

# This Week in The Journal

## ● Cellular/Molecular

### *Trafficking in GABA<sub>A</sub> Receptors in C. elegans*

Aaron M. Rowland, Janet E. Richmond, Jason G. Olsen, David H. Hall, and Bruce A. Bamber

(see pages 1711–1720)

As for most synaptic receptors, GABA<sub>A</sub> receptors cluster at sites of innervation by GABAergic nerve terminals. In *Caenorhabditis elegans*, postsynaptic muscles receive both acetylcholine and GABA inputs. This week, Rowland et al. controlled whether these excitatory and inhibitory motor neurons reached their targets. To do this, they manipulated expression of the netrin receptor that is necessary for motor neurons to project dorsally and innervate dorsal muscles. When GABAergic inputs were eliminated because of a lack of netrin receptors in GABAergic neurons, postsynaptic GABA<sub>A</sub> receptors became diffusely distributed in the muscle membrane. When GABAergic axons reached the muscle, clusters were normal even in the absence of acetylcholine inputs. However, when both inputs were absent, GABA receptors disappeared from the cell surface altogether and were concentrated in intracellular autophagosomes. Acetylcholine receptors were not targeted to these structures. The results suggest that presynaptic input can affect both receptor localization and stabilization in the membrane.

## ▲ Development/Plasticity/Repair

### *Reelin/Dab1 Signaling in Cortical Dendritogenesis*

Eric C. Olson, Seonhee Kim, and Christopher A. Walsh

(see pages 1767–1775)

Neuronal migration and positioning in the developing mammalian cortex depends on molecules of the Reelin signaling system, including the downstream adapter protein Dab1. This week, Olson et al. suppressed expression of Dab1 in migrating cortical neurons *in vivo* using

RNAi. They transfected migrating neurons with a green fluorescent protein expression vector and RNAi plasmids by *in utero* electroporation. Normally, cells migrated through the cell-dense cortical plate (CP) and entered the marginal zone (MZ), where the leading process typically became branched. At embryonic day 20 in lateral neocortex and cingulate cortex, Dab1-deficient neuronal cell bodies were displaced away from the CP/MZ border compared to controls. The leading processes and postnatal dendrite of Dab1-deficient cells failed to contact the MZ and were less branched compared to controls. Reelin/Dab1 signaling may transform the leading process into a dendrite that affects migration and cell positioning.

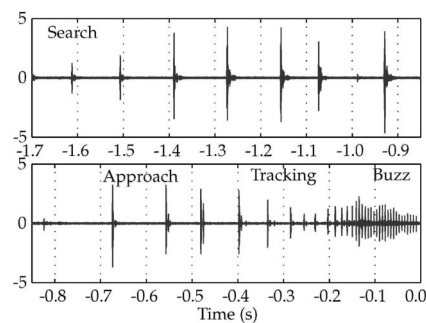
## ■ Behavioral/Systems/Cognitive

### *The Flight Plan of the Echolocating Bat*

Kaushik Ghose and Cynthia F. Moss

(see pages 1704–1710)

A bat has the advantage of navigating and hunting in the dark, but the complication that echoes of the emitted ultrasonic pulses must be decoded as the animal “gazes” and grazes. This involves continuous conversion of the binaural frequency and times of the echoes into spatial location, thus directing a flight plan. Ghose and Moss examined such sensorimotor



Trains of pulses produced by a bat catching an insect in the laboratory. Capture occurred at time 0. Note the change in pattern and frequency of the sonar pulses during search, pursuit and capture. The small signals following the large amplitude initial pulses represent echoes. See Ghose and Moss for details.

integration in the big brown bat, *Eptesicus fuscus*. Infrared cameras and microphones tracked a bat in a dark, empty room as it located and retrieved a suspended mealworm. The sonar pulses vary from 4 Hz in search mode to 150–200 Hz during prey detection. The authors describe a gain factor that links acoustic gaze (sonar direction) to locomotion (flight turn rate) that changed with behavioral state. Much like a visually guided animal, the bat can operate in scan mode without altering its movement direction, but in attack mode, the gain is high, and movement is determined by prey location.

## ◆ Neurobiology of Disease

### *A 5-HT<sub>1A</sub> Polymorphism and Transcriptional Regulation*

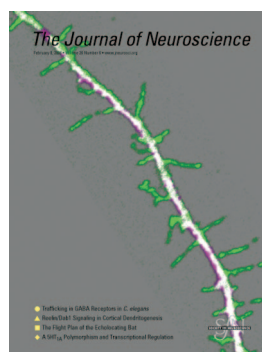
Margaret Czesak, Sylvie Lemonde, Erica A. Peterson, Anastasia Rogava, and Paul R. Albert

(see pages 1864–1871)

Serotonergic neurons of the raphe nuclei express 5-HT<sub>1A</sub> autoreceptors that inhibit 5-HT release. Neurons that express 5-HT<sub>1A</sub> receptors are implicated in mood and emotion, and clinical depression has been linked to reduced serotonin activity. For example, serotonin-selective reuptake inhibitors may in part act by desensitizing the autoreceptors on serotonergic neurons, thereby increasing 5-HT output. The expression of these receptors may genetically predispose patients to mood disorders, based on suggestive evidence from a functional polymorphism in the 5-HT<sub>1A</sub> promoter. This week, Czesak et al. examined the C(-1019)G polymorphism in the 5-HT<sub>1A</sub> promoter region in a serotonergic cell line RN46A. In serotonergic neurons, the Deaf-1 transcription repressed 5-HT<sub>1A</sub> expression at the C(-1019) allele, but it enhanced transcription in nonserotonergic cell lines that expressed 5-HT<sub>1A</sub>. In the G(-1019) allele, both of these actions were blocked. Hes5 repressed transcription in all cell types. These cell- and allele-specific actions of Deaf-1 may contribute to regulation of neuronal 5-HT<sub>1A</sub> receptors.

# The Journal of Neuroscience

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**Cover picture:** A cultured hippocampal neuron at 14 d in vitro was double labeled with anti-telencephalin (TLCN) and anti-microtubule-associated protein 2 (MAP-2) antibodies. The picture shows a pseudocolored image of a dendritic adhesion molecule TLCN (green) and MAP-2 (magenta) in a dendritic segment. TLCN protein is abundantly present in dendritic filopodia and shaft, whereas MAP-2 is confined to dendritic shaft. TLCN plays an important role in the formation and maintenance of dendritic filopodia. For details, see the article by Matsuno et al. in this issue (pages 1776–1786).

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## Preoptic Glutamate Facilitates Male Sexual Behavior

Juan M. Dominguez, Mario Gil, and Elaine M. Hull

Department of Psychology and Neuroscience Program, Florida State University, Tallahassee, Florida 32306-1270

The medial preoptic area (MPOA) is a critical regulatory site for the control of male sexual behavior. We first measured glutamate in 2 min microdialysate samples from the MPOA before, during, and after copulation by male rats. There was a slight [ $\sim 140\%$  of baseline (BL)] rise in extracellular glutamate when the female was presented, a significant increase ( $\sim 170\%$  of BL) during periods of mounting and intromitting, and a very large increase in samples collected during ejaculation ( $\sim 300\%$  of BL). A precipitous fall in levels occurred in the first postejaculatory sample; the magnitude of this fall was highly correlated with the length of the postejaculatory interval of quiescence. In experiment 2, we reverse-dialyzed a mixture of glutamate uptake inhibitors into the MPOA before and during mating; control animals received artificial CSF. The mixture increased extracellular glutamate ( $\sim 280\%$  of BL), increased the number of ejaculations in the 40 min test, decreased ejaculation latency, and decreased the postejaculatory latency to resume copulation. These data, together with other findings that glutamate in the MPOA can elicit genital reflexes in anesthetized rats and that glutamate receptor antagonists in the MPOA impair copulation, strongly suggest that MPOA glutamate is a major facilitator of copulation and that the postejaculatory fall in glutamate regulates the postejaculatory interval.

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## Articles

### CELLULAR/MOLECULAR

## Single $I_h$ Channels in Pyramidal Neuron Dendrites: Properties, Distribution, and Impact on Action Potential Output

Maarten H. P. Kole,<sup>1\*</sup> Stefan Hallermann,<sup>2\*</sup> and Greg J. Stuart<sup>1</sup>

<sup>1</sup>Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra 0200, Australian Capital Territory, Australia, and <sup>2</sup>Physiologisches Institut I, Universität Freiburg, D-79104 Freiburg, Germany

The hyperpolarization-activated cation current ( $I_h$ ) plays an important role in regulating neuronal excitability, yet its native single-channel properties in the brain are essentially unknown. Here we use variance-mean analysis to study the properties of single  $I_h$  channels in the apical dendrites of cortical layer 5 pyramidal neurons *in vitro*. In these neurons, we find that  $I_h$  channels have an average unitary conductance of  $680 \pm 30$  fS ( $n = 18$ ). Spectral analysis of simulated and native  $I_h$  channels showed that there is little or no channel flicker below 5 kHz. In contrast to the uniformly distributed single-channel conductance,  $I_h$  channel number increases exponentially with distance, reaching densities as high as  $\sim 550$  channels/ $\mu\text{m}^2$  at distal dendritic sites. These high channel densities generate significant membrane voltage noise. By incorporating a stochastic model of  $I_h$  single-channel gating into a morphologically realistic model of a layer 5 neuron, we show that this channel noise is higher in distal dendritic compartments and increased threefold with a 10-fold increased single-channel conductance (6.8 pS) but constant  $I_h$  current density. In addition, we demonstrate that voltage fluctuations attributable to stochastic  $I_h$  channel gating impact on action potential output, with greater spike-timing precision in models with the experimentally determined single-channel conductance. These data suggest that, in the face of high current densities, the small single-channel conductance of  $I_h$  is critical for maintaining the fidelity of action potential output.

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## Presynaptic Terminals Independently Regulate Synaptic Clustering and Autophagy of GABA<sub>A</sub> Receptors in *Caenorhabditis elegans*

Aaron M. Rowland,<sup>1</sup> Janet E. Richmond,<sup>2</sup> Jason G. Olsen,<sup>1</sup> David H. Hall,<sup>3</sup> and Bruce A. Bamber<sup>1</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112, <sup>2</sup>Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607, and <sup>3</sup>Center for *Caenorhabditis elegans* Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461

Synaptic clustering of GABA<sub>A</sub> receptors is important for the function of inhibitory synapses, influencing synapse strength and, consequently, the balance of excitation and inhibition in the brain. Presynaptic terminals are known to induce GABA<sub>A</sub> receptor clustering during synaptogenesis, but the mechanisms of cluster formation and maintenance are not known. To study how presynaptic neurons direct the formation of GABA<sub>A</sub> receptor clusters, we have investigated GABA<sub>A</sub> receptor localization in postsynaptic cells that fail to receive presynaptic contacts in *Caenorhabditis elegans*. Postsynaptic muscles in *C. elegans* receive acetylcholine and GABA motor innervation, and GABA<sub>A</sub> receptors cluster opposite GABA terminals. Selective loss of GABA inputs caused GABA<sub>A</sub> receptors to be diffusely distributed at or near the muscle cell surface, confirming that GABA presynaptic terminals induce GABA<sub>A</sub> receptor clustering. In contrast, selective loss of acetylcholine innervation had no effect on GABA<sub>A</sub> receptor localization. However, loss of both GABA and acetylcholine inputs together caused GABA<sub>A</sub> receptors to traffic to intracellular autophagosomes. Autophagosomes normally transport bulk cytoplasm to the lysosome for degradation. However, we show that GABA<sub>A</sub> receptors traffic to autophagosomes after endocytic removal from the cell surface and that acetylcholine receptors in the same cells do not traffic to autophagosomes. Thus, autophagy can degrade cell-surface receptors and can do so selectively. Our results

show that presynaptic terminals induce GABA<sub>A</sub> receptor clustering by independently controlling synaptic localization and surface stability of GABA<sub>A</sub> receptors. They also demonstrate a novel function for autophagy in GABA<sub>A</sub> receptor degradative trafficking.

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## A Novel Role for Sema3A in Neuroprotection from Injury Mediated by Activated Microglia

Henry H. Majed,<sup>1\*</sup> Siddharthan Chandran,<sup>1\*</sup> Simone P. Niclou,<sup>2</sup> Richard S. Nicholas,<sup>1</sup> Alastair Wilkins,<sup>1</sup> Mark G. Wing,<sup>1</sup> Kate E. Rhodes,<sup>1</sup> Maria Grazia Spillantini,<sup>1</sup> and Alastair Compston<sup>1</sup>

<sup>1</sup> Department of Clinical Neurosciences and Centre for Brain Repair, University of Cambridge, Forvie Site, Cambridge CB2 2PY, United Kingdom, and

<sup>2</sup> Graduate School of Neurosciences Amsterdam, Netherlands Institute for Brain Research, 1105 AZ Amsterdam, The Netherlands

Microglia exist under physiological conditions in a resting state but become activated after neuronal injury. Recent studies have highlighted the reciprocal role of neurons in controlling both the number and activity of microglia. In this study, microglia derived from newborn rat cortices were cultured and activated by interferon- $\gamma$  (IFN $\gamma$ ) treatment, then exposed to recombinant Sema3A or conditioned medium derived from stressed embryonic cortical neurons. We found that activation of microglia by IFN $\gamma$  induced differential upregulation of the semaphorin receptors Plexin-A1 and Neuropilin-1. This result was confirmed by Northern blotting, reverse transcription-PCR, and Western blotting. Furthermore, recombinant Sema3A induced apoptosis of microglia when added to the *in vitro* culture, and a similar result was obtained on activated microglia when Sema3A was produced by stressed neurons. Using an *in vivo* model of microglia activation by striatal injection of lipopolysaccharide demonstrated a corresponding upregulation of Plexin-A1 and Neuropilin-1 in activated microglia and enhanced production of Sema3A by stressed adult neurons. These results suggest a novel semaphorin-mediated mechanism of neuroprotection whereby stressed neurons can protect themselves from further damage by activated microglia.

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## Synaptic Transmission Mediated by Internal Calcium Stores in Rod Photoreceptors

Anuradha Suryanarayanan and Malcolm M. Slaughter

Department of Physiology and Biophysics, University at Buffalo, Buffalo, New York 14214

Retinal rod photoreceptors are depolarized in darkness to approximately  $-40$  mV, a state in which they maintain sustained glutamate release despite low levels of calcium channel activation. Blocking voltage-gated calcium channels or ryanodine receptors (RyRs) at the rod presynaptic terminal suppressed synaptic communication to bipolar cells. Spontaneous synaptic events were also inhibited when either of these pathways was blocked. This indicates that both calcium influx and calcium release from internal stores are required for the normal release of transmitter of the rod. RyR-independent release can be evoked by depolarization of a rod to a suprathreshold potential ( $-20$  mV) that activates a large fraction of voltage-gated channels. However, this calcium channel-mediated release depletes rapidly if RyRs are blocked, indicating that RyRs support prolonged glutamate release. Thus, the rod synapse couples a small transmembrane calcium influx with a RyR-dependent amplification mechanism to support continuous vesicle release.

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## Presynaptic I<sub>1</sub>-Imidazoline Receptors Reduce GABAergic Synaptic Transmission in Striatal Medium Spiny Neurons

Mitsuo Tanabe, Yurika Kino, Motoko Honda, and Hideki Ono

Laboratory of CNS Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467-8603, Japan

Imidazoline receptors are expressed widely in the CNS. In the present study, whole-cell patch-clamp recordings were made from medium spiny neurons in dorsal striatum slices from the rat brain, and the roles of I<sub>1</sub>-imidazoline receptors in the modulation of synaptic transmission were studied. Moxonidine, an I<sub>1</sub>-imidazoline receptor agonist, decreased the GABA<sub>A</sub> receptor-mediated IPSCs in a concentration-dependent manner. However, glutamate-mediated EPSCs were hardly affected. The depression of IPSCs by moxonidine was antagonized by either idazoxan or efaroxan, which are both imidazoline receptor antagonists containing an imidazoline moiety. In contrast, yohimbine and SKF86466 (6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine), which are  $\alpha$ 2-adrenergic receptor antagonists with no affinity for imidazoline receptors, did not affect the moxonidine-induced inhibition of IPSCs. Moxonidine increased the paired-pulse ratio and reduced the frequency of miniature IPSCs without affecting their amplitude, indicating that this agent inhibits IPSCs via presynaptic mechanisms. Moreover, the sulfhydryl alkylating agent *N*-ethylmaleimide (NEM) significantly reduced the moxonidine-induced inhibition of IPSCs. Thus, the activation of presynaptic I<sub>1</sub>-imidazoline receptors decreases GABA-mediated inhibition of medium spiny neurons in the striatum, in which NEM-sensitive proteins such as G<sub>βγ</sub>-type G-proteins play an essential role. The adenylate cyclase activator forskolin partly opposed IPSC inhibition elicited by subsequently applied moxonidine. Furthermore, the protein kinase C (PKC) activator phorbol 12,13-dibutyrate attenuated and the PKC inhibitor chelerythrine potentiated the moxonidine-induced inhibition of IPSCs. These results suggest that IPSC inhibition via presynaptic I<sub>1</sub>-imidazoline receptors involves intracellular adenylate cyclase activity and is influenced by static PKC activity in the striatum.

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# Integrins Control Dendritic Spine Plasticity in Hippocampal Neurons through NMDA Receptor and Ca<sup>2+</sup>/Calmodulin-Dependent Protein Kinase II-Mediated Actin Reorganization

Yang Shi and Iryna M. Ethell

Division of Biomedical Sciences, University of California Riverside, Riverside, California 92521-0121

The formation of dendritic spines during development and their structural plasticity in the adult brain are critical aspects of synaptogenesis and synaptic plasticity. Many different factors and proteins have been shown to control dendritic spine development and remodeling (Ethell and Pasquale, 2005). The extracellular matrix (ECM) components and their cell surface receptors, integrins, have been found in the vicinity of synapses and shown to regulate synaptic efficacy and play an important role in long-term potentiation (Bahr et al., 1997; Chavis and Westbrook, 2001; Chan et al., 2003; Lin et al., 2003; Bernard-Trifilo et al., 2005). Although molecular mechanisms by which integrins affect synaptic efficacy have begun to emerge, their role in structural plasticity is poorly understood. Here, we show that integrins are involved in spine remodeling in cultured hippocampal neurons. The treatment of 14 d *in vitro* hippocampal neurons with arginine-glycine-aspartate (RGD)-containing peptide, an established integrin ligand, induced elongation of existing dendritic spines and promoted formation of new filopodia. These effects were also accompanied by integrin-dependent actin reorganization and synapse remodeling, which were partially inhibited by function-blocking antibodies against  $\beta 1$  and  $\beta 3$  integrins. This actin reorganization was blocked with the NMDA receptor (NMDAR) antagonist MK801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate]. The Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN93 (*N*-[2-[*N*-(4-chlorocinnamyl)-*N*-methylaminomethyl]phenyl]-*N*-(2-hydroxyethyl)-4-methoxybenzenesulfonamide) also suppressed RGD-induced actin reorganization and synapse remodeling. Our findings show that integrins control ECM-mediated spine remodeling in hippocampal neurons through NMDAR/CaMKII-dependent actin reorganization.

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# Loss of Leukemia Inhibitory Factor Receptor $\beta$ or Cardiotrophin-1 Causes Similar Deficits in Preganglionic Sympathetic Neurons and Adrenal Medulla

Stephan Oberle,<sup>1</sup> Andreas Schober,<sup>1</sup> Verena Meyer,<sup>1</sup> Bettina Holtmann,<sup>2</sup> Christopher Henderson,<sup>3</sup> Michael Sendtner,<sup>2</sup> and Klaus Unsicker<sup>1</sup>

<sup>1</sup>Neuroanatomy and Interdisciplinary Center for Neurosciences, University of Heidelberg, D-69120 Heidelberg, Germany, <sup>2</sup>Department of Clinical Neurobiology, University of Würzburg, D-97080 Würzburg, Germany, and <sup>3</sup>Hammer Health Sciences, Columbia University, New York, New York 10032

Leukemia inhibitory factor (LIF) receptor  $\beta$  (LIFR $\beta$ ) is a receptor for a variety of neurotrophic cytokines, including LIF, ciliary neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1). These cytokines play an essential role for the survival and maintenance of developing and postnatal somatic motoneurons. CNTF may also serve the maintenance of autonomic, preganglionic sympathetic neurons (PSNs) in the spinal cord, as suggested by its capacity to prevent their death after destruction of one of their major targets, the adrenal medulla. Although somatic motoneurons and PSNs share a common embryonic origin, they are distinct in several respects, including responses to lesions. We have studied PSNs in mice with targeted deletions of the LIFR $\beta$  or CT-1 genes, respectively. We show that LIF, CNTF, and CT-1 are synthesized in embryonic adrenal gland and spinal cord and that PSNs express LIFR $\beta$ . In embryonic day 18.5 LIFR $\beta$  (–/–) and CT-1 (–/–) mice, PSNs were reduced by ~20%. PSNs projecting to the adrenal medulla were more severely affected (–55%). Although LIFR $\beta$  (–/–) mice revealed normal numbers of adrenal chromaffin cells and axons terminating on chromaffin cells, levels of adrenaline and numbers of adrenaline-synthesizing cells were significantly reduced. We conclude that activation of LIFR $\beta$  is required for normal development of PSNs and one of their prominent targets, the adrenal medulla. Thus, both somatic motoneurons and PSNs in the spinal cord depend on LIFR $\beta$  signaling for their development and maintenance, although PSNs seem to be overall less affected than somatic motoneurons by LIFR $\beta$  deprivation.

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# Disinhibition Opens the Gate to Pathological Pain Signaling in Superficial Neurokinin 1 Receptor-Expressing Neurons in Rat Spinal Cord

Carole Torsney<sup>1</sup> and Amy B. MacDermott<sup>1,2</sup>

<sup>1</sup>Department of Physiology and Cellular Biophysics and <sup>2</sup>Center for Neurobiology and Behavior, Columbia University, New York, New York 10032

Blockade of local spinal cord inhibition mimics the behavioral hypersensitivity that manifests in chronic pain states. This suggests that there is a pathway capable of mediating allodynia/hyperalgesia that exists but is normally under strong inhibitory control. Lamina I and III neurokinin 1 (NK1) receptor expressing (NK1R+) dorsal horn neurons, many of which are projection neurons, are required for the development of this hypersensitivity and are therefore likely to be a component of this proposed pathway. To investigate, whole-cell patch-clamp recordings were made from lamina I and III NK1R+ neurons in the spinal cord slice preparation with attached dorsal root. Excitatory postsynaptic currents were recorded in response to electrical stimulation of the dorsal root. Lamina I NK1R+ neurons were shown to receive high-threshold (A $\delta$ /C fiber) monosynaptic input, whereas lamina III NK1R+ neurons received low-threshold (A $\beta$  fiber) monosynaptic input. In contrast, lamina I neurons lacking NK1 receptor (NK1R–) received polysynaptic A fiber input. Blockade of local GABAergic and glycinergic inhibition with bicuculline (10  $\mu$ M) and strychnine (300 nM), respectively, revealed significant A fiber input to lamina I NK1R+ neurons that was predominantly A $\beta$  fiber mediated. This novel A fiber input was polysynaptic in nature and required NMDA receptor activity to be functional. In lamina I NK1R– and lamina III NK1R+ neurons, disinhibition enhanced control-evoked responses, and this was also NMDA receptor dependent. These disinhibition-induced changes, in particular the novel polysynaptic low-threshold input onto lamina I NK1R+ neurons, may be an underlying component of the hypersensitivity present in chronic pain states.

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# Site of Action Potential Initiation in Layer 5 Pyramidal Neurons

Lucy M. Palmer and Greg J. Stuart

Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra 0200, Australia

Fundamental to an understanding of how neurons integrate synaptic input is the knowledge of where within a neuron this information is converted into an output signal, the action potential. Although it has been known for some time that action potential initiation occurs within the axon of neurons, the precise location has remained elusive. Here, we provide direct evidence using voltage-sensitive dyes that the site of action potential initiation in cortical layer 5 pyramidal neurons is  $\sim 35 \mu\text{m}$  from the axon hillock. This was the case during action potential generation under a variety of conditions, after axonal inhibition, and at different stages of development. Once initiated action potentials propagated down the axon in a saltatory manner. Experiments using local application of low-sodium solution and TTX, as well as an investigation of the influence of axonal length on action potential properties, provided evidence that the initial  $40 \mu\text{m}$  of the axon is essential for action potential generation. To morphologically identify the relationship between the site of action potential initiation and axonal myelination, we labeled oligodendrocytes supplying processes to the proximal region of the axon. These experiments indicated that the axon initial segment was  $\sim 40 \mu\text{m}$  in length, and the first node of Ranvier was  $\sim 90 \mu\text{m}$  from the axon hillock. Experiments targeting the first node of Ranvier suggested it was not involved in action potential initiation. In conclusion, these results indicate that, in layer 5 pyramidal neurons, action potentials are generated in the distal region of the axon initial segment.

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# Astrocytes Induce Hemeoxygenase-1 Expression in Microglia: A Feasible Mechanism for Preventing Excessive Brain Inflammation

Kyoung-Jin Min,<sup>1,2,3</sup> Myung-soon Yang,<sup>2,3</sup> Seung-Up Kim,<sup>1,3,5</sup> Ilo Jou,<sup>2,4</sup> and Eun-hye Joe<sup>1,2,3</sup>

<sup>1</sup>Neuroscience Graduate Program, <sup>2</sup>Department of Pharmacology, <sup>3</sup>Brain Disease Research Center, and <sup>4</sup>Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon 442-721, Korea, and <sup>5</sup>Department of Neurology, University of British Columbia, Vancouver, British Columbia, Canada V5Z 4E3

Microglia are the major inflammatory cells in the brain, in which microglial inflammatory responses are modulated by interactions with other brain cells. Here, we show that astrocytes, the most abundant cells in the brain, can secrete one or more factors capable of modulating microglial activation by regulating the microglial levels of reactive oxygen species (ROS). Treatment of microglia with astrocyte culture-conditioned media (ACM) increased the expression level and activity of hemeoxygenase-1 (HO-1). ACM also induced nuclear translocation of the nuclear factor E2-related factor 2 transcription factor, increased the binding activity of the antioxidant response element (ARE), and enhanced HO-1 promoter activity in an ARE-dependent manner. Furthermore, treatment with ACM suppressed interferon- $\gamma$  (IFN- $\gamma$ )-induced ROS production, leading to reduced inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) release. In agreement with these results, mimickers of HO-1 products, such as bilirubin, ferrous iron, and a carbon monoxide-releasing molecule, reduced IFN- $\gamma$ -induced iNOS expression and/or NO release. Finally, we found that the active component(s) in ACM was heat labile and smaller than 3 kDa. Together, these results suggest that astrocytes could cooperate with microglia to prevent excessive inflammatory responses in the brain by regulating microglial expression of HO-1 and production of ROS.

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## DEVELOPMENT/PLASTICITY/REPAIR

# Distinct Roles for Ras-Guanine Nucleotide-Releasing Factor 1 (Ras-GRF1) and Ras-GRF2 in the Induction of Long-Term Potentiation and Long-Term Depression

Shaomin Li,<sup>1\*</sup> Xuejun Tian,<sup>1\*</sup> Dean M. Hartley,<sup>2</sup> and Larry A. Feig<sup>1</sup>

<sup>1</sup>Departments of Biochemistry and Neuroscience, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, Massachusetts 02111, and <sup>2</sup>Department of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

NMDA-type glutamate receptors (NMDARs) contribute to many forms of long-term potentiation (LTP) and long-term depression (LTD). NMDARs are heteromers containing calcium-permeating neuronal receptor 1 (NR1) subunits and a variety of NR2 subunits. Evidence suggests that, in the CA1 region of the hippocampus, NR2A-containing NMDARs promote LTP whereas NR2B-containing receptors promote LTD. However, the calcium sensors that distinguish between these signals to promote the appropriate form of synaptic plasticity are not known. Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) and Ras-GRF2 are highly similar calcium-stimulated exchange factors that activate Ras and Rac GTPases. Here, using a set of Ras-GRF knock-out mice, we show that Ras-GRF2 contributes predominantly to the induction of NMDAR-dependent LTP, whereas Ras-GRF1 contributes predominantly to the induction of NMDAR-dependent LTD in the CA1 region of the hippocampus of postpubescent mice (postnatal days 25–36). In contrast, neither Ras-GRF protein influences synaptic plasticity in prepubescent mice (postnatal days 14–18). Ras-GRF2 mediates signaling from (R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-[(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid-sensitive (NVP-AAM077-sensitive) (NR2A-containing) NMDARs to the Ras effector extracellular signal-related protein kinase 1/2 (Erk1/2) mitogen-activated protein (MAP) kinase, a promoter of NMDAR-induced LTP at this site. In contrast, Ras-GRF1 mediates signaling from ifenprodil-sensitive (NR2B-containing) NMDARs to the Rac effector p38 MAP kinase, a promoter of LTD. These findings show that, despite their similar functional domain organization, Ras-GRF1 and Ras-GRF2 mediate opposing forms of synaptic plasticity by coupling different classes of NMDARs to distinct MAP kinase pathways. Moreover, the postnatal appearance of Ras-GRF-dependent LTP and LTD coincides with the emergence of hippocampal-dependent behavior, implying that Ras-GRF proteins contribute to forms of synaptic plasticity that are required specifically for mature hippocampal function.

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# Impaired Neuronal Positioning and Dendritogenesis in the Neocortex after Cell-Autonomous Dab1 Suppression

**Eric C. Olson, Seonhee Kim, and Christopher A. Walsh**

Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center, Department of Neurology and Program in Neuroscience, Harvard Medical School, Boston, Massachusetts 02115

Reelin and Disabled 1 (Dab1) are essential for positioning migrating neurons in the developing neocortex. Cell-autonomous RNA interference-mediated suppression of Dab1 in migrating neurons destined for layer 2/3 shifted the median position of these cells to deeper positions within the cortex. At the time of migration arrest [embryonic day 20 (E20) to E21], Dab1-suppressed cells were underrepresented in the upper  $\sim 40\ \mu\text{m}$  of the cortex compared with controls, suggesting that Dab1 is essential for somal translocation through the cell-dense cortical plate. Closer examination of the morphology of Dab1-suppressed neurons at E20 revealed simplified leading processes that are less likely to contact the marginal zone (MZ), in which high levels of Reelin are expressed. Examination of Dab1-suppressed cells 3 d later (postnatal day 2) revealed simplified dendrites that are also less likely to contact the MZ. These data reveal a cell-autonomous role of Dab1 in dendritogenesis in the neocortex and suggest that remodeling of the leading process of a migrating neuron into a nascent dendrite by Reelin/Dab1 signaling plays an important role in cell positioning.

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# Telencephalin Slows Spine Maturation

**Hitomi Matsuno,<sup>1,2</sup> Shigeo Okabe,<sup>4</sup> Masayoshi Mishina,<sup>5</sup> Toshio Yanagida,<sup>2,3</sup> Kensaku Mori,<sup>6</sup> and Yoshihiro Yoshihara<sup>1,7</sup>**

<sup>1</sup>Laboratory for Neurobiology of Synapse, RIKEN Brain Science Institute, Saitama 351-0198, Japan, <sup>2</sup>Department of Systems and Human Science, Graduate School of Engineering Science, and <sup>3</sup>Nanobiology Laboratories, Graduate School of Frontier Biosciences, Osaka University, Osaka 560-8531, Japan,

<sup>4</sup>Department of Anatomy and Cell Biology, School of Medicine, Tokyo Medical and Dental University, Tokyo 113-8519, Japan, Departments of <sup>5</sup>Molecular Neurobiology and Pharmacology and <sup>6</sup>Physiology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan, and <sup>7</sup>Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Osaka 560-0082, Japan

Dendritic filopodia are highly dynamic structures, and morphological maturation from dendritic filopodia to spines is intimately associated with the stabilization and strengthening of synapses during development. Here, we report that telencephalin (TLCN), a cell adhesion molecule belonging to the Ig superfamily, is a negative regulator of spine maturation. Using cultured hippocampal neurons, we examined detailed localization and functions of TLCN in spine development and synaptogenesis. At early stages of synaptogenesis, TLCN immunoreactivity gradually increased and was present in dendritic shafts and filopodia. At later stages, TLCN tended to be excluded from mature spine synapses in which PSD-95 (postsynaptic density-95) clusters were apposed to presynaptic synaptophysin clusters. To elucidate the function of TLCN in spine maturation, we analyzed the dendrite morphology of TLCN-overexpressing and TLCN-deficient neurons. Overexpression of TLCN caused a dramatic increase in the density of dendritic filopodia and a concomitant decrease in the density of spines. Conversely, TLCN-deficient mice showed a decreased density of filopodia and an acceleration of spine maturation *in vitro* as well as *in vivo*. These results demonstrate that TLCN normally slows spine maturation by promoting the filopodia formation and negatively regulating the filopodia-to-spine transition. In addition, we found that spine heads of mature neurons were wider in TLCN-deficient mice compared with wild-type mice. Thus, the preservation of immature synapses by TLCN may be an essential step for refinement of functional neural circuits in the telencephalon, that take charge of higher brain functions such as learning, memory, and emotion.

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# Molecular Reconstruction of Nodes of Ranvier after Remyelination by Transplanted Olfactory Ensheathing Cells in the Demyelinated Spinal Cord

**Masanori Sasaki, Joel A. Black, Karen L. Lankford, Hajime A. Tokuno, Stephen G. Waxman, and Jeffery D. Kocsis**

Department of Neurology and Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, Connecticut 06510, and Rehabilitation Research Center, Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut 06516

Myelin-forming glial cells transplanted into the demyelinated spinal cord can form compact myelin and improve conduction properties. However, little is known of the expression and organization of voltage-gated ion channels in the remyelinated central axons or whether the exogenous cells provide appropriate signaling for the maturation of nodes of Ranvier. Here, we transplanted olfactory ensheathing cells from green fluorescent protein (GFP)-expressing donor rats [GFP-olfactory ensheathing cells (OECs)] into a region of spinal cord demyelination and found extensive remyelination, which included the development of mature nodal, paranodal, and juxtaparanodal domains, as assessed by ultrastructural, immunocytochemical, and electrophysiological analyses. In remyelinated axons,  $\text{Na}_v1.6$  was clustered at nodes, whereas  $\text{K}_v1.2$  was aggregated in juxtaparanodal regions, recapitulating the distribution of these channels within mature nodes of uninjured axons. Moreover, the recruitment of  $\text{Na}_v$  and  $\text{K}_v$  channels to specific membrane domains at remyelinated nodes persisted for at least 8 weeks after GFP-OEC transplantation. *In vivo* electrophysiological recordings demonstrated enhanced conduction along the GFP-OEC-remyelinated axons. These findings indicate that, in addition to forming myelin, engrafted GFP-OECs provide an environment that supports the development and maturation of nodes of Ranvier and the restoration of impulse conduction in central demyelinated axons.

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## Steering by Hearing: A Bat's Acoustic Gaze Is Linked to Its Flight Motor Output by a Delayed, Adaptive Linear Law

Kaushik Ghose<sup>1,2</sup> and Cynthia F. Moss<sup>1,2,3</sup>

<sup>1</sup>Neuroscience and Cognitive Science Program, <sup>2</sup>Department of Psychology, and <sup>3</sup>Institute for Systems Research, University of Maryland, College Park, Maryland 20742

Adaptive behaviors require sensorimotor computations that convert information represented initially in sensory coordinates to commands for action in motor coordinates. Fundamental to these computations is the relationship between the region of the environment sensed by the animal (gaze) and the animal's locomotor plan. Studies of visually guided animals have revealed an anticipatory relationship between gaze direction and the locomotor plan during target-directed locomotion. Here, we study an acoustically guided animal, an echolocating bat, and relate acoustic gaze (direction of the sonar beam) to flight planning as the bat searches for and intercepts insect prey. We show differences in the relationship between gaze and locomotion as the bat progresses through different phases of insect pursuit. We define acoustic gaze angle,  $\theta_{gaze}$ , to be the angle between the sonar beam axis and the bat's flight path. We show that there is a strong linear linkage between acoustic gaze angle at time  $t$  [ $\theta_{gaze}(t)$ ] and flight turn rate at time  $t + \tau$  into the future [ $\dot{\theta}_{flight}(t + \tau)$ ], which can be expressed by the formula  $\dot{\theta}_{flight}(t + \tau) = k\theta_{gaze}(t)$ . The gain,  $k$ , of this linkage depends on the bat's behavioral state, which is indexed by its sonar pulse rate. For high pulse rates, associated with insect attacking behavior,  $k$  is twice as high compared with low pulse rates, associated with searching behavior. We suggest that this adjustable linkage between acoustic gaze and motor output in a flying echolocating bat simplifies the transformation of auditory information to flight motor commands.

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## Block of Inferior Olive Gap Junctional Coupling Decreases Purkinje Cell Complex Spike Synchrony and Rhythmicity

Timothy A. Blenkinsop and Eric J. Lang

Department of Physiology and Neuroscience, School of Medicine, New York University, New York, New York 10016

Inferior olivary (IO) neurons are electrotonically coupled by gap junctions. This coupling is thought to underlie synchronous complex spike (CS) activity generated by the olivocerebellar system in Purkinje cells, and also has been hypothesized to be necessary for IO neurons to generate spontaneous oscillatory activity. These characteristics of olivocerebellar activity have been proposed to be central to the role of this system in motor coordination. However, the relationship of gap junction coupling between IO neurons to synchronous and rhythmic CS activity has never been directly tested. Thus, to address this issue, multiple electrode recordings were obtained from crus 2a Purkinje cells, and carbenoxolone, a gap junction blocker, was injected into the IO. Carbenoxolone reduced CS synchrony by 50% overall, but in some experiments, >80% reductions were achieved. Carbenoxolone also reduced the average firing rate by 50%, suggesting that electrical coupling is a significant source of excitation for IO neurons. Moreover, carbenoxolone caused a reduction in the ~10 Hz rhythmicity of CS activity, and this reduction was correlated with the extent to which the injection reduced CS synchrony. Lastly, carbenoxolone was found to reverse or prevent changes in synchrony that are normally induced by injection of GABA<sub>A</sub> and glutamate receptor antagonists into the IO, suggesting that the effects of these drugs on CS synchrony patterns require electrical coupling of IO neurons. In sum, our results provide direct evidence that electrical coupling of IO neurons underlies synchronous CS activity, and suggest important roles for this coupling in shaping other aspects of IO spiking patterns.

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## Direct GABAergic and Glycinergic Inhibition of the Substantia Gelatinosa from the Rostral Ventromedial Medulla Revealed by *In Vivo* Patch-Clamp Analysis in Rats

Go Kato,<sup>1,2</sup> Toshiharu Yasaka,<sup>1</sup> Toshihiko Katafuchi,<sup>1</sup> Hidemasa Furue,<sup>1</sup> Masaharu Mizuno,<sup>3</sup> Yukihide Iwamoto,<sup>2</sup> and Megumu Yoshimura<sup>1</sup>

Departments of <sup>1</sup>Integrative Physiology and <sup>2</sup>Orthopedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan, and <sup>3</sup>Division of Higher Brain Functions, Department of Brain Science and Engineering, Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan

Stimulation of the rostral ventromedial medulla (RVM) is believed to exert analgesic effects through the activation of the serotonergic system descending to the spinal dorsal horn; however, how nociceptive transmission is modulated by the descending system has not been fully clarified. To investigate the inhibitory mechanisms affected by the RVM, an *in vivo* patch-clamp technique was used to record IPSCs from the substantia gelatinosa (SG) of the spinal cord evoked by chemical (glutamate injection) and electrical stimulation (ES) of the RVM in adult rats. In the voltage-clamp mode, the RVM glutamate injection and RVM-ES produced an increase in both the frequency and amplitude of IPSCs in SG neurons that was not blocked by glutamate receptor antagonists. Serotonin receptor antagonists were unexpectedly without effect, but a GABA<sub>A</sub> receptor antagonist, bicuculline, or a glycine receptor antagonist, strychnine, completely suppressed the RVM stimulation-induced increase in IPSCs. The RVM-ES-evoked IPSCs showed fixed latency and no failure at 20 Hz stimuli with a conduction velocity of >3 m/s (3.1–20.7 m/s), suggesting descending monosynaptic GABAergic and/or glycinergic inputs from the RVM to the SG through myelinated fibers. In the current-clamp mode, action potentials elicited by noxious mechanical stimuli applied to the receptive field of the ipsilateral hindlimb were suppressed by the RVM-ES in more than half of the neurons tested (63%; 10 of 16). These findings suggest that the RVM-mediated antinociceptive effects on noxious inputs to the SG may be exerted preferentially by the direct GABAergic and glycinergic pathways to the SG.

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# Small-Conductance Ca<sup>2+</sup>-Activated K<sup>+</sup> Channel Type 2 (SK2) Modulates Hippocampal Learning, Memory, and Synaptic Plasticity

Rebecca S. Hammond,<sup>1</sup> Chris T. Bond,<sup>3</sup> Timothy Strassmaier,<sup>3</sup> Thu Jennifer Ngo-Anh,<sup>3</sup> John P. Adelman,<sup>3</sup> James Maylie,<sup>2</sup> and Robert W. Stackman<sup>1</sup>

Departments of <sup>1</sup>Behavioral Neuroscience and <sup>2</sup>Obstetrics and Gynecology and <sup>3</sup>Vollum Institute, Oregon Health and Science University, Portland, Oregon 97239-3089

Apamin-sensitive, small-conductance, Ca<sup>2+</sup>-activated K<sup>+</sup> channels (SK channels) modulate neuronal excitability in CA1 neurons. Blocking all SK channel subtypes with apamin facilitates the induction of hippocampal synaptic plasticity and enhances hippocampal learning. In CA1 dendrites, SK channels are activated by Ca<sup>2+</sup> through NMDA receptors and restrict glutamate-mediated EPSPs. Studies of SK channel knock-out mice reveal that of the three apamin-sensitive SK channel subunits (SK1–SK3), only SK2 subunits are necessary for the apamin-sensitive currents in CA1 hippocampal neurons. To determine the specific influence of SK2 channels on hippocampal synaptic plasticity, learning, and memory, we used gene targeting through homologous recombination in embryonic stem cells to generate transgenic mice that overexpress SK2 subunits by 10-fold (SK2+/T). In these mice, the apamin-sensitive current in CA1 neurons was increased by approximately fourfold, relative to wild-type (WT) littermates. In addition, the amplitude of synaptically evoked EPSPs recorded from SK2+/T CA1 neurons increased twice as much in response to SK channel blockade relative to EPSPs recorded from WT CA1 neurons. Consistent with this, SK2 overexpression reduced long-term potentiation after high-frequency stimulation compared with WT littermates and severely impaired learning in both hippocampus- and amygdala-dependent tasks. We conclude that SK2 channels regulate hippocampal synaptic plasticity and play a critical role in modulating mechanisms of learning and memory.

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# Increased Vulnerability to Nicotine Self-Administration and Relapse in Alcohol-Naive Offspring of Rats Selectively Bred for High Alcohol Intake

A. D. Lê,<sup>1,2,3</sup> Z. Li,<sup>1</sup> D. Funk,<sup>1</sup> M. Shram,<sup>1,2</sup> T. K. Li,<sup>4</sup> and Y. Shaham<sup>5</sup>

<sup>1</sup>Department of Neuroscience, Center for Addiction and Mental Health, Toronto, Ontario, Canada M5S 2S1, Departments of <sup>2</sup>Pharmacology and <sup>3</sup>Psychiatry, University of Toronto, Toronto, Ontario, Canada M5S 1A8, <sup>4</sup>Office of the Director, National Institute on Alcohol Abuse and Alcoholism–National Institutes of Health (NIH)–Department of Health and Human Services, Bethesda, Maryland 20892, and <sup>5</sup>Behavioral Neuroscience Branch, Intramural Research Program–National Institute on Drug Abuse–NIH–Department of Health and Human Services, Baltimore, Maryland 21224

The prevalence of smoking in human alcoholics is substantially higher than in the general population, and results from twin studies suggest that a shared genetic vulnerability underlies alcohol and nicotine addiction. Here, we directly tested this hypothesis by examining nicotine-taking behavior in alcohol-naive offspring of alcohol-preferring (P) rats and alcohol-nonpreferring (NP) rats that had been selectively bred for high and low alcohol intake. The self-administration of intravenous nicotine (0.015–0.060 mg/kg per infusion) in P rats was more than twice that of NP rats. Nicotine seeking induced by reexposure to nicotine cues in extinction tests was also substantially greater in P rats than in NP rats. In a subsequent relapse test, priming nicotine injections reinstated drug seeking in P rats but not NP rats. P rats also self-administered higher amounts of oral sucrose (1–20%) than NP rats, a finding consistent with previous reports. In contrast, self-administration of intravenous cocaine (0.1875–1.125 mg/kg per infusion) was remarkably similar in the P and NP rats; however, P–NP differences in cocaine seeking emerged in subsequent extinction and cocaine priming-induced reinstatement tests. In both cases, lever responding was higher in P rats than in NP rats. Thus, alcohol-naive offspring of rats genetically selected for high alcohol intake are highly susceptible to nicotine self-administration and relapse, and this susceptibility is not likely caused by general reward deficits in NP rats. The present findings provide experimental evidence for the hypothesis that a shared genetic determinant accounts for the co-abuse of nicotine and alcohol.

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## NEUROBIOLOGY OF DISEASE

# MTH1, an Oxidized Purine Nucleoside Triphosphatase, Suppresses the Accumulation of Oxidative Damage of Nucleic Acids in the Hippocampal Microglia during Kainate-Induced Excitotoxicity

Kosuke Kajitani,<sup>1</sup> Hiroo Yamaguchi,<sup>1</sup> Yukihiro Dan,<sup>1</sup> Masato Furuichi,<sup>1,2</sup> Dongchon Kang,<sup>3</sup> and Yusaku Nakabeppu<sup>1</sup>

<sup>1</sup>Division of Neurofunctional Genomics, Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, <sup>2</sup>Radioisotope Center, Kyushu University, and <sup>3</sup>Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Enhanced oxidative stress has been implicated in the excitotoxicity of the CNS, and 8-oxo-7,8-dihydro-guanine (8-oxoG), a major type of oxidative damage in nucleic acids, was reported to be accumulated in the rat hippocampus after kainate administration. We herein showed that the 8-oxoG levels in mitochondrial DNA and cellular RNA increased significantly in the CA3 subregion of the mouse hippocampus 6–12 h after kainate administration but returned to basal levels within a few days. Laser-scanning confocal microscopy revealed the 8-oxoG accumulation in mitochondrial DNA to be remarkable in CA3 microglia, whereas that in nuclear DNA or cellular RNA was also detected in the CA3 pyramidal cells and astrocytes. 8-oxoG accumulation in cellular DNA or RNA should be suppressed by MutT homolog 1 (MTH1) with 8-oxo-dGTPase (8-oxo-7,8-dihydro-2'-deoxyguanosine triphosphatase) activity and 8-oxoG-DNA glycosylase 1 (OGG1) with 8-oxoG DNA glycosylase activity. We thus examined the

expression level of MTH1 and OGG1 in the mouse hippocampus after kainate administration. The *Mth1* mRNA level decreased soon after kainate administration and then quickly recovered beyond the basal level, and a continuously increased MTH1 protein level was observed, whereas the *Ogg1* mRNA level remained constant. MTH1-null and wild-type mice exhibited a similar degree of CA3 neuron loss after kainate administration; however, the 8-oxoG levels that accumulated in mitochondrial DNA and cellular RNA in the CA3 microglia significantly increased in the MTH1-null mice in comparison with wild-type mice, thus demonstrating that MTH1 efficiently suppresses the accumulation of 8-oxoG in both cellular DNA and RNA in the hippocampus, especially in microglia, caused by excitotoxicity.

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## Chronic Intermittent Ethanol-Induced Switch of Ethanol Actions from Extrasynaptic to Synaptic Hippocampal GABA<sub>A</sub> Receptors

Jing Liang,<sup>1,2</sup> Nianhui Zhang,<sup>3</sup> Elisabetta Cagetti,<sup>2</sup> Carolyn R. Houser,<sup>3</sup> Richard W. Olsen,<sup>2</sup> and Igor Spigelman<sup>1</sup>

<sup>1</sup>Division of Oral Biology and Medicine, School of Dentistry, University of California, Los Angeles, and Departments of <sup>2</sup>Molecular and Medical Pharmacology and <sup>3</sup>Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California 90095

Alcohol withdrawal syndrome (AWS) symptoms include hyperexcitability, anxiety, and sleep disorders. Chronic intermittent ethanol (CIE) treatment of rats with subsequent withdrawal of ethanol (EtOH) reproduced AWS symptoms in behavioral assays, which included tolerance to the sleep-inducing effect of acute EtOH and its maintained anxiolytic effect. Electrophysiological assays demonstrated a CIE-induced long-term loss of extrasynaptic GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) responsiveness and a gain of synaptic GABA<sub>A</sub>R responsiveness of CA1 pyramidal and dentate granule neurons to EtOH that we were able to relate to behavioral effects. After CIE treatment, the  $\alpha 4$  subunit-preferring GABA<sub>A</sub>R ligands 4,5,6,7 tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol, La<sup>3+</sup>, and Ro15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5- $\alpha$ ][1,4]benzodiazepine-3-carboxylate) exerted decreased effects on extrasynaptic currents but had increased effects on synaptic currents. Electron microscopy revealed an increase in central synaptic localization of  $\alpha 4$  but not  $\delta$  subunits within GABAergic synapses on the dentate granule cells of CIE rats. Recordings in dentate granule cells from  $\delta$  subunit-deficient mice revealed that this subunit is not required for synaptic GABA<sub>A</sub>R sensitivity to low [EtOH]. The profound alterations in EtOH sensitivity and  $\alpha 4$  subunit localization at hippocampal GABA<sub>A</sub>Rs of CIE rats suggest that such changes in these and other relevant brain circuits may contribute to the development of tolerance to the sleep-inducing effects and long-term dependence on alcohol.

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## Cell-Specific Repressor or Enhancer Activities of Deaf-1 at a Serotonin 1A Receptor Gene Polymorphism

Margaret Czesak,\* Sylvie Lemonde,\* Erica A. Peterson, Anastasia Rogueva, and Paul R. Albert

Ottawa Health Research Institute (Neuroscience), Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada K1H 8M5

The serotonin-1A (5-HT<sub>1A</sub>) receptor is the primary somatodendritic autoreceptor that inhibits the activity of serotonergic raphe neurons and is also expressed in nonserotonergic cortical and limbic neurons. Alterations in 5-HT<sub>1A</sub> receptor levels are implicated in mood disorders, and a functional C(-1019)G 5-HT<sub>1A</sub> promoter polymorphism has been associated with depression, suicide, and panic disorder. We examined the cell-specific activity of identified transcription factors, human nuclear deformed epidermal autoregulatory factor-1 (DEAF-1)-related (NUDR)/Deaf-1 and Hes5, at the 5-HT<sub>1A</sub> C(-1019) site. In serotonergic raphe RN46A cells, Deaf-1 and Hes5 repressed the 5-HT<sub>1A</sub> receptor gene at the C(-1019)-allele but not the G(-1019)-allele. However, in nonserotonergic cells that express 5-HT<sub>1A</sub> receptors (septal SN48, neuroblastoma SKN-SH, and neuroblastoma/glioma NG108–15 cells), Deaf-1 enhanced 5-HT<sub>1A</sub> promoter activity at the C(-1019)-allele but not the G-allele, whereas Hes5 repressed in all cell types. The enhancer activity of Deaf-1 was orientation independent and competed out Hes5 repression. To test whether Deaf-1 activity is intrinsic, the activity of a Gal4DBD (DNA binding domain)-Deaf-1 fusion protein at a heterologous Gal4 DNA element was examined. Gal4DBD-Deaf-1 repressed transcription in RN46A cells but enhanced transcription in SN48 cells, indicating that these opposite activities are intrinsic to Deaf-1. Repressor or enhancer activities of Deaf-1 or Gal4DBD-Deaf-1 were blocked by histone deacetylase inhibitor trichostatin A. Thus, the intrinsic activity of Deaf-1 at the 5-HT<sub>1A</sub> promoter is opposite in presynaptic versus postsynaptic neuronal cells and requires deacetylation. Cell-specific regulation by Deaf-1 could underlie region-specific alterations in 5-HT<sub>1A</sub> receptor expression in different mood disorders.

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