the object was lighter or heavier than predicted, two different types of preprogrammed force corrections occurred. There was a slow force increase when the weight of the object was heavier than predicted. This corrective response was associated with activity in the left primary motor and somatosensory cortices. The fast termination of the excessive force when the object was lighter than predicted activated the right cerebellum. These findings show how the parietal cortex, cerebellum, and motor cortex are involved in the signaling of the discrepancy between predicted and actual sensory feedback and the associated corrective mechanisms.

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# Transgenic Mice Overexpressing Glycogen Synthase Kinase 3 $\beta$ : A Putative Model of Hyperactivity and Mania

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Lithium is used as treatment for bipolar disorder with particular efficacy in the treatment of mania. Lithium inhibits glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ) directly or indirectly via stimulation of the kinase Akt-1. We therefore investigated the possibility that transgenic mice overexpressing GSK-3 $\beta$  could be of relevance to model bipolar disorder. Transgenic mice showed hypophagia, an increased general locomotor activity, and decreased habituation as assessed in an open field, an increased acoustic startle response, and again decreased habituation. The forced swim test revealed a reduced immobility in transgenic mice, but this is probably related to the hyperactivity of the animals. There were no differences in baseline and stress-induced increases of plasma adrenocorticotrophic hormone and corticosterone levels. Molecular analysis suggests compensatory mechanisms in the striatum of these transgenic mice for the overload of active GSK-3 $\beta$  by dimming the endogenous GSK-3 $\beta$  signaling pathway via upregulation of Akt-1 expression. Brain-derived neurotrophic factor protein levels were increased in the hippocampus of the transgenic mice. This suggests some kind of compensatory mechanism to the observed reduction in brain weight, which has been related previously to a reduced size of the somatodendritic compartment. Together, in mice overexpressing GSK-3 $\beta$ , specific intracellular signaling pathways are affected, which is accompanied by altered plasticity processes and increased activity and reactivity, whereas habituation processes seem to be decreased. The behavioral observations led to the suggestion that the model at hand recapitulates hyperactivity as observed in the manic phase of bipolar disorder.

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# Decoding Stimulus Variance from a Distributional Neural Code of Interspike Intervals

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The spiking output of an individual neuron can represent information about the stimulus via mean rate, absolute spike time, and the time intervals between spikes. Here we discuss a distinct form of information representation, the local distribution of spike intervals, and show that the time-varying distribution of interspike intervals (ISIs) can represent parameters of the statistical context of stimuli. For many sensory neural systems the mapping between the stimulus input and spiking output is not fixed but, rather, depends on the statistical properties of the stimulus, potentially leading to ambiguity. We have shown previously that for the adaptive neural code of the fly H1, a motion-sensitive neuron in the fly visual system, information about the overall variance of the signal is obtainable from the ISI distribution. We now demonstrate the decoding of information about variance and show that a distributional code of ISIs can resolve ambiguities introduced by slow spike frequency adaptation. We examine the precision of this distributional code for the representation of stimulus variance in the H1 neuron as well as in the Hodgkin–Huxley model neuron. We find that the accuracy of the decoding depends on the shapes of the ISI distributions and the speed with which they adapt to new stimulus variances.

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# Firing Properties of Anatomically Identified Neurons in the Medial Septum of Anesthetized and Unanesthetized Restrained Rats

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Cholinergic and GABAergic neurons in the medial septum-diagonal band of Broca (MS-DB) project to the hippocampus where they are involved in generating theta rhythmicity. So far, the functional properties of neurochemically identified MS-DB neurons are not fully characterized. In this study, MS-DB neurons recorded in urethane anesthetized rats and in unanesthetized restrained rats were labeled with neurobiotin and processed for immunohistochemistry against glutamic acid decarboxylase (GAD), parvalbumin (PV), and choline acetyltransferase (ChAT). The majority of the 90 labeled neurons (75.5%) were GAD+. Among them, 34.0% were also PV+, but none were ChAT+. Only 8.8% of the labeled neurons were found ChAT+. Remaining neurons (15.5%) were not identified. In anesthetized rats, all of the PV/GAD+ and 65% of GAD+ neurons exhibited burst-firing activity at the theta frequency. PV/GAD+ neurons displayed higher discharge rate and longer burst duration compared with GAD+ neurons. At variance, all of the ChAT+ neurons were slow-firing. Cluster-firing and tonic-firing were observed in GAD+ and unidentified neurons. In unanesthetized rats, during wakefulness or rapid eye movement sleep with hippocampal theta, the bursting neurons were PV/GAD+ or GAD+, whereas all of the ChAT+ neurons were slow-firing. Across the sleep-wake cycle, the GABAergic component of the septohippocampal pathway was always more active than the cholinergic one. The fact that cholinergic MS-DB neurons do not display theta-related bursting or tonic activity but have a very low firing rate questions how acetylcholine exerts its activating role in the septohippocampal system.

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## NEUROBIOLOGY OF DISEASE

# Enhanced Presynaptic Neurotransmitter Release in the Anterior Cingulate Cortex of Mice with Chronic Pain

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The anterior cingulate cortex (ACC) is a forebrain structure known for its roles in learning and memory. Recent studies show that painful stimuli activate the prefrontal cortex and that brain chemistry is altered in this area in patients with chronic pain. Components of the CNS that are involved in pain transmission and modulation, from the spinal cord to the ACC, are very plastic and undergo rapid and long-term changes after injury. Patients suffering from chronic pain often complain of memory and concentration difficulties, but little is known about the neural circuitry underlying these deficits. To address this question, we analyzed synaptic transmission in the ACC from mice with chronic pain induced by hindpaw injection of complete Freund's adjuvant (CFA). *In vitro* whole-cell patch-clamp recordings revealed a significant enhancement in neurotransmitter release probability in ACC synapses from mice with chronic pain. Trace fear memory, which requires sustained attention and the activity of the ACC, was impaired in CFA-injected mice. Using knock-out mice, we found that calmodulin-stimulated adenylyl cyclases, AC1 and/or AC8, were crucial in mediating the long-lasting enhanced presynaptic transmitter release in the ACC of mice with chronic pain. Our findings provide strong evidence that presynaptic alterations caused by peripheral inflammation contribute to memory impairments after injury.

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# Mutations in the K<sup>+</sup>/Cl<sup>-</sup> Cotransporter Gene *kazachoc* (*kcc*) Increase Seizure Susceptibility in *Drosophila*

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During a critical period in the developing mammalian brain, there is a major switch in the nature of GABAergic transmission from depolarizing and excitatory, the pattern of the neonatal brain, to hyperpolarizing and inhibitory, the pattern of the mature brain. This switch is believed to play a major role in determining neuronal connectivity via activity-dependent mechanisms. The GABAergic developmental switch may also be particularly vulnerable to dysfunction leading to seizure disorders. The developmental GABA switch is mediated primarily by KCC2, a neuronal K<sup>+</sup>/Cl<sup>-</sup> cotransporter that determines the intracellular concentration of Cl<sup>-</sup> and, hence, the reversal potential for GABA. Here, we report that *kazachoc* (*kcc*) mutations that reduce the level of the sole K<sup>+</sup>/Cl<sup>-</sup> cotransporter in the fruitfly *Drosophila melanogaster* render flies susceptible to epileptic-like seizures. *Drosophila kcc* protein is widely expressed in brain neuropil, and its level rises with developmental age. Young *kcc* mutant flies with low *kcc* levels display behavioral seizures and demonstrate a reduced threshold for seizures induced by electroconvulsive shock. The *kcc* mutation enhances a series of other *Drosophila* epilepsy mutations indicating functional interactions leading to seizure disorder. Both genetic and pharmacological experiments suggest that the increased

seizure susceptibility of *kcc* flies occurs via excitatory GABAergic signaling. The *kcc* mutants provide an excellent model system in which to investigate how modulation of GABAergic signaling influences neuronal excitability and epileptogenesis.

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## NEUROBIOLOGY OF DISEASE

# Glucocorticoids Increase Amyloid- $\beta$ and Tau Pathology in a Mouse Model of Alzheimer's Disease

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Various environmental and genetic factors influence the onset and progression of Alzheimer's disease (AD). Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, which controls circulating levels of glucocorticoid hormones, occurs early in AD, resulting in increased cortisol levels. Disturbances of the HPA axis have been associated with memory impairments and may contribute to the cognitive decline that occurs in AD, although it is unknown whether such effects involve modulation of the amyloid  $\beta$ -peptide (A $\beta$ ) and tau. Using *in vitro* and *in vivo* experiments, we report that stress-level glucocorticoid administration increases A $\beta$  formation by increasing steady-state levels of amyloid precursor protein (APP) and  $\beta$ -APP cleaving enzyme. Additionally, glucocorticoids augment tau accumulation, indicating that this hormone also accelerates the development of neurofibrillary tangles. These findings suggest that high levels of glucocorticoids, found in AD, are not merely a consequence of the disease process but rather play a central role in the development and progression of AD.

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# Accumulation of Amyloid Precursor Protein in the Mitochondrial Import Channels of Human Alzheimer's Disease Brain Is Associated with Mitochondrial Dysfunction

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Mitochondrial dysfunction is one of the major intracellular lesions of Alzheimer's disease (AD). However, the causative factors involved in the mitochondrial dysfunction in human AD are not well understood. Here we report that nonglycosylated full-length and C-terminal truncated amyloid precursor protein (APP) accumulates exclusively in the protein import channels of mitochondria of human AD brains but not in age-matched controls. Furthermore, in AD brains, mitochondrially associated APP formed stable ~480 kDa complexes with the translocase of the outer mitochondrial membrane 40 (TOM40) import channel and a super complex of ~620 kDa with both mitochondrial TOM40 and the translocase of the inner mitochondrial membrane 23 (TIM23) import channel TIM23 in an "N<sub>in mitochondria</sub>-C<sub>out cytoplasm</sub>" orientation. Accumulation of APP across mitochondrial import channels, which varied with the severity of AD, inhibited the entry of nuclear-encoded cytochrome *c* oxidase subunits IV and Vb proteins, which was associated with decreased cytochrome *c* oxidase activity and increased levels of H<sub>2</sub>O<sub>2</sub>. Regional distribution of mitochondrial APP showed higher levels in AD-vulnerable brain regions, such as the frontal cortex, hippocampus, and amygdala. Mitochondrial accumulation of APP was also observed in the cholinergic, dopaminergic, GABAergic, and glutamatergic neuronal types in the category III AD brains. The levels of translocationally arrested mitochondrial APP directly correlated with mitochondrial dysfunction. Moreover, apolipoprotein genotype analysis revealed that AD subjects with the E3/E4 alleles had the highest content of mitochondrial APP. Collectively, these results suggest that abnormal accumulation of APP across mitochondrial import channels, causing mitochondrial dysfunction, is a hallmark of human AD pathology.

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